Mechanisms of sex differences in rat cardiac myocyte response to β-adrenergic stimulation

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The results of many investigations document gender differences in basal cardiac function (14, 15, 29, 30), response to increased loading (exercise) (1, 7, 8, 16, 17, 29) and pathological pressure overload (6, 24, 43). The sympathetic nervous system plays a central role in regulating heart function and response to load stress through β-adrenergic receptor stimulation. Binding of β-adrenergic agonists to receptors in the heart activates adenyl cyclase via a stimulatory G protein. The resultant production of cAMP activates protein kinase A and the kinase phosphorylates cellular proteins including the α1c subunit of the L-type Ca2+ channel (10, 19). The result is an increase in the inward Ca2+ current (I_{Ca}) and increased force of contraction.

Animal studies (4, 27, 34, 35, 36) confirm gender differences in myocardial function and response to exercise and pathophysiological load and provide models for investigation of mechanisms responsible for these sex differences. Using an isolated atrial model, our laboratory (37) recently described sex differences in contractile function and in response to β-adrenergic stimulation. The primary purpose of this study is to investigate sex differences in isolated rat ventricular myocyte response to β-adrenergic stimulation. Our study is the first to use isolated ventricular cells to study sex differences in response to β-adrenergic stimulation in the absence of neurohormonal and hemodynamic influences. Specifically, we investigated potential sex differences in 1) control (field stimulated in the absence of drug) and isoproterenol-stimulated ventricular myocyte shortening, 2) β-adrenergic receptor and L-type Ca2+ channel density and affinity, 3) control and isoproterenol-stimulated myocyte cAMP content, and 4) control and isoproterenol-stimulated I_{Ca}. Theoretically, sex differences in response to β-adrenergic agonists could contribute to gender differences in heart function and pathology.

MATERIALS AND METHODS

Animals. Sexually mature, weight-matched (270–290 g weight range) male and female Sprague-Dawley rats were used in all the studies. Same-sex animals were housed three to a cage with free access to water and rat chow and were maintained on 12-h day/night cycles. Treatment of animals conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1985).

Myocyte isolation methods. Ventricular myocytes for shortening studies were isolated by an enzymatic dissociation procedure as previously described by Pyo and Wahler (31). Thirty minutes before the heart was removed, animals were anesthetized with pentobarbital sodium (50 mg/kg ip) and heparinized (200 U ip) to prevent coagulation. The heart was removed via sternotomy and placed in cold (4°C) nominally Ca2+-free solution containing (in mM) 133.5 NaCl, 1.2
NaH2PO4, 4.0 KCl, 1.2 MgSO4, 10.0 HEPES, 11.1 glucose, and 1 mg/ml fatty acid-free albumin (pH 7.4). The heart was retrogradely perfused for 5 min with oxygenated (100% O₂) nominally Ca²⁺-free solution (37°C), followed by perfusion (16.5 min/g wet wt) with the same solution containing 5 μM CaCl₂ and 50 U/ml of type II collagenase (pH 7.4) (Worthington Biochemical; Freehold, NJ). Ventricular tissue was then removed and periodically triturated for 10 min in warm (37°C) storage solution (Ca²⁺-free solution + 50 μM CaCl₂) to obtain single myocytes. Isolated myocytes were resuspended in centrifuge tubes and allowed to settle for 15 min, and the supernatant was then discarded. This procedure was repeated once more, and the final cell pellet was resuspended in storage solution.

Ventricular myocytes for electrophysiologic studies were isolated using a similar method with the following modifications (20). Isolated hearts were briefly perfused with a modified warmed, oxygenated (37°C, 95% O₂-5%CO₂) nominally Ca²⁺-free buffer containing (in mM) 118.5 NaCl, 14.5 NaHCO₃, 2.6 KCl, 1.18 MgCl₂, 11.1 glucose, and 1 mg/ml fatty acid-free albumin (pH 7.4). The heart was then minced and triturated in storage solution containing (in mM) 118.5 NaCl, 14.5 NaHCO₃, 2.6 KCl, 1.18 MgCl₂, 11.1 glucose, and 1 mg/ml fatty acid-free albumin (pH 7.4). The ventricles were homogenized (in 0.25 M sucrose with 0.1% sodium dodecyl sulfate for measurement of total protein content using bicinchoninic acid protein assay reagent kit (Pierce; Rockford, IL)).

