Gender Differences in Cardioprotection against Ischemia/Reperfusion Injury in Adult Rat Hearts: Focus on Akt and Protein Kinase C Signaling

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Received June 9, 2005; accepted August 9, 2005

ABSTRACT
Previous studies have reported the sex differences in heart susceptibility to ischemia/reperfusion (I/R) injury, but the mechanisms are not understood. The present study tested the hypothesis that Akt and protein kinase C (PKC) play an important role in the sexual dimorphism of heart susceptibility to I/R injury. Isolated hearts from 2-month-old male and female rats were subjected to I/R in the Langendorff preparation. The post-ischemic recovery of left ventricular function was significantly smaller in female than in male hearts. There were no differences in myocardial protein levels of heat shock protein 70, Akt, and PKC\textsubscript{\varepsilon}, respectively, between male and female rats. However, the ratio of phosphorylated (p)-Akt/Akt (0.58 \pm 0.05 versus 0.22 \pm 0.04; \textit{P} < 0.05) and p-PKC\textsubscript{\varepsilon}/PKC\textsubscript{\varepsilon} (0.35 \pm 0.03 versus 0.22 \pm 0.02; \textit{P} < 0.05) was significantly higher in female than in male hearts. In addition, there were significant increases in p-Akt and p-PKC\textsubscript{\varepsilon} levels during reperfusion in female but not in male hearts. The results suggest that increased p-Akt and p-PKC\textsubscript{\varepsilon} levels in female hearts contribute to the gender-related differences in heart susceptibility to I/R and play an important role in cardioprotection against I/R injury in females.

It is becoming increasingly appreciated that gender differences exist in susceptibility to ischemia/reperfusion (I/R) injury, but the mechanisms are not understood. The present study tested the hypothesis that Akt and protein kinase C (PKC\textsubscript{\varepsilon}) play an important role in the sexual dimorphism of heart susceptibility to I/R injury. Isolated hearts from 2-month-old male and female rats were subjected to I/R in the Langendorff preparation. The post-ischemic recovery of left ventricular function was significantly better, and infarct size was significantly smaller in female (37.1 \pm 1.9\%) than in male (48.3 \pm 2.3\%) hearts after 25-min ischemia followed by 2-h reperfusion. Inhibition of phosphatidylinositol 3-kinase/Akt pathway by wortmannin or PKC by chelerythrine chloride before ischemia significantly reduced postischemic recovery and increased infarct size in female but not male hearts. There were no differences in myocardial protein levels of heat shock protein 70, Akt, and PKC\textsubscript{\varepsilon} respectively, between male and female rats. However, the ratio of phosphorylated (p)-Akt/Akt (0.58 \pm 0.05 versus 0.22 \pm 0.04; \textit{P} < 0.05) and p-PKC\textsubscript{\varepsilon}/PKC\textsubscript{\varepsilon} (0.35 \pm 0.03 versus 0.22 \pm 0.02; \textit{P} < 0.05) was significantly higher in female than in male hearts. In addition, there were significant increases in p-Akt and p-PKC\textsubscript{\varepsilon} levels during reperfusion in female but not in male hearts. The results suggest that increased p-Akt and p-PKC\textsubscript{\varepsilon} levels in female hearts contribute to the gender-related differences in heart susceptibility to I/R and play an important role in cardioprotection against I/R injury in females.

This work was supported in part by National Institutes of Health Grants HL-67745, HL-57787, and HD-31226 and by Loma Linda University School of Medicine.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.105.090803.

ABBREVIATIONS: HSP, heat shock protein; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; LV, left ventricle; LVEDP, left ventricle end diastolic pressure; LVDP, left ventricle developed pressure; HR, heart rate; PKC\textsubscript{\varepsilon}-TIP, PKC\textsubscript{\varepsilon} translocation inhibitor peptide; DMSO, dimethyl sulfoxide; p-PKC, phosphorylated protein kinase; p-Akt, phosphorylated Akt; ANOVA, analysis of variance.
left ventricle contractile recovery after transient ischemia (Fujio et al., 2000; Matsui et al., 2001). Interestingly, sexually mature female mice showed higher levels of activated Akt in nuclei than that in male mice (Camper-Kirby et al., 2001). In addition to Akt, it has been demonstrated that PKCε plays a pivotal role of cardioprotection in response to cardiac ischemia and reperfusion injury (Murriel and Mochly-Rosen, 2003). Studies in a PKCε knock-out mouse model have demonstrated that PKCε expression is not required for the maintenance of cardiac function under normal conditions but that PKCε activation is necessary and sufficient for acute cardioprotection during cardiac ischemia and reperfusion (Gray et al., 2003).

