Ionic and Cellular Basis for the Predominance of the Brugada Syndrome Phenotype in Males

José M. Di Diego, MD; Jonathan M. Cordeiro, PhD; Robert J. Goodrow, BS; Jeffrey M. Fish, DVM; Andrew C. Zygmunt, PhD; Guillermo J. Pérez, PhD; Fabiana S. Scornik, PhD; Charles Antzelevitch, PhD

Background—The Brugada syndrome displays an autosomal dominant mode of transmission with low penetrance. Despite equal genetic transmission of the disease, the clinical phenotype is 8 to 10 times more prevalent in males than in females. The basis for this intriguing sex-related distinction is unknown. The present study tests the hypothesis that the disparity in expression of the Brugada phenotype is a result of a more prominent $I_{Na}$-mediated action potential notch in the right ventricular (RV) epicardium of males versus females.

Methods and Results—We studied epicardial tissue slices, arterially perfused wedge preparations, and dissociated epicardial myocytes isolated from male and female canine hearts. RV epicardium action potential phase 1 amplitude was 64.8±2.0% of that of phase 2 in males compared with 73.8±4.4% in females ($P<0.05$) at a cycle length of 2000 ms. $I_{Na}$ density was 26% smaller and time constant for inactivation 17% smaller at +40 mV in female versus male RV epicardial cells ($P<0.05$). The other functional characteristics of $I_{Na}$, including the voltage dependence of inactivation and time course of reactivation, were no different between the sexes. Pinacidil caused loss of action potential dome in male, but not female, RV epicardial tissue slices. Terfenadine (5 μmol/L) induced phase 2 reentry in 6 of 7 male but only 2 of 7 female arterially perfused wedge preparations. Two of 6 male and 1 of 2 female preparations developed polymorphic ventricular tachycardia/ventricular fibrillation.

Conclusions—Our results suggest that the predominance of the Brugada phenotype in males is a result of the presence of a more prominent $I_{Na}$ in males versus females. (Circulation. 2002;106:2004-2011.)

Key Words: ion channels • pinacidil • sex • epicardium

A syndrome of sudden death characterized by ST-segment elevation in right precordial leads V₁ to V₃, unrelated to ischemia or structural heart disease but at times accompanied by a right bundle-branch block morphology of the ECG, was described as a new clinical entity by Pedro and Josep Brugada in 1992.¹ The arrhythmic syndrome, named the Brugada syndrome in 1996,²,³ was first linked to mutations in SCN5A, the α-subunit of the sodium channel, and in the late 1990s was shown to cause loss of function.⁴,⁵ To date, more than 2 dozen distinct SCN5A mutations have been linked to the Brugada syndrome and shown to reduce sodium channel current by a variety of mechanisms (see Antzelevitch and Dumaine,⁶ Antzelevich,⁷ Balser,⁸ and Bezzina et al⁽⁹⁾ for references). Although SCN5A mutations appear to account for no more than 20% of cases of Brugada syndrome¹⁰ and another locus has been identified on chromosome 3,¹¹ the genetic diversity of the syndrome remains to be more fully delineated.

The Brugada syndrome displays an autosomal dominant mode of transmission with low penetrance (ie, the abnormal gene is inherited by 50% of the offspring, and both males and females equally inherit the defective gene, but not all will develop the disease). Despite equal genetic transmission of the disease, the clinical phenotype is 8 to 10 times more prevalent in males than in females.¹² The basis for this intriguing sex-related distinction is unknown.

