Estrus cycle: influence on cardiac function following trauma-hemorrhage


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Estrus cycle: influence on cardiac function following trauma-hemorrhage. Am J Physiol Heart Circ Physiol 291: H2807–H2815, 2006. First published July 28, 2006; doi:10.1152/ajpheart.00195.2006.—Since cardiac function is depressed in males but not in proestrus (PE) females following trauma-hemorrhage (T-H), we examined whether different estrus cycles influence cardiac function in female rats under those conditions. We hypothesized that females in the PE cycle only will have estrus cycles influence cardiac function in female rats under those conditions. We hypothesized that females in the PE cycle only will have normal cardiac function following T-H and resuscitation. Sham operation or T-H was performed in five groups of rats (250–275 g) including PE, estrus (E), metestrus (ME), diestrus (DE), and ovariectomized (OVX) females. Cardiac function was determined 2 h after T-H, following which cardiomyocytes were isolated and nuclei extracted. Cardiomyocyte IL-6 and NF-κB expressions were measured using Western blotting. Moreover, plasma IL-6, estradiol, and progesterone levels were measured using ELISA or ELA kits. Results (1-way ANOVA) indicated that following T-H, cardiac function was depressed in DE, E, ME, and OVX groups but maintained in the PE group; 2) the PE group had the highest plasma estrogen level; 3) plasma IL-6 levels increased significantly in DE, E, ME, and OVX groups, but the increase was attenuated in the PE group; 4) cardiomyocyte IL-6 protein level increased significantly in DE, E, ME, and OVX groups after TH, but the increase was attenuated in the PE group; and 5) cardiomyocyte NF-κB expression increased significantly but was attenuated in the PE group. These data collectively suggest that the estrus cycle plays an important role in cardiac function following TH. The salutary effect seen in PE following TH is likely due to a decrease in NF-κB-dependent cardiac IL-6 pathway.

female reproductive cycle; ovariectomy; estrogen; trauma-hemorrhagic shock; interleukin-6; nuclear factor-κB

PREVIOUS STUDIES HAVE SHOWN that cardiac function is depressed and circulating IL-6 levels increase in both males and females following trauma-hemorrhage, except in females in proestrus cycle in which these changes were not apparent (2, 8, 16, 39, 40, 42). Although some studies have indicated that sexual dimorphism did not influence outcome of traumatic brain injury (5) and that women are at high risk of adverse outcome in long-term posttraumatic stress disorders (14), more and more clinical and experimental studies have demonstrated that sex plays an important role in response to trauma and sepsis (2, 11, 16, 36, 37). In this regard, studies suggest that female mice in the proestrus stage of their estrus cycle are more tolerant to trauma than male mice (22, 37). Furthermore, female mice exhibit enhanced immune responses, as opposed to depressed immune functions in males, following trauma-hemorrhage (1, 2, 8, 37). Studies also have indicated that such a sex dimorphism following trauma-hemorrhage appears to be hormonally regulated and that the hormones involved originate primarily from the gonads and secondarily from the thymus and the hypothalamus-pituitary gland (2, 29, 30).

It has been shown that high estrogen levels in female rats in the proestrus cycle maintain cardiac function following trauma-hemorrhage (16). However, the estrogen levels vary in different estrus cycles, and thus, depending on the stage of estrus cycle, females may exhibit variations in their responses to trauma-hemorrhage. Studies also have shown a correlation between the sustained elevation in IL-6 levels and poor outcome following hypoxia (26, 36, 37). It also has been demonstrated that IL-6 gene expression that is induced by hypoxia is mainly through the activation of NF-κB in cardiomyocytes (24). Furthermore, the transcription factors NF-IL-6 and NF-κB are known to synergistically activate the transcription of inflammatory cytokines, chemokines, and adhesion molecules (27). More recently, we (40) found an increase in cardiac IL-6 protein levels and also increased cardiomyocyte IL-6 gene expression following trauma-hemorrhage. In this study we investigated whether different estrus cycles have an influence on cardiac function following trauma-hemorrhage and whether cardiac function under those conditions is related to cardiac IL-6 production. Thus we hypothesized that downregulation of cardiac IL-6 might be one of the mechanisms by which proestrus females have protective effects on cardiac function following trauma-hemorrhage. Since NF-κB is involved in IL-6 regulation, we also examined whether the different female reproductive cycles have any effects on NF-κB/IκB-α.