Ventricular membrane preparation. Ligand binding, (–) [³²P]iodocyanopindolol ([³²P]ICYP) and isradipine ([³²P]PN200–110), was determined in ventricular membrane preparations. Isolated hearts from male and female rats were placed in cold (4°C) normal saline, and the left ventricle was dissected out. Wet weights were obtained on a combined low-salt buffer containing (in mM) 50 Tris-HCl, 3 MgCl₂, 1 EDTA, and proteases (pH 7.4). The ventricles were homogenized with the use of Polytron for two 5-s bursts at a setting of 5. The homogenate was then centrifuged at 100,000 g for 60 min at 4°C. The resulting pellet was resuspended in homogenizing buffer at a protein concentration of 200 μg/100 μl. Total pellet protein content was determined by bicinchoninic acid assay (Sigma).

Myocyte shortening measurement. Isolated ventricular myocytes were placed in a bath chamber, on the stage of an inverted microscope (Diaphot-TMD, Nikon), and allowed to attach to the glass bottom of the chamber. Myocyte shortening was measured in healthy, rod-shaped cells characterized by clear striations and no spontaneous contractile activity. Cells were continuously superfused with control bathing solution (37°C) containing (in mM) 133.5 NaCl, 4.0 CsCl, 1.0 CaCl₂, 1.2 MgSO₄, 10 HEPES, and 11.1 glucose (pH 7.4) and electrically paced with field stimulation (0.8 Hz, 12-ms duration, voltage intensity was twofold greater than that needed to elicit shortening). Unloaded cell shortening was measured with a video edge-detection system (51). An image of the cell was projected onto a television monitor by a charge-coupled device camera (TM-640 Sequential Scanning Camera, Pulnix). An oscilloscope monitored the signal emitted by cell edge movement, and a tracing of the signal was printed on a chart recorder (Dash II, Astromed; W. Warwick, RI). Maximal control (shortening in response to field stimulation and in the absence of drug) and isoproterenol-stimulated myocyte shortening was calculated from the chart recording and normalized to resting cell length.

Recording of whole cell Ca²⁺ current. Isolated myocytes were placed in an experimental bath chamber and allowed to attach to the glass bottom. The cells were superfused with the following bath solution (in mM): 133.5 NaCl, 4.0 CsCl, 1.0 CaCl₂, 1.2 MgCl₂, 11.1 glucose, and 10.0 HEPES, pH 7.4. Standard whole cell voltage clamp methods were used to record I_Ca(13), as routinely used in our laboratory (20). Patch pipettes with resistances of 2–4 MΩ were pulled from borosilicate glass capillary tubes with a two-stage pull technique, fire-polished, and filled with pipette solution composed of (in mM) 120 CsCl, 10 EGTA, 5 Na₂ATP, and 10 HEPES, pH 7.2. The junction potential was electronically zeroed just before the formation of a seal between the pipette and the cell membrane. Cell membrane capacitance was estimated by integrating the area under the capacitative transient (generated by a 5-mV pulse) and by dividing by the voltage pulse. The L-type I_Ca was elicited by 200 ms pulses to 0 mV from a holding potential of ~80 mV (after a brief prepulse to ~40 mV) at a frequency of 0.2 Hz. Prepubilising the membrane to ~40 mV eliminated the Na⁺ current (and any T-type Ca²⁺ currents). K⁺ currents were eliminated by substitution of K⁺ in the bath and pipette solution with Cs⁺. Control I_Ca was measured in the absence of drug, and the isoproterenol-stimulated I_Ca was measured in the presence of isoproterenol (1 μM) in the bath solution.
Current-voltage (I-V) curves were generated by increasing command potentials in 10 mV steps from -40 to +50 mV from a holding potential of -80 mV. Voltage-clamp protocols, data acquisition, and storage were accomplished by the use of pCLAMP software (version 5.1, Axon Instruments, Foster City, CA) and a digital-to-analog converter (Labmaster). Membrane currents were sampled at 5 kHz and stored on a computer. For $I_{Ca}$ analysis, currents were filtered at 2 kHz. $I_{Ca}$ was measured as the difference between the peak current and current at the end of the 200-ms pulse. The $I_{Ca}$ recorded in male and female myocytes was expressed as the $I_{Ca}$ density ($I_{Ca}$ normalized to cell capacitance).