We have previously proposed a cellular mechanism for the Brugada syndrome in which accentuation of the epicardial action potential notch and eventual loss of the epicardial action potential dome results in ST-segment elevation, phase 2 reentry, and polymorphic ventricular tachycardia/ventricular fibrillation (VT/VF).⁷,¹³–¹⁵ The proposed mechanism involves a rebalancing of the currents available at the end of phase 1 of the epicardial action potential. Diminution of inward currents ($I_{Na}$ and $I_{Na}$) or enhancement of outward currents ($I_{K}$, $I_{K,ATP}$, $I_{K,Ca}$) can result in an accentuation of the epicardial action potential notch as well as all-or-none repolarization at the end of phase 1. Although changes in $I_{Na}$ may not be responsible for precipitating the syndrome in most cases of Brugada syndrome, the presence of a prominent $I_{Na}$-mediated action potential notch appears to be...
a prerequisite. Indeed, the presence of a much greater \( I_c \) in right versus left ventricular epicardium (RV versus LV epicardium) accounts for the RV nature of the disease.\(^{16}\)

The present study tests the hypothesis that the disparity in penetrance of the Brugada phenotype between the sexes is caused by a more prominent \( I_c \)-mediated action potential notch in the RV epicardium of males versus females.

**Methods**

**Isolated Ventricular Epicardial Preparations**

RV and LV epicardial tissues (≈1.0 × 0.5 × 0.1 cm) were isolated from hearts retrieved from anesthetized (sodium pentobarbital, 35 mg/kg) mongrel male and female dogs. The preparations consisted of dermatomic shavings (Davol Simon Dermatome Power Handle 3293 with cutting head 3295) obtained from the anterobasal regions of the RV free wall. The tissues were superfused with oxygenated (95% \( \text{O}_2 \)/5% \( \text{CO}_2 \)) Tyrode’s solution maintained at 36.5°C to 37°C. The composition of the Tyrode’s solution was (in mmol/L): NaCl 129, KCl 4.0, NaH\(_2\)PO\(_4\) 0.9, NaHCO\(_3\) 20.0, CaCl\(_2\) 1.8, MgSO\(_4\) 0.5, and \( \text{d-glucose} \) 5.5; pH 7.4. All preparations were studied in the same bath solution by use of a roller pump. Temperature was maintained at 35°C and pressure between 40 and 50 mm Hg.

**Arterially Perfused Canine RV Wedge Preparations**

The methods used for isolation, perfusion, and recording of transmembrane activity from the arterially perfused canine RV (anterior wall) wedge preparation, as well as the viability and electrical stability of the preparation, have been detailed in previous studies.\(^{17,19}\) Briefly, transmural wedges with dimensions of approximately 2.0 × 1.0 to 2.5 × 1.5 × 1.2 cm were dissected from the RV of males and females. The descending branch of the right coronary artery was cannulated and perfused with cardioplegic solution. Unperfused tissue was carefully removed with a razor blade. The preparations were then placed in a small tissue bath and arterially perfused with Tyrode’s solution by use of a roller pump. Temperature was maintained at 35°C ± 0.5°C and pressure between 40 and 50 mm Hg. Temperature was maintained at 35°C ± 0.5°C and pressure between 40 and 50 mm Hg.

The endocardial surface of the wedge preparation was stimulated at phase 1 frequencies of 2000 ms and allowed to equilibrate 1 to 2 hours until it became electrically stable. A transmural ECG was recorded with electrodes consisting of AgCl half-cells placed in the Tyrode’s solution bathing the preparation, 1.0 to 1.5 cm from the epicardial and endocardial surfaces of the preparation, along the same axis as the transmembrane recordings (epicardium: + pole). Transmembrane action potentials were simultaneously recorded from 2 epicardial sites and 1 endocardial site with floating microelectrodes filled with 2.7 mol/L KCl.

**Isolated Myocyte Experiments**

Myocytes were prepared from canine hearts using techniques described previously.\(^{20,21}\)