MATERIALS AND METHODS

Experimental protocol. The aim of the present study was to determine whether different estrus cycles influence cardiac function following trauma-hemorrhage in female rats and to clarify the relationship between sex hormones and cardiac function following trauma-hemorrhage. Sham operation or trauma-hemorrhage was performed in five groups of female rats: proestrus (PE), estrus (E), metestrus (ME), diestrus (DE), and ovariectomized (OVX). Cardiac function was determined at 2 h after trauma-hemorrhage, cardiomyocytes were isolated, and nuclei were then extracted. Plasma sex hormones including estradiol and progesterone were measured. Systemic IL-6 levels also were measured. Moreover, cardiomyocyte IL-6 and NF-κB expression levels were determined by Western blot analysis.

Trauma-hemorrhagic shock model and experimental groups. Trauma-hemorrhagic shock (34) was induced in PE, E, ME, DE, and OVX (2 wk before trauma-hemorrhage was induced) female adult (225–275 g) Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) as described previously by our group, with some modifications (34, 35). In brief, all experimental rats were fasted overnight but allowed water ad libitum before the experiment. After determination of the estrus cycle, both the right and left femoral arteries and right
 vein were cannulated with PE-50 tubing under anesthesia with isoflu- 

eurane inhalation. The tubing in the right femoral artery was connected 

to a blood pressure analyzer (BPA; Digi-Med, Louisville, KY) for 

measuring and monitoring mean arterial pressure (MAP) and heart 

rate (HR); the tubing in the left femoral artery was used for blood 

withdrawal; and the tubing in the right femoral vein was used for fluid 

resuscitation. The total circulating blood volume was calculated ac- 

cording to our previous studies (i.e., total circulating blood volume = 

body weight × 6%). MAP was monitored continuously before the 

onset of hemorrhage until the end of resuscitation. We allowed 

~10–15 min after cannulation for the animal to awaken completely 

from anesthesia. The animals were bled rapidly under conscious 

conditions to reach MAP of 35–40 mmHg within 10 min, and MAP 

was maintained at that level by further withdrawing blood until 

maximum bleed out (MBO) occurred. The amount of blood volume 

withdrawn to reach MBO was ~60% of the total circulating blood 

volume. The rats were then maintained at the MAP of 40 mmHg 

with infusions of small volumes of Ringer lactate for 45 min, followed by 

fluid resuscitation with Ringer lactate (4× MBO volume) over 1 h. 

The total time for trauma-hemorrhage model preparation was ~2.5–3 h. 

The animals were then returned to cages and allowed food and 

water ad libitum.

In the OVX group, ovariectomy was performed 2 wk before 

trauma-hemorrhage. Ovariectomy was performed by placing the ani- 

mal in a prone position. A 1-cm-long incision was made on both sides 

of the waist under isoflurane inhalation, and ovaries on both sides 

were removed, blood vessels secured, and incisions closed. The 

experiments described were performed in adherence to the National 

Institutes of Health Guidelines for the Use and Care of Laboratory 

Animals. This project was approved by the Institutional Animal Care 

and Use Committee of the University of Alabama at Birmingham.

Estrus cycle determination. Estrus cycles were determined in early 

morning on the experimental day by vaginal smear. Three types of 

cells are found in vaginal smears: polymorphonuclear (PMN), nuc- 

leated epithelial, and cornified epithelial cells (ECs). Primarily nuc- 

leated ECs with a few cornified ECs were detected in proestrus; 

nucleated ECs with a few cornified ECs were found in diestrus; and 

primarily cornified ECs with a few nucleated ECs were found in 

estrus. Primarily nucleated PMNs with a few nucleated cornified ECs 

were detected in metestrus, and very few cells of all types were found in 

diestrus (21, 33).