In the present study, we investigated the gender differences in cardioprotective mechanisms against ischemia and reperfusion injury in age-matched adult male and female rats and tested the hypothesis that the activation of PI3K/Akt-dependent pathway and PKCε plays an important role in the gender dichotomy in cardiac responses to ischemia and reperfusion.

Materials and Methods

Rat Hearts Subjected to Ischemia and Reperfusion. Two-month-old Sprague-Dawley male and female rats were purchased from Charles River Breeding Laboratories (Portage, MI). Rats were anesthetized by intramuscular injections of ketamine (75 mg/kg) and xylazine (5 mg/kg). Heparin (300 U/kg) was injected into the peritoneum 5 min before heart removal. Hearts were isolated rapidly and retrogradely perfused via the aorta in the Langendorff mode under normothermic conditions. Hearts were pretreated with 5 μM PKCε translocation inhibitor peptide (PKCε-TIP; Calbiochem) for 20 min before ischemia and reperfusion with no wash-out period.

Myocardial Infarct Size. Myocardial infarct size was measured as described previously (Bae et al., 2005). Briefly, at the end of reperfusion, left ventricles were collected, cut into four slices, incubated with 1% triphenyltetrazolium chloride solution for 15 min at 37°C, and immersed in formalin for 30 min. Each slice was then photographed (Kodak digital camera; Eastman Kodak, Rochester, NY) separately, and the areas of myocardial infarction in each slice were analyzed by computerized planimetry (Image-Pro Plus; Media Cybernetics, Inc., Silver Spring, MD), corrected for the tissue weight, summed for each heart, and expressed as a percentage of the total LV weight.

Western Blot Analysis. Protein levels of HSP70, PKCε, phospho-PKCε (p-Ser189), Akt, and phospho-Akt (p-Ser473) in LV were determined by Western blot analysis. In brief, tissues were homogenized in ice-cold homogenization buffer containing 150 mM NaCl, 50 mM Tris-HCl, 10 mM EDTA, 0.1% Tween 20, 0.1% β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, and 5 μg/ml apro tin, pH 7.4. Homogenates were then incubated on ice for 10 min in the homogenization buffer containing 1% Triton X-100 followed by centrifugation at 10,000g for 30 min at 4°C. Protein was quantified in the supernatant using protein assay kit from Bio-Rad (Hercules, CA). Samples with equal protein were loaded on 10% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and incubated with primary antibodies against HSP70, PKCε, phospho-PKCε (Upstate Biotechnology; Lake Placid, NY), Akt, and phospho-Akt (Sigma-Aldrich), respectively. After washing, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Proteins were then visualized with an enhanced chemiluminescence detection system. Results were quantified with Kodak electrophoresis documentation and analysis system and Kodak 1D image analysis software.

Statistical Analysis. Data were expressed as means ± S.E.M. Statistical significance (P < 0.05) was determined by analysis of variance (ANOVA) or Student’s t test, where appropriate.