**Solutions**

All solutions were made with Milli-Q grade water. Nominally Ca\(^{2+}\)-free dissecting buffer had the following composition (mmol/L): NaCl 129, KCl 4.0, MgSO\(_4\) 2.0, NaH\(_2\)PO\(_4\) 0.9, glucose 5.5, and NaHCO\(_3\) 20.0. This solution was bubbled with 95% \( \text{O}_2 \)/5% \( \text{CO}_2 \). The modified storage solution had the following composition (mmol/L): NaCl 129, CaCl\(_2\) 5.0, MgSO\(_4\) 2.0, NaH\(_2\)PO\(_4\) 0.9, glucose 5.5, NaHCO\(_3\) 20.0, BSA 1.5%, and CaCl\(_2\) 0.5. Myocytes were superfused with a HEPES buffer of the following composition (mmol/L): NaCl 140, KCl 4.0, MgCl\(_2\) 1.0, CaCl\(_2\) 2.0, HEPES 10, and glucose 10. pH was adjusted to 7.4 with NaOH. In addition, 300 \( \mu\text{mol/L} \) CdCl\(_2\) was added to the HEPES buffer to block L-type Ca\(^{2+}\) current.

**Recording Techniques**

Voltage-clamp recordings of the Ca\(^{2+}\)-dependent transient outward current (\( I_c \)) were made from epicardial cells isolated from hearts of both male and female dogs. Patch pipettes were filled with pipette solution of the following composition (mmol/L): KCl 10, aspartate 125, MgATP 5, MgCl\(_2\) 1.0, HEPES 10, NaCl 10, and EGTA 5, with pH adjusted to 7.1 with KOH. The pipette resistance ranged from 1 to 4 \( \Omega \) when filled with the internal solution. \( I_c \) was recorded with an Axopatch 200 amplifier (Axon Instruments Inc), and series resistance errors were reduced by 65% to 70% with electronic compensation. Membrane currents were acquired at 10 kHz (Digidata 1200, Axon Instruments) and analyzed with a microcomputer running pClamp 8.0 software (Axon Instruments).

**Drugs**

Terfenadine (5 \( \mu\text{mol/L} \)) was used to induce the Brugada syndrome in the arterially perfused wedge. To facilitate loss of the epicardial action potential dome, the preparations were paced at a cycle length of 500 ms for up to 30 seconds, then 400 ms for up to 30 seconds, then either 600 ms or 800 ms for up to 30 seconds, followed by a return to cycle length of 2000 ms. The occurrence of a closely coupled extrasystole via a phase 2 reentrant mechanism was defined as a positive arrhythmogenic response. Preparations that failed to exhibit an arrhythmia with the above protocol after 2 hours of exposure to terfenadine were considered to show a negative result.

Pinacidil was used to induce phase 2 reentry in isolated tissues. The drug (Leo Pharmaceutical Products) was dissolved in ethanol (2% vol/vol) to yield a stock solution of 1 mmol/L.

**Statistics**

Statistical analysis was performed with either unpaired or paired \( t \) test or ANOVA coupled with Student-Newman-Keuls for all pairwise multiple comparisons, as appropriate. Summary data are reported as mean±SEM.

**Results**

The distinctions in the early phases and other characteristics of the epicardial action potential recorded from tissue slices isolated from male and female canine RV myocardium are presented in Figure 1 and Table 1. Superimposed action potentials recorded at BCLs of 300, 500, 800, and 2000 ms (Figure 1, A and B) and average data on the rate dependence of phase 1 amplitude and voltage at the end of phase 1 (V/phase 1, mV) in male versus female RV epicardium (Figure 1C) are shown. Phase 1 amplitude is significantly smaller in males (60.8±1.07 versus 73.25±2.55 mV in females at a BCL of 2000 ms), and the voltage at the end of phase 1 is correspondingly more negative (−26.2±1.4 versus −12.9±2.6 mV in females). Both parameters display a prominent rate dependence owing to the relatively slow recovery of \( I_c \) from inactivation. The sex-mediated distinctions and the rate dependence of these parameters were largely abolished with the addition of 2 mmol/L 4-aminopyridine to inhibit \( I_c \) (data not shown). Interestingly, we did not observe any significant difference in action potential duration at 90% repolarization between males and females in RV, as previously reported for LV.

Figure 2 contrasts data relative to the early phases of the action potential obtained from RVs and LVs of male and female dogs. Representative action potentials are depicted in the insets, highlighting the great diversity of epicardial action.
potential morphologies present in the ventricular myocardium. Phase 1 amplitude was larger and the voltage at the end of phase 1 correspondingly less negative in LV versus RV epicardium. Phase 1 amplitude was significantly smaller and the voltage at the end of phase 1 more negative in males versus females in RV, but not in LV.