Cardiac function determination. At 2 h after trauma-hemorrhage or 

sham operation, cardiac output (CO) was determined using the indo- 

estrus (21, 33).

Briefly, the heart was quickly removed from the chest and perfused in 

a retrograde manner via the aorta at 37°C and at a constant rate (12 

mg·min⁻¹·g tissue⁻¹) for 5 min with a calcium-free Krebs buffer 

containing (in mM) 118 NaCl, 4.7 KCl, 25 NaHCO₃, 1.2 KH₂PO₄, 1.2 

MgSO₄, 10 HEPES, and 11 glucose, gassed with 95%O₂-5%CO₂. After the wash-perfusion, the calcium-free Krebs buffer was replaced by 

an enzymatic digestion buffer containing collagenase type II 

(Worthington, Lakewood, NJ), 0.1% fat-free BSA, 100 μM CaCl₂, 

and 10 mM taurine and perfused at 37°C at a constant rate (5 

mg·min⁻¹·g tissue⁻¹) for 9 min. When the heart became swollen and 

hard, the left ventricle was removed and cut into small chunks (~1 mm³) and further digested with the incubation buffer containing the 
enzymatic digesting buffer and 2% fat-free BSA in a shaker (60–70 rpm) 

water bath at 37°C for 10 min. The supernatant containing the 
dispersed cardiomyocytes was filtered through a 300-μl filter into a 
50-ml sterilized tube and gently centrifuged at 500 rpm for 1 min. The upper portion of the supernatant was discarded, and ~30 ml BSA-free buffer (calcium-free Krebs buffer + 50 μM CaCl₂) was added and 

centrifuged at 480 rpm for 1 min. The upper portion of the supernatant 

d was discarded again, and a 10-ml cell suspension was carefully 

dispersed onto high BSA (4%) medium and centrifuged (480 rpm, 1 min). The number of cardiomyocytes was then counted by 
suspending cardiomyocytes in 0.02% Trypan blue under a light microscope. All 

the buffers were filtered (0.2-μM filter) and equilibrated with 95%O₂- 

5%CO₂ for at least 20 min before use. To reduce bacterial and viral 

contamination, the perfusion setup was washed each day with 70% 

alcohol and then washed with sterilized distilled water before perfu- 

sion.

Cardiomyocyte nuclear extraction. Isolated cardiomyocytes 

(~1.5 × 10⁶) were rapidly suspended in 1 ml of hypotonic buffer 

including (in mM) 10 HEPES (pH 7.9), 1.5 MgCl₂, 10 KCl, 0.5 DTT, 

0.1 PMSF, and 10 μg/ml aprotinin. The cardiomyocytes were cen- 

trifuged at 1,500 rpm for 5 min at 4°C, and the supernatant was 
discarded. The packed cardiomyocytes were resuspended in hypotonic 

buffer and allowed to swell for 10 min on ice. The cardiomyocyte 

integrity was then broken using a 26.5 needle with six repeats of 

up-and-down pushing. The cardiomyocyte nuclei were then collected 

by centrifuging at 4,000 rpm for 15 min at 4°C. Finally, the packed 

cells were resuspended in an equal volume of high-salt buffer 

including (in mM) 20 HEPES (pH 7.9), 1.5 MgCl₂, 0.6 KCl, 0.2 

EDTA, 0.5 DTT, 0.1 PMSF, 25% glycerol, and 10 μg/ml aprotinin for 

60 min with constant gentle mixing to extract the nuclei. The 

dispersed nuclei nuclei were collected by centrifuging at 14,000 rpm for 15 min at 4°C. The extracted cardiomyocyte nuclei were 

stored at ~70°C until used.