To determine the effect of extracellular Ca$^{2+}$ on $I_{Ca}$ in male and female myocytes, the L-type $I_{Ca}$ was measured in the presence of 0.5, 1.0, 2.0, and 4.0 mM extracellular Ca$^{2+}$, according to the whole cell voltage clamp methods as previously described. The control $I_{Ca}$ was recorded in the presence of 1.0 mM CaCl$_2$.

Radioligand binding studies. β-Adrenergic receptor binding experiments were performed at 37°C in a final volume of 500 μl containing 100 μg membrane protein, 50 mM MgCl$_2$, 10 mM Tris·HCl (pH 7.4), and [125I]ICYP (0–220 pM) (specific activity 2,000 Ci/mmol). Nonspecific binding was determined in the presence of propranolol (1 μM) in duplicate sets of tubes. The reaction was terminated by rapid filtration through presoaked GF/B filters (0.3% polyethylenimine) (Whatman; Clifton, NJ) using a cell harvester (Brandel; Gaithersburg, MD). Filters were washed three times with 4 ml of ice-cold assay buffer. Bound [125I]ICYP was measured by gamma counting. Specific binding was determined by subtracting nonspecific binding from total binding. The dissociation constant ($K_d$) and maximum binding capacity ($B_{max}$) were determined using a nonlinear regression method for a one-site binding fit (Pism; GraphPad Software; San Diego, CA).

Dihydropyridine receptor binding experiments were performed under sodium lighting at 26°C in a final volume of 500 μl containing 200 μg membrane protein, 50 mM MgCl$_2$, 1 mM EDTA (pH 7.4) and tritiated [3H]PN200–110 (specific activity 2,000 Ci/mmol). Nonspecific binding was determined with the addition of nifedipine (1 μM) to duplicate sets of tubes. The reaction was terminated by rapid filtration as described above. Bound [3H]PN200–110 was determined by liquid scintillation counting. The $K_d$ and $B_{max}$ were determined as previously described.

cAMP determination. Isolated myocytes were incubated for 2 min in “bath solution” in the absence and presence of isoproterenol (1 μM). The cells were then centrifuged at a high speed (30 s; model 5415C, Eppendorf), and the supernatant was discarded. Ice-cold 65% ethanol (600 μl) was added to the cell pellet and mixed by vortexing. The cells were centrifuged again (20 min, high speed, 4°C, Eppendorf 5414C). The supernatant was stored in glass test tubes at 4°C until determination of cAMP content by a commercially available radioimmunoassay kit (Biomedical Technologies; Stoughton, MA). The cell pellet was resuspended in 1% sodium dodecyl sulfate for protein determination. The control and isoproterenol-stimulated cAMP content was expressed as pmol cAMP/mg cell protein.

Drugs and materials. All chemical compounds and drugs, except for radiolabeled receptor ligands, were from Sigma. [3H]PN200–110 and [125I]ICYP were purchased from Amer sham Pharmacia Biotech (Piscataway, NJ). All drug stock solutions and homogenizing and assay buffer solutions were prepared daily. A stock solution of 10–3 M isoproterenol was prepared in MilliQ water and stored in a dark, capped storage container to protect from light and air exposure and was used to prepare a final drug solution of 1 μM isoproterenol in bath solution. All responses to isoproterenol were measured in the presence of 1 μM of the drug. Propranolol, [125I]ICYP, and [3H]PN200–110 were prepared in their respective assay buffer solutions. A stock solution of 10–3 M nifedipine was prepared in dimethyl sulfoxide and used to make a final 10–6 M drug solution in assay buffer. [3H]PN200–110 and nifedipine solutions were prepared under sodium lighting.