Because the characteristics of phase 1 of the action potential are determined primarily by \( I_{\text{to}} \), in another series of experiments we used whole-cell patch-clamp techniques to quantify \( I_{\text{to}} \) in myocytes enzymatically dissociated from RV and LV epicardium of male and female hearts (Figure 3). \( I_{\text{to}} \) density as a function of the test potential, the voltage pulse protocol, and representative current traces are shown in Figure 3A. Average peak \( I_{\text{to}} \) density was significantly greater in male than female RV epicardium at membrane potentials of +10 mV or more (\( P<0.05 \)). In contrast, \( I_{\text{to}} \) density was considerably smaller in LV, and sex-related differences were not observed. These results are consistent with the distinc-

**Figure 1.** Transmembrane action potentials recorded from isolated canine RV epicardial male (A) and female (B) tissue slices. BCLs=300, 500, 800, and 2000 ms. C, Rate dependence of phase 1 amplitude and voltage at end of phase 1 (V/phase 1, mV) in males (solid squares) vs females (solid circles).

**TABLE 1. Action Potential Parameters of Male and Female Canine RV Epicardium**

<table>
<thead>
<tr>
<th></th>
<th>Male RV epicardium (n=5)</th>
<th>Female RV epicardium (n=8)</th>
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<tbody>
<tr>
<td></td>
<td>BCL, ms</td>
<td></td>
</tr>
<tr>
<td>Phase 0, mV</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>Phase 1 amplitude, mV</td>
<td>95.8±2.5</td>
<td>97.4±2.7</td>
</tr>
<tr>
<td>Phase 2, mV</td>
<td>70.0±2.2*</td>
<td>65.2±1.8†</td>
</tr>
<tr>
<td>Overshoot 0, mV</td>
<td>86.4±1.0</td>
<td>91.0±1.6</td>
</tr>
<tr>
<td>Phase 1 magnitude, mV</td>
<td>11.0±2.7</td>
<td>11.8±2.7</td>
</tr>
<tr>
<td>V/phase 1, mV</td>
<td>25.4±1.8*</td>
<td>32.0±1.4†</td>
</tr>
<tr>
<td>APD90, ms</td>
<td>14.8±2.5*</td>
<td>20.4±2.0†</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>101.6±4.5</td>
<td>168.2±5.1</td>
</tr>
<tr>
<td>Rusher potential, mV</td>
<td>84.8±0.7</td>
<td>85.6±0.5</td>
</tr>
</tbody>
</table>

*\( P<0.05 \) vs female RV epicardium; †\( P<0.01 \) vs female RV epicardium.
tions in the characteristics of the epicardial action potential notch between RV and LV as well as between males and females (see Figures 1 and 2). The time course of reactivation (Figure 3B) and voltage dependence of inactivation (Figure 3C) were similar between the sexes; no significant differences were observed in RV epicardial myocytes from males versus females. The times constant of decay of $I_{K_{\text{ATP}}}$ was 17% smaller in females ($7.95 \pm 1.25$ ms in females and $9.56 \pm 2.28$ ms in males, $P<0.001$). Consequently, the total charge carried by the current was 62% greater in males than females ($414.7 \pm 101.8$ versus $256.3 \pm 43.3$ fC/pF, $P<0.05$).

In another series of experiments, we used isolated RV tissue slices and arterially perfused wedge preparations to assess the functional significance of these sex-related differences in action potential morphology. In previous studies, we demonstrated that interventions capable of increasing the magnitude of the epicardial action potential notch, either by increasing outward currents (ie, $I_{K_{\text{ATP}}}$ and/or $I_{\text{Na}}$ and/or $I_{\text{Ca}}$), can alter the balance of currents active during the early phases of the action potentials and lead to all-or-none repolarization at the end of phase 1. In the present study, we used the $I_{K_{\text{ATP}}}$ opener pinacidil in an attempt to induce phase 2 reentry.