Western blot analysis of cardiomyocyte IL-6, estrogen receptor 

(ER)-α/ER-β, NF-κB, and IκB-α/phospho-IκB-α expression. Approxi- 

mately 1.5–2.0 × 10⁶ cardiomyocytes were homogenized in 0.5–1 ml 

of lysis buffer containing (in mM) 20 HEPES (pH 7.9), 1.5 MgCl₂, 20 

KCl, 0.2 EDTA, 2 Na₃VO₄, 10 NaF, 0.2 PMSF, 20% glycerol, 1% 

Triton X-100, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. Cardiomyo- 

cyte lysate was centrifuged at 10,000 g for 10 min at 4°C, and the protein 

concentration of supernatant was measured (Bio-Rad Laboratories, 

Hercules, CA). The lysates were analyzed on SDS-PAGE 

(4–20%), and the proteins were transferred to nitrocellulose mem- 

branes. For IL-6, ERs, and IκB-α, 40 μg of lysate per lane were loaded, whereas for NF-κB, 20 μg of cardiomyocyte nuclear extract 

per lane were loaded. After the transfer, the membranes were immu-

noblotted with anti IL-6 (Biosource International, Camarillo, CA), 

ER-α/ER-β, NF-κB, and IκB-α/phospho-IκB-α antibodies (Santa 

Cruz Biotechnology, Santa Cruz, CA), respectively, followed by the addi- 

tion of horseradish peroxidase-conjugated secondary antibody. 

After the final wash, membranes were probed using enhanced chemi- 

luminescence dye (ECL; Amersham Pharmacia Biotech, Piscataway, 

NJ) and autoradiographed. The density of the bands was determined 

using densitometric analysis. In addition, the membranes were re-

probed using glyceraldehyde-3-phosphate dehydrogenase (Abcam,
RESULTS

Alterations in cardiac function and systemic hemodynamic parameters following trauma-hemorrhage and resuscitation. Table 1 shows cardiac functions in sham animals in various stages of estrus cycle, because the plasma sex hormone levels are different in various estrus cycles and these levels may influence the basal cardiac function. However, when conducting the experiment, we found that there was no difference in the basal cardiac function from various estrus cycles, including OVX females included in the subsequent data presentations where comparisons are made between sham and trauma-hemorrhage in various stages of estrus cycle.

CO decreased by 26.2, 27.8, 35.9, and 51.1% in E, ME, DE, and OVX females compared with shams, respectively, at 2 h after trauma-hemorrhage (P < 0.05, Fig. 1A); however, CO was normal in PE females and was significantly higher compared with that in E, ME, DE, and OVX groups at 2 h after trauma-hemorrhage (P < 0.05, Fig. 1A). Moreover, SV decreased by 16.3, 17.0, 19.6, and 36.4% in E, ME, DE, and OVX females compared with shams, respectively, after trauma-hemorrhage (P < 0.05, Fig. 1B); however, no significant difference in SV was observed in the PE group compared with sham. Furthermore, SV in the PE trauma-hemorrhage group was significantly higher compared with all other trauma-hemorrhage groups (P < 0.05, Fig. 1B). In contrast, TPR increased by 21.5% in OVX females following trauma-hemorrhage compared with shams (P < 0.05, Fig. 1C); however, TPR was not significantly altered in PE, E, ME, and DE groups under those conditions (Fig. 1C). Similarly, +dP/dt decreased by 28.6, 29.2, 44.1, and 54.2%, and −dP/dt decreased by 25.4, 36.3, 53.4, and 66.5%, in E, ME, DE, and OVX females, respectively, at 2 h after trauma-hemorrhage compared with shams (P < 0.05, Fig. 1, D and E). Both +dP/dt and −dP/dt were maintained in PE females at 2 h after trauma-hemorrhage (Fig. 1, D and E). There was a significant decrease in MAP in E, ME, DE, and OVX females and in HR in DE and OVX females after trauma-hemorrhage compared with shams; however, both MAP and HR were maintained in the PE group (Table 2). The hemoglobin and hematocrit results indicate that the trauma-hemorrhagic shock models were consistent in various groups (Table 2). Although 60% of total circulating blood was withdrawn and hemoglobin and hematocrit significantly decreased in all trauma-hemorrhage groups, systemic O₂ delivery in PE significantly improved in the PE compared with DE and OVX groups. Moreover, the increase in circulating lactate was prevented in the PE trauma-hemorrhage group. This finding suggests that acidosis following trauma-hemorrhage was prevented and the metabolic situation was improved in this group (Table 2). In addition, the mortality rate in the present study was 10.0% (1 of 10), 18.2% (2 of 11), and 33.3% (4 of 12) in E, DE, and OVX females within 2 h after trauma-hemorrhage, respectively. In contrast, all PE and ME females were alive at 2 h after trauma-hemorrhage in this study.