Statistical analysis. Values are expressed as the means ± SE for the following measures in female and male rats: 1) body weight, heart and left ventricular wet weights, cell length, and capacitance; 2) control and isoproterenol-stimulated myocyte shortening; 3) control and isoproterenol-stimulated peak $I_{Ca}$; 4) control and isoproterenol-stimulated cAMP content; and 5) $B_{max}$ and $K_d$ for ICYP and [3H]PN200–110. Significant sex differences in rat and cell characteristics and the $B_{max}$ and $K_d$ from binding studies were determined by unpaired t-tests. Significant sex- and drug-dependent differences were determined by two-way ANOVA. Post hoc analysis to determine specific sex- and drug-dependent effects was determined by independent Student’s t-tests. The level of significance was set at $P \leq 0.05$ with Bonferroni correction when appropriate.

RESULTS

Female and male rat physical characteristics are presented in Table 1. Body and heart wet weights were not significantly different between males and females. Male myocytes were significantly longer than female myocytes. No significant difference was found in cell capacitance, an indirect measure of cell membrane area.

Effect of isoproterenol on myocyte shortening. Isoproterenol (1 μM) elicited a significant increase in myocyte shortening compared with control in both male and female cells (Fig. 1A) indicating β-adrenergic-mediated enhancement of myocyte contractile function in both sexes ($P \leq 0.01$). Fractional shortening in the absence of drug tended to be greater in female than in male cells (9.7 ± 1.1 and 8.4 ± 0.6%, respectively); however, the difference was not significant. The isoproterenol-induced change in myocyte shortening (expressed as % change from control shortening) was significantly greater in males compared with females ($P \leq 0.05$) (Fig. 1B). This indicates a sex difference in β-adrenergic-mediated enhancement of myocyte contractile function.

Table 1. Body weight, heart weight, and cell characteristics of myocytes isolated from male and female rats

<table>
<thead>
<tr>
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<th>Female</th>
<th>Male</th>
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<tr>
<td>Body weight, g</td>
<td>269 ± 3.0</td>
<td>273 ± 3.0</td>
</tr>
<tr>
<td>Heart wet weight, g</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Cell length, μm</td>
<td>101.0 ± 5.0</td>
<td>127.0 ± 5.0*</td>
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<tr>
<td>Cell capacitance, pF</td>
<td>154.0 ± 8.0</td>
<td>160.0 ± 7.0</td>
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Values are means ± SE. Body and heart weight was determined in 23 male and 24 female rats. Cell length was determined in 26 female cells from 6 female hearts and 24 male cells from 5 male hearts. Cell capacitance was determined in 23 cells from 8 female hearts and in 22 cells from 7 male hearts. *$P \leq 0.001$, significantly different from female.

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The relationship between membrane potential and Ca\(^{2+}\) current (the I-V relationship) in the absence of isoproterenol was bell shaped as is typically found (Fig. 2), and there was no significant difference between the male and female I-V relationship. The maximum current density occurred at 0 mV for both of the sexes. In the presence of 1 mM extracellular Ca\(^{2+}\), the maximal current density was \(-7.2 \pm 0.7\) pA/pF in females and the maximal current density was \(-6.2 \pm 0.8\) pA/pF in males.

**Effect of isoproterenol on female and male myocyte Ca\(^{2+}\) current.** Figure 3A shows superimposed Ca\(^{2+}\) current tracings from a male myocyte recorded in the presence and absence of isoproterenol (control conditions). The control L-type \(I_{\text{Ca}}\) was significantly less in male myocytes compared with the \(I_{\text{Ca}}\) recorded in female myocytes in the absence of β-adrenergic receptor stimulation (Fig. 3B). The mean \(I_{\text{Ca}}\) density in females was \(-5.6 \pm 0.5\) pA/pF, whereas in males, the mean \(I_{\text{Ca}}\) density was \(-3.9 \pm 0.6\) pA/pF. Isoproterenol (1 μM)
significantly increased the amplitude of $I_{Ca}$ in both sexes. However, the magnitude of the drug-induced response was significantly greater in male compared with female myocytes (Fig. 3C).