Results

Gender Differences in Postischemic Recovery of LV Function. The average body weight was significantly higher in 2-month-old male than female rats (382.4 ± 16.7 g versus 239.6 ± 6.8 g; P < 0.05). However, females showed significantly higher heart/body weight ratio (milligrams per gram) than that in males (4.5 ± 0.01 versus 4.2 ± 0.01; P < 0.05). Table 1 shows the preischemic values of LV function and coronary flow rate in isolated male and female hearts in the Langendorff preparation. Compared with male hearts, female hearts showed significantly reduced LVDP (Table 1). As expected, female hearts showed significantly lower LVDP and HR, as well as a reduced dP/dtmax and dP/dtmin. The LVDP and HR were calculated using the left ventricular pressure (milliliters of mercury) and heart rate (beats per minute), respectively.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LVDP (mm Hg)</th>
<th>HR (bpm)</th>
<th>dP/dtmax (mm Hg/s)</th>
<th>dP/dtmin (mm Hg/s)</th>
<th>CF (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>111.2 ± 1.5</td>
<td>272.5 ± 8.2</td>
<td>3153.0 ± 172.8</td>
<td>2256.0 ± 84.4</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>Female</td>
<td>103.8 ± 2.4*</td>
<td>263.2 ± 4.4</td>
<td>2962.0 ± 163.1</td>
<td>2155.0 ± 67.7</td>
<td>8.1 ± 0.3</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. male.
Fig. 1. Postischemic recovery of LV function in male and female hearts. Hearts were isolated from 2-month-old male, and female rats and were subjected to 15 (A, male, \( n = 4 \); female, \( n = 5 \)) and 25 (B, male, \( n = 12 \); female, \( n = 12 \)) min of ischemia followed by 60 min of reperfusion in the Langendorff preparation. PRP, pressure-rate product (LVDP × HR). Data were analyzed by two-way ANOVA with ischemia-reperfusion as one factor and gender as the other. *, significant difference (\( P < 0.05 \)) from female for the entire curve.
and expressed as a percentage of the total left ventricular weight, as infarct size was determined by 1% triphenyltetrazolium chloride staining.

Therefore, we determined the effect of selective inhibition of PKC isozymes with opposite functions in cardioprotection. Because it has been reported that PKC plays a pivotal role in cardioprotection in response to cardiac ischemia and reperfusion injury in the hearts, the finding that chelerythrine had no effect on postischemic recovery of left ventricular function in male hearts was intriguing. Given that chelerythrine is a nonselective PKC inhibitor, the absence of the effect of chelerythrine in male hearts may be due to its inhibition of different PKC isozymes with opposite functions in cardioprotection. Therefore, we determined the effect of selective inhibition of PKC.

**Effect of Wortmannin on Postischemic Recovery of LV Function.** To determine whether the activation of PI3K/Akt pathway contributes to the cardioprotection observed in female hearts, male and female hearts were treated with the PI3K inhibitor wortmannin (100 nM) or the vehicle (0.04% DMSO) as the control before ischemia. Wortmannin significantly increased preischemic values of LVDP, dP/dt\text{max}, and dP/dt\text{min} in females and decreased dP/dt\text{max} and coronary flow rate in male hearts (Table 2, top). Compared with the control, the postischemic recovery of LVDP, dP/dt\text{max}, dP/dt\text{min}, and the pressure-rate product was significantly reduced in the wortmannin-treated females (Fig. 3A). In male hearts, wortmannin treatment decreased postischemic recovery of LVDP but it had no effects on dP/dt\text{max}, dP/dt\text{min}, and the pressure-rate product (Fig. 3B). As shown in Fig. 5, wortmannin significantly increased ischemia and reperfusion-induced infarct size (50.4 ± 4.7 versus 37.1 ± 2.7%; P < 0.05) in female hearts but not in male hearts. In the absence of wortmannin, the infarct size was significantly smaller in female than in male hearts. However, in the presence of wortmannin, there was no significant difference in myocardial infarct size between male and female hearts (Fig. 5).

**Effect of Chelerythrine on Postischemic Recovery of LV Function.** To further determine whether PKC contributes to the cardioprotection observed in female hearts, hearts were pretreated with the nonselective PKC inhibitor chelerythrine (2 μM) or the vehicle (0.04% DMSO) as the control before ischemia. Unlike wortmannin, inhibition of PKC with chelerythrine did not affect the preischemic baseline values of LV function in both male and female hearts (Table 2, bottom). Similar to the results obtained with wortmannin, chelerythrine selectively decreased the postischemic recovery of left ventricular function in female but not male hearts (Fig. 4) and selectively increased myocardial infarct size in female hearts (Fig. 5). In the presence of chelerythrine, there was no significant difference in the infarct size between male and female hearts (Fig. 5).