Alterations in plasma levels of sex hormones. Plasma levels of estradiol were highest at the start of the experiment in the PE group (9.34 ± 0.76 pg/mg protein) and were significantly lower in the E (5.34 ± 0.16 pg/mg protein), ME (3.29 ± 0.27 pg/mg protein), DE (2.32 ± 0.11 pg/mg protein), and OVX groups (0.59 ± 0.12 pg/mg protein) (P < 0.05, Fig. 2A). Moreover, the highest plasma progesterone levels were found in the E and ME groups (Fig. 2B).

Alterations in systemic IL-6 levels. Plasma levels of IL-6 were significantly increased in the ME, DE, and OVX groups after trauma-hemorrhage compared with shams (P < 0.05) (Fig. 3A); however, plasma IL-6 levels were markedly lower in PE females after trauma-hemorrhage.

Alterations in cardiomyocyte IL-6 expression. A significant increase occurred in IL-6 protein level in the cardiomyocytes homogenates prepared from the DE and OVX groups after trauma-hemorrhage compared with the homogenates from sham-operated animals; however, the trauma-hemorrhage-me-

Table 1. Cardiac function, systemic $\dot{D}O_2$ and $\dot{V}O_2$, Hb, systemic hematocrit, and plasma lactate at 2 h after sham operation in different estrus cycles

<table>
<thead>
<tr>
<th></th>
<th>PE</th>
<th>E</th>
<th>ME</th>
<th>DE</th>
<th>OVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO, ml/min $\cdot$100 g body wt $^{-1}$</td>
<td>41.1±0.4</td>
<td>40.6±0.4</td>
<td>40.2±0.3</td>
<td>40.2±0.3</td>
<td>40.0±0.4</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>15338±1160</td>
<td>14698±600</td>
<td>15081±1150</td>
<td>13983±1045</td>
<td>14078±988</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>9705±591</td>
<td>9222±564</td>
<td>9324±120</td>
<td>9303±443</td>
<td>9111±814</td>
</tr>
<tr>
<td>SV, µl-beat $^{-1}$·100 g tissue $^{-1}$</td>
<td>100.3±0.9</td>
<td>100.9±0.7</td>
<td>99.7±0.8</td>
<td>100.1±0.3</td>
<td>99.7±0.9</td>
</tr>
<tr>
<td>TPR, mmHg·mg$^{-1}$·100 g body wt $^{-1}$</td>
<td>2.98±0.04</td>
<td>2.98±0.03</td>
<td>3.01±0.14</td>
<td>2.98±0.12</td>
<td>3.01±0.16</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>123±3</td>
<td>121±2</td>
<td>121±6</td>
<td>120±4</td>
<td>120±6</td>
</tr>
<tr>
<td>HR, beat/min</td>
<td>410±27</td>
<td>403±2</td>
<td>403±4</td>
<td>402±2</td>
<td>401±2</td>
</tr>
<tr>
<td>$\dot{D}O_2$, ml/min $^{-1}$·100 g body wt $^{-1}$</td>
<td>7.7±0.2</td>
<td>7.7±0.1</td>
<td>7.7±0.1</td>
<td>7.8±0.2</td>
<td>7.7±0.1</td>
</tr>
<tr>
<td>$\dot{V}O_2$, ml/min $^{-1}$·100 g body wt $^{-1}$</td>
<td>2.4±0.3</td>
<td>2.4±0.2</td>
<td>2.2±0.3</td>
<td>2.4±0.1</td>
<td>2.4±0.4</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>14.4±0.5</td>
<td>14.5±0.2</td>
<td>14.9±0.1</td>
<td>14.7±0.4</td>
<td>14.7±0.2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.0±1.0</td>
<td>44.6±0.4</td>
<td>45.0±0.8</td>
<td>44.8±1.8</td>
<td>44.6±0.7</td>
</tr>
<tr>
<td>Lactate, mg/dl</td>
<td>8.2±2</td>
<td>7±1</td>
<td>8±1</td>
<td>9±1</td>
<td>8±1</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE (n = 4/group) and were compared using 1-way ANOVA and Tukey’s test. PE, proestrus; E, estrus; ME, metestrus; DE, diestrous; OVX, ovariectomized. CO, cardiac output; +dP/dt, rate of pressure increase; −dP/dt, rate of pressure decrease; SV, stroke volume; TPR, total peripheral resistance; MAP, mean arterial pressure; HR, heart rate; $\dot{D}O_2$, oxygen delivery; $\dot{V}O_2$, oxygen consumption; Hb, hemoglobin; hematocrit, systemic hematocrit.
diated increase in IL-6 expression was markedly attenuated in PE females (Fig. 3, B and C).