Effect of changes in extracellular Ca$^{2+}$ on female and male myocyte Ca$^{2+}$ current. $I_{Ca}$ was examined in male and female myocytes in the presence of 0.5, 1.0, 2.0, and 4.0 mM extracellular Ca$^{2+}$. At every concentration of extracellular Ca$^{2+}$, a significantly greater $I_{Ca}$ was recorded in female myocytes compared with male myocytes ($P \leq 0.05$) (Fig. 4A). However, the extracellular Ca$^{2+}$-induced change in the $I_{Ca}$ (expressed as percent change from $I_{Ca}$ measured at 1.0 mM extracellular Ca$^{2+}$) was not significantly different between males and females (Fig. 4B). In addition, no sex difference was found if the results were expressed as percentage of maximal $I_{Ca}$ (data not shown). This indicates that the relationship between the extracellular Ca$^{2+}$ concentration and $I_{Ca}$ was not significantly different between the sexes.

ICYP and PN200–110 binding. Table 2 summarizes the results of β-adrenergic ([125I]ICYP) and Ca$^{2+}$ channel ([3H]PN200–110) binding studies. The $B_{max}$ for the β-adrenergic ligand ICYP was twofold greater in male left ventricular membranes compared with females indicating a greater left ventricular β-adrenergic receptor density in males. The mean receptor density in males was 36.3 ± 7.0 fmol/mg protein compared with 18 ± 3.0 fmol/mg protein in females. No significant sex difference was found in the $K_d$ of specific ICYP binding to left ventricular membranes indicating no sex difference in left ventricular β-adrenergic receptor affinity.

Left ventricular dihydropyridine receptor density ($B_{max}$ of [3H]PN200–110 binding) was significantly greater in females compared with males indicating a greater L-type Ca$^{2+}$ channel abundance in female left ventricular membranes (Table 2). The affinity of [3H]PN200–110 for the dihydropyridine receptor was similar between females and males.

Effect of isoproterenol on myocyte cAMP production. In both male and female myocytes, isoproterenol significantly increased cellular cAMP content (Fig. 5). However, the isoproterenol-induced change in cAMP content was twofold larger in males compared with females ($P \leq 0.05$). In males, cAMP increased by 5.8 ± 1.1 pmol/mg protein, compared with 2.8 ± 0.8 pmol/mg protein in females.

DISCUSSION

Short-term stimulation of myocardial function is primarily mediated by catecholamine-induced activation of the β-adrenergic signaling pathway. Our previous

Table 2. Female and male β-adrenergic and dihydropyridine receptor density and affinity

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<th>ICYP</th>
<th>PN200-110</th>
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<tr>
<td></td>
<td>$K_d$ pM</td>
<td>$B_{max}$ fmol/ mg prot</td>
</tr>
<tr>
<td>Female</td>
<td>43.8 ± 2.3</td>
<td>18.2 ± 3.0</td>
</tr>
<tr>
<td>Male</td>
<td>42.5 ± 3.7</td>
<td>36.3 ± 7.0*</td>
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Values are means ± SE; $n$ = 5 assays for iodocyanopindolol (ICYP) binding in male and female left ventricular membranes; $n$ = 4 assays for dihydropyridine (PN200-110) binding in male and female left ventricular membranes. $K_d$ dissociation constant; $B_{max}$ maximum binding capacity. *$P \leq 0.05$ significantly different from female.

Fig. 5. Isoproterenol-induced change in cAMP content in female and male myocytes. Bars represent the isoproterenol-induced change in female and male myocyte CAMP content. Values are means ± SE; $n$ = 10 batches of cells from 5 female and 5 male hearts respectively. *$P \leq 0.05$, significantly different from female.
SEX DIFFERENCES IN MYOCYTE RESPONSE TO ISOPROTERENOL

studies (37) demonstrated that the inotropic response to the β-adrenergic agonist isoproterenol is greater in the male rat atrium compared with the female rat atrium. The present study extends this finding to rat heart isolated ventricular cells. Here, we are the first to demonstrate that the isoproterenol-elicted increase in cell shortening is significantly greater in isolated male ventricular myocytes than drug-induced shortening in ventricular cells from female hearts. The finding implies an inherent sex difference in components of the β-adrenergic signaling pathway.