**Effect of Selective PKCε Inhibition on Postischemic Recovery of LV Function in Male Hearts.** Because it has been reported that PKCε plays a pivotal role in cardioprotection in response to cardiac ischemia and reperfusion injury in the hearts, the finding that chelerythrine had no effect on postischemic recovery of left ventricular function in male hearts was intriguing. Given that chelerythrine is a nonselective PKC inhibitor, the absence of the effect of chelerythrine in male hearts may be due to its inhibition of different PKC isozymes with opposite functions in cardioprotection. Therefore, we determined the effect of selective inhibition of PKCε.

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**TABLE 2**

Preischemic left ventricle functional parameters of 2-month-old male rats after wortmannin (top) or chelerythrine (bottom) treatments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (6)</th>
<th>Wortmannin (6)</th>
<th>Control (5)</th>
<th>Wortmannin (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDP (mm Hg)</td>
<td>115.0 ± 2.4</td>
<td>108.0 ± 2.0</td>
<td>106.7 ± 2.8</td>
<td>123.6 ± 2.7*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>275.2 ± 14.2</td>
<td>281.0 ± 7.2</td>
<td>280.8 ± 5.8</td>
<td>272.9 ± 11.0</td>
</tr>
<tr>
<td>dP/dt\text{max} (mm Hg/s)</td>
<td>2194.0 ± 75.5</td>
<td>1912.0 ± 69.6*</td>
<td>2104.0 ± 75.5</td>
<td>2430.0 ± 72.5*</td>
</tr>
<tr>
<td>dP/dt\text{min} (mm Hg/s)</td>
<td>2811.0 ± 85.4</td>
<td>2632.0 ± 50.2</td>
<td>2678.0 ± 78.5</td>
<td>2912.0 ± 53.4*</td>
</tr>
<tr>
<td>CF (ml/min)</td>
<td>10.5 ± 0.4</td>
<td>8.9 ± 0.5*</td>
<td>7.9 ± 0.4</td>
<td>8.1 ± 0.1</td>
</tr>
<tr>
<td>LVDP (mm Hg)</td>
<td>106.9 ± 6.1</td>
<td>104.2 ± 3.3</td>
<td>102.0 ± 3.2</td>
<td>109.0 ± 11.0</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>269.8 ± 9.5</td>
<td>275.3 ± 12.1</td>
<td>266.0 ± 7.4</td>
<td>265.5 ± 7.2</td>
</tr>
<tr>
<td>dP/dt\text{min} (mm Hg/s)</td>
<td>2319.0 ± 155.1</td>
<td>2267.0 ± 150.0</td>
<td>2215.0 ± 122.4</td>
<td>2151.0 ± 257.9</td>
</tr>
<tr>
<td>dP/dt\text{max} (mm Hg/s)</td>
<td>3495.0 ± 278.1</td>
<td>3637.0 ± 105.2</td>
<td>3302.0 ± 289.7</td>
<td>3328.0 ± 388.8</td>
</tr>
<tr>
<td>CF (ml/min)</td>
<td>11.0 ± 0.9</td>
<td>9.9 ± 0.5</td>
<td>8.9 ± 0.6</td>
<td>7.3 ± 0.7</td>
</tr>
</tbody>
</table>