Alterations in cardiomyocyte ERs. Cardiac ER-α expression decreased significantly in the DE and OVX groups after trauma-hemorrhage compared with shams (P<0.05, Figs. 4, A and B); however, no difference in cardiomyocyte ER-α levels was observed in the PE group compared with shams (Fig. 4, A and B). Similarly, cardiac ER-β expression decreased significantly in the DE and OVX groups after trauma-hemorrhage (P<0.05, Fig. 4, A and C); however, the levels in the PE group after trauma-hemorrhage were similar to those in shams (P<0.05, Fig. 4, A and C).

Table 2. Alterations in MAP, HR, systemic DO2 and VO2, Hb, systemic hematocrit, and plasma lactate at 2 h after trauma-hemorrhage and resuscitation

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>PE-TH</th>
<th>E-TH</th>
<th>ME-TH</th>
<th>DE-TH</th>
<th>OVX-TH</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>121±4</td>
<td>100±2</td>
<td>81±6*</td>
<td>88±5*</td>
<td>76±3†</td>
<td>73±8†</td>
</tr>
<tr>
<td>HR, beat/min</td>
<td>403±5</td>
<td>360±12</td>
<td>357±12</td>
<td>365±16</td>
<td>322±8*</td>
<td>316±22*</td>
</tr>
<tr>
<td>DO2, ml/min/100 g body wt⁻¹</td>
<td>7.6±0.1</td>
<td>2.9±0.1*</td>
<td>2.4±0.1*§</td>
<td>2.5±0.1*§</td>
<td>2.0±0.1*†</td>
<td>1.7±0.1†</td>
</tr>
<tr>
<td>VO2, ml/min/100 g body wt⁻¹</td>
<td>2.4±0.3</td>
<td>1.5±0.1*</td>
<td>1.2±0.1*</td>
<td>1.0±0.1*</td>
<td>1.1±0.1*</td>
<td>1.0±0.1*</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>14.2±0.3</td>
<td>5.4±0.3*</td>
<td>5.6±0.2*</td>
<td>5.8±0.2*</td>
<td>5.7±0.1*</td>
<td>6.2±0.3*</td>
</tr>
<tr>
<td>Hematocritsys</td>
<td>43±1</td>
<td>17±1*</td>
<td>18±1*</td>
<td>18±1*</td>
<td>18±1*</td>
<td>19±1*</td>
</tr>
<tr>
<td>Lactate, mg/dl</td>
<td>7±1</td>
<td>10±1</td>
<td>18±2§</td>
<td>21±4*§</td>
<td>35±5†</td>
<td>38±3*</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE (n=6–7/group) and were compared using 1-way ANOVA and Tukey’s test. *P<0.05 vs. sham. †P<0.05 vs. PE-TH. ‡P<0.05 vs. DE-TH. §P<0.05 vs. OVX-TH. T-H, trauma-hemorrhage.
trauma-hemorrhage in the E, ME, DE, and OVX groups (P < 0.05); however, there was no significant difference in NF-κB in the PE group following trauma-hemorrhage compared with the sham group (P < 0.05, Fig. 6, A and B).