Pinacidil (5 $\mu$mol/L) abolished the action potential dome in 4 of 4 male and 0 of 3 female RV epicardial tissue slices (Figure 4). Figure 4A shows superimposed action potentials recorded at BCLs of 300 to 2000 ms from male (top) and female preparations before (left) and after pinacidil (5 $\mu$mol/L; 45 minutes). Pinacidil caused all-or-none repolarization at the end of phase 1, leading to loss of the dome and marked abbreviation of the action potential in the male preparations, but only a slight depression of the action potential I plateau (phase 2) in the female preparation (Figure 4B). Table 2 summarizes the differential response of male and female preparations to the potassium channel opener. At the high concentration of pinacidil (5 $\mu$mol/L) used, loss of the dome occurred throughout the male RV epicardial tissue slice. With lower concentrations of the drug, loss of the dome in the male RV epicardium was generally heterogeneous, leading to the development of phase 2 reentry. Figure 4C illustrates an example of phase 2 reentry induced by 3 $\mu$mol/L pinacidil. Propagation of the dome from a site at which it is maintained to a site at which it is lost results in reexcitation of the preparation and the development of a closely coupled extrasystole.

In a final series of experiments, we sought to determine whether the sex-related electrophysiological differences and arrhythmogenic manifestations are observed in the intact wall of the canine RV. In this series, we used terfenadine, a combined sodium and calcium channel blocker, to induce ST-segment elevation, phase 2 reentry, and VT/VF in RV wedge preparations isolated from the hearts of male and female dogs (Figure 5). Figure 5A shows the effect of terfenadine (5 $\mu$mol/L) to cause loss of the action potential dome at some epicardial sites, but not at others and not in endocardium. Accentuation of the action potential notch and loss of the dome in epicardium but not endocardium results in an ST-segment elevation in the ECG. The wide notch in the epicardial action potentials in which the dome is maintained serves to delay repolarization of the epicardial response.
beyond that of endocardium, resulting in inversion of the T wave. Propagation of the dome from the site at which it is maintained to a site at which it is lost (arrow) results in a closely coupled phase 2 reentrant extrasystole. Note that the Q wave of the phase 2 reentrant beat fuses with the inverted T wave to accentuate its appearance. Figure 5B illustrates a typical response recorded in a female RV wedge preparation under identical conditions. Terfenadine (5 μmol/L) induced phase 2 reentry in 6 of 7 male, but only 2 of 7 female, wedge preparations (Figure 5D). In 2 of the male preparations, phase 2 reentry triggered a polymorphic VT (Figure 5C). The I_{to} blocker 4-aminopyridine (2 mmol/L) restored the action potential dome, normalized the ECG, and prevented the development of terfenadine-induced phase 2 reentry and VT (n=3, not shown).

**Discussion**

The sex-related difference in the phenotypic expression of the Brugada syndrome is more pronounced than with any other autosomally transmitted arrhythmic syndrome. Although the genotype is transmitted in equal proportion to males and females, the manifestation of the clinical syndrome is observed ≈10 times more often in males than in females. This low penetrance of the disease in females led to the Southeast Asian custom of men dressing in women’s clothes at bedtime to fool the “evil spirits” that were presumed to target males in their sleep. The basis for this disparity between the sexes has long defied explanation.12,22–26 Our study provides insights into the cellular and ionic bases for the sex-related distinctions, showing that the presence of a more prominent I_{to} in males underlies their predisposition to development of the Brugada phenotype. Our study points to clear differences in I_{to} density and inactivation kinetics in RV epicardial cells isolated from male versus female canine hearts. The less prominent I_{to} in females is probably secondary to the more rapid inactivation kinetics exhibited by the channels in females as well as differences in channel density, although the latter remains to be determined. The other functional characteristics of I_{to}, including the voltage dependence of inactivation and time course of reactivation, are no different between the sexes (Figures 1 to 3).