* CF, coronary flow.
* P < 0.05 vs. control in the same gender.
Fig. 3. Effect of wortmannin on postischemic recovery of LV function. Hearts were isolated from 2-month-old male (control, n = 6; wortmannin, n = 6) and female (control, n = 5; wortmannin, n = 4) rats and were pretreated in the presence of wortmannin (100 nM) or vehicle (0.04% DMSO) for 20 min before subjecting to 25 min of ischemia and 60 min of reperfusion in the Langendorff preparation. PRP, pressure-rate product. Data were analyzed by two-way ANOVA with ischemia-reperfusion as one factor and wortmannin treatment as the other. ∗, significant difference (P < 0.05) from control for the entire curve.
Fig. 4. Effect of chelerythrine on postischemic recovery of LV function. Hearts were isolated from 2-month old male (control, n = 6; chelerythrine, n = 5) and female (control, n = 5; chelerythrine, n = 5) rats and were pretreated in the presence of chelerythrine (2 μM) or vehicle (0.04% DMSO) for 20 min before subjecting to 25 min of ischemia and 60 min of reperfusion in the Langendorff preparation. PRP, pressure-rate product. Data were analyzed by two-way ANOVA with ischemia-reperfusion as one factor and chelerythrine treatment as the other. * significant difference (P < 0.05) from control for the entire curve.
PKCε on posts ischemic recovery of LV function in male hearts, using a selective PKCε translocation inhibitor peptide, PKCε-TIP (Murriel and Mochly-Rosen, 2003; Przyklenk et al., 2003). As shown in Table 3, PKCε-TIP had no significant effects on left ventricular function at baseline levels. Figure 6 shows the effect of PKCε-TIP on posts ischemic recovery of LV function. Compared with the control, there were significant decreases in posts ischemic recovery of LVDP, dP/dt\text{max}, dP/dt\text{min}, and the pressure-rate product in hearts pretreated with PKCε-TIP. Posts ischemic recovery of heart rate and coronary flow was not significantly different between the two groups. Consistent with the decreased posts ischemic recovery of LV function, PKCε-TIP significantly increased ischemia and reperfusion-induced myocardial infarct size in the left ventricle (Fig. 7).

**Gender Differences in Myocardial HSP70, Akt, and PKCε.** Western blot analyses showed that there were no significant differences in basal levels of HSP70 (Fig. 8), Akt, and PKCε (Fig. 9) between male and female hearts. However, the active forms of Akt (phospho-Akt\text{Ser473}) and PKCε (phospho-PKCε\text{Ser729}) in the left ventricle were significantly increased in female hearts compared with those in male hearts (Fig. 10). The elevated phospho-Akt\text{Ser473} and phospho-PKCε\text{Ser729} in female hearts persisted at the end of 25-min ischemia and before reperfusion (Fig. 10). In addition, we determined the effect of reperfusion on the levels of activated Akt and PKCε in male and female hearts. As shown in Fig. 11, the levels of phospho-Akt\text{Ser473} were significantly increased during reperfusion in female but not in male hearts. Similarly, reperfusion increased phospho-PKCε\text{Ser729} levels in female but not in male hearts (Fig. 12).

**Discussion**

The present study demonstrated in adult rats that female hearts had greater resistance to ischemia and reperfusion injury by improving posts ischemic left ventricular function and reducing infarct size after ischemia and reperfusion compared with males. This is in agreement with the recent studies showing the gender differences in heart susceptibility to myocardial ischemia and reperfusion injury (Wang et al., 2005). The finding that myocardial HSP70 content was not significantly different between male and female hearts in the present study is consistent with the previous finding in adult rats of the same age (Paroo et al., 2002). However, studies in older rats (12–16 weeks old) showed higher expression levels of HSP70 in female than male hearts (Voss et al., 2003). Although the age may contribute to the different results, other differences in methodology and sample collection are also important factors. In the present study, we measured HSP70 content by Western blots in the left ventricle compared with the studies by Voss et al. (2003), in which HSP70 levels in the whole heart were measured by an enzyme-linked immunosorbent assay.

In the present study, the inhibition of the PI3K/Akt pathway with wortmannin significantly reduced posts ischemic recovery of left ventricular function and increased myocardial infarct size in female but not in male hearts. In the presence of wortmannin, there were no significant gender differences in the posts ischemic recovery and myocardial infarct size. Wortmannin has been widely used to inhibit PI3K-Akt in animal models of ischemia and reperfusion injury (Cai and Semenza, 2004; Okumura et al., 2005). The finding that myocardial HSP70 content was not significantly different between male and female hearts in the present study is consistent with the previous finding in adult rats of the same age (Paroo et al., 2002). However, studies in older rats (12–16 weeks old) showed higher expression levels of HSP70 in female than male hearts (Voss et al., 2003). Although the age may contribute to the different results, other differences in methodology and sample collection are also important factors. In the present study, we measured HSP70 content by Western blots in the left ventricle compared with the studies by Voss et al. (2003), in which HSP70 levels in the whole heart were measured by an enzyme-linked immunosorbent assay.