The results of our study revealed a twofold greater density of β-adrenergic receptors and greater isoproterenol-stimulated cAMP production in male myocytes compared with female myocytes. β-Adrenergic stimulation is known to result in cAMP/protein kinase A-induced phosphorylation the α1c-subunit of the L-type Ca2+ channel (19) and enhancement of ICa through these channels (32, 41). Consistent with this, our results showed a greater isoproterenol-induced increase in ICa in male myocytes. This suggests that there is an enhanced β-adrenergic signaling in male myocytes compared with female myocytes. The possibility that a higher density of L-type Ca2+ channels in male myocytes could contribute to the larger positive inotropic response to β-adrenergic stimulation was ruled out by the results of the PN200–110 binding studies (Table 2). In fact, female myocytes had greater maximal binding of PN200–110 binding to the α1c-subunit of the channel than male myocytes suggesting higher Ca2+ channel density in females compared with males. This is consistent with the greater ICa detected in females in the absence of isoproterenol (Fig. 3B). The possibility that L-type Ca2+ channels may be functionally different between males than females cannot be ruled out; but this is unlikely based on the similar KD of PN200–110 binding and on the similarity of the Ca2+ I-V relationship in male and female myocytes (Fig. 2). Moreover, whereas the mean current density was greater in females than in males in the absence of drug (control conditions), the relationship between change in Ca2+ concentration and percent change in ICa was the same (Fig. 4). This indicates that proportional increases in Ca2+ influx occurred with each change in extracellular Ca2+ and is more consistent with a difference in the number of Ca2+ channels than with a difference in channel conductance.

Several of the findings in this study would lead us to expect that in the absence of β-adrenergic stimulation (control conditions), cell shortening would be greater in female myocytes than male myocytes. First, female myocytes exhibited augmented ICa compared with male myocytes at several extracellular Ca2+ concentrations (Fig. 4). Second, when the Ca2+ current/voltage relationship was determined, the peak current in females was somewhat larger than in males (Figs. 2 and 3B). Finally, Ca2+ channel density was higher in female myocytes. Although our previous study (37) did find that contraction was greater in female than male atrium (37), this was not the case in the current study. While fractional shortening of female myocytes tended to be greater than male myocytes, the difference was not statistically significant. Several factors could explain this seemingly incongruent observation. One possibility is that the small sex difference in shortening observed in a single myocyte could summate in a multicellular preparation (such as the atrium or papillary muscle), thereby revealing comparatively greater contraction in female hearts. We are examining sex differences in contractile parameters in the rat papillary model to determine whether this is the case. In addition, contractile force or shortening is regulated by altering the delivery of free Ca2+ to the contractile proteins and by altering the sensitivity of contractile proteins to Ca2+ (38) and the mechanisms responsible for alterations in Ca2+ delivery and Ca2+ sensitivity are multifactorial.

Of the many mechanisms that modulate delivery of Ca2+ to the contractile proteins, Ca2+ influx is only one. The amount of Ca2+ released from the sarcoplasmic reticulum that contributes to contraction for a given Ca2+ influx, depends on the concentration of Ca2+ in the sarcoplasmic reticulum and on the relationship between ICa and the probability of ryanodine receptor channel opening (9). Potentially, sarcoplasmic reticular Ca2+ uptake via Ca2+-ATPase could be less efficient in females, thereby resulting in reduced Ca2+ uptake and storage. Sarcoplasmic reticular release mechanisms in female myocytes could also be less sensitive to trigger Ca2+. In support of this proposition, Bowling et al. (2) found that ovariectomy in rats produces a decrease in the K0 of ryanodine binding that could be reversed by estrogen replacement. This observation suggests that estrogen may modulate a change in ryanodine receptor properties and indirectly suggests a potential for estrogen-mediated differences in Ca2+-stimulated Ca2+ release. Other regulators of Ca2+ homeostasis could differ between males and females such as the Na+/Ca2+ exchanger. No other studies have examined sex differences these proposed mechanisms; therefore, their contribution to differences in Ca2+ homeostasis remains speculative.