Our data also indicate that I_{to} is still smaller in LV than in RV epicardium but that sex-related differences are not significant in the LV. These distinctions in I_{to} between LV and RV epicardium, previously reported,16 form the basis for why the Brugada syndrome is an RV disease.7 The functional significance of a prominent I_{to} has long been recognized. In previous studies, we and others have shown that the presence of a prominent I_{to}-mediated spike-and-dome morphology in RV epicardium but not endocardium is in large part responsible for the differential response of these 2 tissue types to simulated ischemia, sodium channel blockers, flecainide, pinacidil, and elevated extracellular calcium combined with rapid pacing.27,28 The present study demonstrates similar distinctions between male and female RV epicardium.

A greater contribution of I_{to} to phase 1 of the action potential results in a more negative termination of phase 1. When the end of phase 1 achieves potentials negative to the threshold for activation of the calcium current (I_{Ca}), all-or-none repolarization of the action potential at the end of phase 1 results, causing loss of the action potential dome and marked abbreviation of action potential duration. Agents that cause an outward shift in the balance of current, either by inhibiting I_{Na} (flecainide) or I_{Ca} (verapamil) or by activating I_{K-ATP} (pinacidil) are capable of causing loss of the action potential dome in RV epicardium by causing phase 1 to dip below the threshold for activation of I_{Ca}.29 In the present

**Figure 3.** Sex-based and interventricular differences in I_{to}. A, Mean I-V relationship for I_{to} recorded from RV epicardial cells isolated from hearts of male and female dogs. Inset, Representative I_{to} current traces and voltage protocol. I_{to} density was significantly greater in male vs female RV epicardial cells. No sex differences were observed in LV. B, Recovery of I_{to} in male vs female RV epicardial cells. Inset, Representative current traces and double pulse voltage protocol. C, Voltage dependence of inactivation of I_{to} in male vs female RV epicardial cells. Inset, Representative current traces and voltage protocol. Values are mean±SEM. *P<0.05 vs female RV epicardium.
study, we demonstrate the effect of the $I_{K-ATP}$ activator pinacidil and of terfenadine, an agent capable of inhibiting both $I_{Na}$ and $I_{Ca}$ (in addition to other currents), to more readily cause loss of the action potential dome in male versus female RV epicardium.

Because loss of the dome is usually heterogeneous, agents with this capability create both an epicardial and transmural dispersion of repolarization. The transmural dispersion creates voltage gradients that manifest as a J wave or ST-segment elevation and give rise to a vulnerable window across the ventricular wall. The epicardial dispersion provides the substrate for phase 2 reentry, which in turn provides the extrasystole that captures the transmural vulnerable window to precipitate a reentry, usually in the form of a polymorphic ventricular tachycardia.