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**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>LVDP</th>
<th>HR</th>
<th>dP/dt\text{max}</th>
<th>dP/dt\text{min}</th>
<th>CF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm Hg</td>
<td>bpm</td>
<td>mm Hg/s</td>
<td>mm Hg/s</td>
<td>ml/min</td>
</tr>
<tr>
<td>Control</td>
<td>123.5 ± 3.4</td>
<td>298.0 ± 13.2</td>
<td>3418.6 ± 153.3</td>
<td>2659.4 ± 131.5</td>
<td>13.4 ± 0.4</td>
</tr>
<tr>
<td>PKCε-TIP</td>
<td>119.2 ± 6.7</td>
<td>270.0 ± 6.7</td>
<td>3217.4 ± 203.9</td>
<td>2527.8 ± 161.7</td>
<td>11.8 ± 0.6</td>
</tr>
</tbody>
</table>

*CF, coronary flow.*
ences in basal levels of total Akt protein in left ventricles between male and female hearts. However, the proportion of the Akt that was phosphorylated was significantly higher in female than in male hearts. Ischemia per se did not significantly change Akt activity levels, but reperfusion caused a significant increase in phospho-Akt in female but not in male hearts. This is in agreement with previous findings in cardiomyocytes that Akt was phosphorylated during reperfusion after simulated ischemia but not during simulated ischemia itself (Mockridge et al., 2000). The finding that wortmannin significantly increased preischemic values of LVDP, dP/dt max, and dP/dt min in female but not in male hearts is intriguing and suggests that the increased basal PI3K-Akt activity in female hearts has

Fig. 6. Effect of PKCe-TIP on postischemic recovery of LV function. Hearts were isolated from 2-month-old male rats and were pretreated in the absence or presence of 5 μM PKCe-TIP for 20 min before subjecting to 15 min of ischemia and 30 min of reperfusion in the Langendorff preparation. PRP, pressure-rate product; HR, heart rate; CF, coronary flow rate. Data were analyzed by two-way ANOVA with ischemia-reperfusion as one factor and PKCe-TIP treatment as the other. *, significant difference (P < 0.05) from control for the entire curve (n = 5).
negative regulatory function in the resting heart. Similar findings were obtained in a mouse model of ischemia and reperfusion injury (Gabel et al., 2002).

Unlike wortmannin, the inhibition of PKC with chelerythrine showed no effect on preischemic left ventricular function in either male or female hearts. However, similar to wortmannin, chelerythrine treatment resulted in a significant reduction of posts ischemic recovery of left ventricular function and an increase in myocardial infarct size in female but not in male hearts and eliminated the gender differences in the posts ischemic recovery and myocardial infarct size. In agreement with the present finding, previous studies showed that chelerythrine had no effect on posts ischemic function and infarct size in male hearts (Tian et al., 1999; Ding et al., 2004). The effect of chelerythrine on female hearts has not been previously examined. The finding that selective inhibition of PKCε by PKCε-TIP significantly increased ischemia and reperfusion injury in male hearts suggests that the cardioprotective effect of PKC is likely isozyme-dependent. Given that chelerythrine is a nonselective PKC inhibitor, the absence of the effect of chelerythrine in male hearts may be due to its inhibition of different PKC isozymes with opposite functions in cardioprotection. The potential gender differences in the relative activities of PKC isozymes and their cardioprotective roles in male and female hearts remain an intriguing area for future investigation.