The finding that the larger ICa in female myocytes does not result in significantly greater cell shortening could also be related to sex differences in myofilament Ca2+ sensitivity. A lower sensitivity in females would mitigate the effects of a greater Ca2+ influx. We (37) and others (42) have demonstrated that atria from female rat hearts are more sensitive to extracellular Ca2+ than atria from male hearts. But this cannot be extrapolated to suggest higher myofilament Ca2+ sensitivity in female myocardium. However, using skinned fibers from male and female atria, we were able to demonstrate enhanced responsiveness to Ca2+ in female atria compared with male atria. It is unknown whether ventricular myofilament Ca2+ sensitivity displays a sex difference. However, Watanapermpool (43, 44) demonstrated that ovariectomy increased rat left ventricular myofilament Ca2+ sensitivity and estrogen replacement prevented the increase in Ca2+ sensitivity. The finding suggests an influence of estrogen on cardiac muscle fiber sensitivity.
One limitation of this study is that it is impossible to use rats that are both weight and age matched. We used weight-matched male and female rats (Table 1), and therefore, there is an age disparity. Male rats were ~12 wk old, and the females were ~9 wk old. Both the males and females were sexually mature and not considered old (3). Capasso et al. (4) showed that there is no age-dependent difference in force or kinetics of papillary muscle contraction in this age range. In our previous studies, we used weight-matched animals to avoid issues of normalization of force measurements in atrial cardiac muscle. We continued using weight-matched rats in the current study based on the assumption that weight-matching the animals would increase the probability of obtaining a similar cell length between males and females. Interestingly, however, the average cell length was found to be longer in male myocytes, despite the finding that the cell membrane area (as measured by cell capacitance) was similar between the sexes. The two findings are not contradictory because length is a one-dimensional measure whereas capacitance encompasses three dimensions. However, because of these differences, all measures of $I_{Ca}$ were normalized to take into consideration the membrane area of each cell, whereas the measure of cell shortening is expressed as fractional shortening to take resting cell length into consideration (9).

Few studies have examined sex differences in myocardial β-adrenergic signaling, but several investigations (7, 22, 25) have sought to determine whether sex hormones alter β-adrenergic receptors and response to β-adrenergic agonists. The results of these studies have been equivocal. We did not harvest the cells used in this study at any particular phase of the estrous cycle or attempt to examine the impact of sex hormones. Still, based on the recent identification of estrogen, androgen and progesterone receptors in cardiomyocytes (11, 12, 18, 21, 23, 26, 28, 33, 39), it could be speculated that the sex differences that we found are mediated by sex hormones.

In conclusion, this study is the first to demonstrate a sex difference in β-adrenergic-mediated enhancement of ventricular myocyte contractility and transsarcolemmal Ca$^{2+}$ influx. Whereas myocytes from female hearts were shown to have a greater $I_{Ca}$ in the absence of the drug, male myocytes exhibited a significantly greater isoproterenol-induced increase in the L-type $I_{Ca}$ and cell shortening. The results of this study also suggest that the mechanism responsible for this sex difference in response to β-adrenergic stimulation is, at least in part, a difference in the signal transduction pathway. Specifically, the maximal number of left ventricular β-adrenergic receptors and the isoproterenol-induced change in cAMP were greater in male compared with female myocytes. In addition, the mechanistic basis for the greater L-type $I_{Ca}$ in the absence of β-adrenergic stimulation in females is a higher Ca$^{2+}$ channel density in the female cells. Functionally, these findings are in agreement with our previous report that demonstrated greater developed force in female rat atrium but an enhanced response to β-adrenergic stimulation in the male atrial myocardium.

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