DISCUSSION

Our results indicate that cardiac function was significantly depressed in E, ME, DE, and OVX female rats as demonstrated by significantly decreased CO, SV, and ±dP/dt max following trauma-hemorrhage and resuscitation. Females in the PE cycle, however, maintained all of the above cardiac functional parameters following trauma-hemorrhage. The maintenance of cardiac function following trauma-hemorrhage in PE females was associated with the highest levels of estradiol, whereas all others had significantly lower plasma levels of estradiol. Although estradiol levels were higher in E and ME cycles compared with OVX females, it appears that those levels were not sufficient to prevent cardiac depression following trauma-hemorrhage. Moreover, both cardiomyocyte ER-α and ER-β expression levels were significantly decreased in DE and OVX females following trauma-hemorrhage; however, they were maintained at levels similar to those in shams in the PE group following trauma-hemorrhage. Furthermore, these levels were significantly higher compared with those in DE and OVX females following trauma-hemorrhage. It should also be noted that cardiomyocyte and plasma IL-6 levels were significantly increased in E, ME, DE, and OVX groups at 2 h after trauma-hemorrhage, whereas they were significantly attenuated in the PE group under those conditions. Although high levels of progesterone were found in E and ME females, it appears that progesterone did not have sufficient protective effects on cardiac function following trauma-hemorrhage. This conclusion may appear at variance from previous findings by our group (20), which showed that administration of progesterone (25 mg/kg body wt) in OVX rats following trauma-hemorrhage ameliorated the proinflammatory response and prevented hepatic injury under those conditions. However, it should be noted that the highest level of progesterone observed in the plasma of mice was ~30 pg/mg protein, whereas 25 mg/kg progesterone was administered in the previous study (20). Thus far higher levels of progesterone are needed for progesterone to have salutary effects following trauma-hemorrhage.

Fig. 2. Alterations in plasma estradiol (A) and progesterone (B) levels in PE, E, ME, DE, and OVX females. There were 6–7 animals in each group. Data are expressed as means ± SE and were compared using 1-way ANOVA and Tukey’s test. #P < 0.05 vs. PE. +P < 0.05 vs. OVX. *P < 0.05 vs. DE.

Fig. 3. Plasma (A) and cardiomyocyte IL-6 protein levels (B and C) in sham, PE-TH, E-TH, ME-TH, DE-TH, and OVX-TH females. Representative blot is shown in B. Blots were analyzed densitometrically; densitometric values are pooled from 4 animals in each group and are shown in C. Data are expressed as means ± SE and were compared using 1-way ANOVA and Tukey’s test. *P < 0.05 vs. sham. #P < 0.05 vs. PE-TH. +P < 0.05 vs. OVX-TH.
Our results collectively demonstrate that ovarian and gonadal sex steroids are associated with the sexual dimorphic response to trauma-hemorrhage. Previous studies by our group have shown that immunological functions are markedly depressed in males following trauma-hemorrhage but not in PE females under those conditions (1, 42). The reason for the diverse immunological effects between males and PE females appears to be due to high estradiol levels that are observed in the proestrus state (1, 16). Support for this notion comes from studies showing that OVX females responded to trauma-hemorrhage in a manner even worse than that of males. Furthermore, administration of estradiol in OVX females and even in males normalized the depressed cardiovascular and immunological responses following trauma-hemorrhage (16, 26, 40). These studies therefore suggest that the maintenance or depression of cardiac function is dependent on the estrogen levels prevailing at the time of trauma-hemorrhage. Support for this notion comes from the present study, which indicates that the PE females, which have the highest estrogen levels, maintained cardiac function following trauma-hemorrhage. Although E and ME females showed some improvement in CO, SV, and \( \frac{\partial P}{\partial t} \) compared with OVX females following trauma-hemorrhage, all of the measured parameters were not restored to normal levels. The slight improvement in the above parameters may be due to the relatively higher estrogen levels in E and ME females compared with OVX females. It therefore appears that the protective effects of estrogen on cardiac function following trauma-hemorrhage are likely due to the estrogen levels present at the time of injury.