The findings that female hearts had higher levels of the active form of phospho-PKCε than male hearts and that reperfusion increased phospho-PKCε in female but not male hearts suggest that increased PKCε activity plays a role in the gender difference in cardioprotection. Recent studies have demonstrated that phosphorylation plays a key role in converting nascent PKC isoforms into the mature forms during the process of PKC activation and identified the active form of phospho-PKCε in cardiomyocytes (Parekh et al., 2000; Rybin et al., 2003). The cardioprotective effect of PKCε is proposed to be inhibition of apoptosis and hence reduction of myocardial infarction after ischemia and reperfusion (Liu et al., 2001, 2002; Murriel and Mochly-Rosen, 2003). Ischemia and reperfusion-mediated cell death in the heart occurs through both necrosis and apoptosis (Haunstetter and Izumo, 1998). Many studies have clearly demonstrated that apoptosis plays an important role in ischemia and reperfusion-induced myocardial injury (Yaoita et al., 1998; Condorelli et al., 2001; Li et al., 2003, 2004; Bae and Zhang, 2005; Bae et al., 2005). Increased cytosolic Ca2+, which may trigger or favor apoptosis, occurred in myocardial ischemia and reperfusion, and decreased sarcoplasmic reticulum Ca2+ resulted in a decrease in ischemia and reperfusion injury (James, 1999). It has been demonstrated that activation of PKC during ischemia and early reperfusion reduces cytosolic calcium accumulation in myocardium (Stamm et al., 2001; Stamm and del Nido, 2004). In support of the present finding of gender dimorphism, recent studies demonstrated that females ex-
hibited less sarcoplasmic reticulum Ca\(^{2+}\) loading than males after isoproterenol treatment (Chen et al., 2003), and cardiomyocytes from females were more resistant to ischemia and reperfusion-induced Ca\(^{2+}\) loading compared with cardiomyocytes from males (Ranki et al., 2001). Alternatively, it remains possible that reduced tolerance to ischemic injury in male rats was due in part to an increased energy expenditure for contraction given the finding that developed left ventricular pressure was higher at baseline in males than females.

In conclusion, we have demonstrated in rat model a gender dichotomy in heart susceptibility to ischemia and reperfusion injury and have shown that female hearts have improved postischemic recovery of left ventricular function and decreased myocardial infarct size compared with male hearts. The lack of difference in myocardial HSP70 content between male and female hearts suggests that it may not be important in the regulation of the gender-related heart resistance to ischemia and reperfusion. However, the greater and prolonged activation of Akt and PKCe during reperfusion in female hearts is likely to play an important role in protecting female hearts and to contribute to the gender dimorphism in cardiac vulnerability to ischemia and reperfusion injury.

Fig. 10. Left ventricular phospho-Akt\(^{Ser473}\) and phospho-PKC\(^{eSer729}\) in male and female hearts. Hearts were isolated from 2-month-old male and female rats and were subjected to 25 min of ischemia in the Langendorff preparation. Left ventricles were isolated from the hearts collected before and at the end of ischemia, respectively. Myocardial phospho-Akt\(^{Ser473}\) and phospho-PKC\(^{eSer729}\) levels were determined by Western blots and are expressed as fractions of total Akt and PKCe, respectively. Data are mean ± S.E.M. * P < 0.05 versus female (n = 4).

Fig. 11. Left ventricular phospho-Akt\(^{Ser473}\) during reperfusion in male and female hearts. Hearts were isolated from 2-month-old male and female rats and were subjected to 25 min of ischemia in the Langendorff preparation. Left ventricles were isolated from the hearts collected at 0, 5, 15, 30, and 60 min of reperfusion, respectively, and myocardial phospho-Akt\(^{Ser473}\) levels were determined by Western blots. Data were analyzed by two-way ANOVA with reperfusion as one factor and the gender as the other. *, significant difference (P < 0.05) from male for the entire curve (n = 4).

Fig. 12. Left ventricular phospho-PKC\(^{eSer729}\) during reperfusion in male and female hearts. Hearts were isolated from 2-month-old male and female rats and were subjected to 25 min of ischemia in the Langendorff preparation. Left ventricles were isolated from the hearts collected at 0, 5, 15, 30, and 60 min of reperfusion, respectively, and myocardial phospho-PKC\(^{eSer729}\) levels were determined by Western blots. Data were analyzed by two-way ANOVA with reperfusion as one factor and the gender as the other. *, significant difference (P < 0.05) from male for the entire curve (n = 4).

References