**TABLE 2. Effect of 5 $\mu$mol/L Pinacidil on Action Potential Parameters in Male and Female RV Epicardial Tissues**

<table>
<thead>
<tr>
<th>BCL, ms</th>
<th>Control</th>
<th>Pinacidil</th>
<th>Control</th>
<th>Pinacidil</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male RV epicardium (n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 0 amplitude, mV</td>
<td>97.3±3.7</td>
<td>97.0±3.2</td>
<td>98.0±3.0</td>
<td>96.5±3.2</td>
</tr>
<tr>
<td>Phase 1 amplitude, mV</td>
<td>65.7±2.6</td>
<td>61.7±3.3</td>
<td>60.5±0.9</td>
<td>58.5±0.9</td>
</tr>
<tr>
<td>Phase 2 amplitude, mV</td>
<td>96.0±5.3</td>
<td>61.7±3.3†</td>
<td>100.5±3.3</td>
<td>58.5±0.9†</td>
</tr>
<tr>
<td>V/phase 1, mV</td>
<td>−19.3±2.9</td>
<td>−23.3±2.7†</td>
<td>−25.3±1.9</td>
<td>−26.5±1.2</td>
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<tr>
<td>APD$_{90}$, ms</td>
<td>175.0±4.5</td>
<td>87.3±14.0†</td>
<td>203.3±4.1</td>
<td>93.7±9.1†</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>−85.0±0.6</td>
<td>85.0±0.6</td>
<td>−85.7±1.1</td>
<td>−85.0±0.8</td>
</tr>
<tr>
<td>Female RV epicardium (n=3)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase 0 amplitude, mV</td>
<td>98.0±1.5</td>
<td>95±2.7</td>
<td>96.7±0.9</td>
<td>97.0±3.1</td>
</tr>
<tr>
<td>Phase 1 amplitude, mV</td>
<td>78.3±6.4</td>
<td>77.33±5.9</td>
<td>75.7±6.4</td>
<td>74.0±6.1</td>
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<tr>
<td>Phase 2 amplitude, mV</td>
<td>92.0±5.1</td>
<td>87.33±5.8</td>
<td>92.3±4.3</td>
<td>85.0±8.5</td>
</tr>
<tr>
<td>V/phase 1, mV</td>
<td>−8.0±5.9</td>
<td>−8.0±5.3</td>
<td>−11.0±5.8</td>
<td>−12.0±5.3</td>
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<tr>
<td>APD$_{90}$, ms</td>
<td>157.3±7.5</td>
<td>132.0±6.6*</td>
<td>181.0±6.5</td>
<td>147.0±14.0</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>−86.3±0.7</td>
<td>−85.33±0.7</td>
<td>−86.7±0.7</td>
<td>−86.0±1.1</td>
</tr>
</tbody>
</table>

*P<0.05 vs control RV epicardium; †P<0.01 vs control RV epicardium.

Figure 4. Pinacidil induces phase 2 reentry in male, but not female, RV epicardium. Each panel shows superimposed action potentials recorded at BCLs of 300, 500, 800, and 2000 ms in absence (control) and presence of pinacidil (5 $\mu$mol/L, 45 minutes) in male (A) and female (B) RV epicardial tissue slices. Graphs at right show rate dependence of phase 2 amplitude (peak plateau) in males vs females (n= 4) before (solid squares) and after pinacidil (open squares). C, Pinacidil (3 $\mu$mol/L)-induced phase 2 reentry in male RV epicardium at a BCL of 1000 ms.
VT. Terfenadine induced the arrhythmogenic substrate displaying the characteristics of Brugada syndrome in 6 of 7 male, but only 2 of 7 female, RV wedge preparations (Figure 5). The molecular mechanisms underlying sex-related differences in electrophysiology and predisposition to arrhythmic disease are poorly understood. Kv4.3 has been shown to be downregulated, resulting in reduced \( I_{to} \) in rat myometrium at the end of pregnancy caused by a rise in estrogen levels. This finding suggests the hypothesis that estrogen may regulate the expression of \( I_{to} \) channels by diminishing the transcription of Kv4.3.

The protective nature of a smaller \( I_{to} \) density in the RV epicardium of females compared with males and the direct effect of 4-aminopyridine to abort phase 2 reentry and VT/VF in the perfused wedge model offer further compelling evidence for \( I_{to} \) inhibition as a potential therapeutic strategy for patients afflicted with the Brugada syndrome.

Acknowledgments

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References


Figure 5. Terfenadine induces Brugada phenotype more readily in male than female RV wedge preparations. Each panel shows action potentials recorded from 2 epicardial sites and 1 endocardial site, together with a transmural ECG. Control recordings were obtained at a BCL of 2000 ms, whereas terfenadine data were recorded at a BCL of 800 ms after a brief period of pacing at a BCL of 400 ms. A, Terfenadine (5 \( \mu \)mol/L)-induced, heterogeneous loss of action potential dome, ST-segment elevation, and phase 2 reentry (arrow) in a male RV wedge preparation. B, Terfenadine fails to induce Brugada phenotype in a female RV wedge preparation. C, Polymorphic VT triggered by spontaneous phase 2 reentry in a male preparation. D, Incidence of phase 2 reentry in male (6 of 7) vs female (2 of 7) RV wedge preparations when perfused with 5 \( \mu \)mol/L terfenadine for up to 2 hours.


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