It also appears that not only low levels of estradiol but also the levels of male sex steroids are responsible for the depression of cardiac function following trauma-hemorrhage. In this...
were compared using 1-way ANOVA and Tukey’s test. P

were reprobed with histone H1 for control. Data are expressed as means analyzed densitometrically; densitometric values are pooled from 4 animals in

B. A representative blot is shown in

preparation of various groups were analyzed on SDS-PAGE and immunoblot- tion downregulated trauma-hemorrhage-induced increase in

levels were significantly elevated at 24 h after trauma-hemorrhage (1). Nonetheless, it remains to be determined whether the effects on cell and organ functions following trauma-hemorrhage. Our previous studies have demonstrated that plasma IL-6 levels were increased at 2 h after trauma-hemorrhage and resuscitation, and the elevated plasma and cardiac IL-6 levels in E, ME, DE, and OVX females were associated with adverse effects on cardiac function. However, in PE females the level of IL-6 following trauma-hemorrhage was not significantly different from that observed in sham animals. Since the levels of estrogen are highest in the PE state, it is likely that the elevated level of estrogen in PE females downregulates cardiomyocyte IL-6 production and thus protects cardiac functions following trauma-hemorrhage. To establish the relationship between cardiac IL-6 and cardiac function following trauma-hemorrhage, we administered goat anti-rat-IL-6 monoclonal antibody at the middle of resuscitation after trauma-hemorrhage to neutralize IL-6 and measured the effect of this treatment on cardiac function following trauma-hemorrhage. Our results suggested that treatment of animals with anti-IL-6 antibodies downregulated cardiac IL-6 and improved cardiac function (39).

The protective effects of estrogen in cardiovascular diseases are currently under intensive investigation because of the potential therapeutic hormone effects (7, 25). However, the molecular mechanism by which estrogen downregulates cardiac IL-6 production is still not fully understood. Studies have demonstrated that estradiol attenuates cytokine production by inhibiting the transcription factor (nuclear factor) NF-κB (18). NF-κB is a pleiotropic transcription factor implicated in the regulation of diverse biological phenomena, including the cellular responses to stress, hypoxia, ischemia (18), and hemorrhagic shock (13). Studies have shown that NF-κB is activated in various heart diseases such as myocarditis, congestive heart failure, dilated cardiomyopathy, and heart transplant rejection (18). In the heart, NF-κB is found to be activated following burn, ischemia-reperfusion, and hypoxia (23, 24, 28). Moreover, studies have demonstrated that induction of IL-6 by hypoxia, a condition associated with trauma-hemorrhage, is mediated by NF-κB and NF-IL-6 in cardiomyocytes (28) and that hypoxia induces IL-6 gene expression through NK-κB activation (3). NF-κB and IκB-α interact in an autoregulatory mechanism (24). Transcription factors NF-IL-6 and NF-κB are known to synergistically activate the transcription of inflammatory cytokines (23). Studies also have shown that hypoxia-induced transcriptional activation of IL-6, i.e., IL-6 gene expression, is induced mainly through the activation of NF-κB in cardiomyocytes (29). Studies also have shown that estrogen represses IL-6 gene expression through inhibition of the DNA-binding activities of the transcription factors NF-IL-6 and NF-κB by the ERs (31). The present findings suggest a relationship between the increased cardiac IL-6 and the cardiac NF-κB/IκB-α system following trauma-hemorrhage. Thus the normalized cardiac function following trauma-hemorrhage in PE females also may be due to inhibition of the NF-κB/IκB system. Females in the PE cycle had a significantly attenuated increase in cardiac NF-κB and improvement in cardiac func-
tion under those conditions. It also has been demonstrated that JAK2/STAT3, not ERK1/2, mediates IL-6-induced activation of inducible NO synthase and a decrease in contractility of adult ventricular myocytes (41). Studies also have shown that estrogen inhibits growth hormone (GH) signaling by suppressing GH-induced JAK2 phosphorylation, and the suppressors of cytokine signaling (SOCS) play a central mechanistic role. Moreover, STAT3 is a molecular participant in ER inhibition of the IL-6 signaling pathway (21). In addition, studies have indicated sexual dimorphism in the permeability response of coronary microvessels to adenosine (15). Nonetheless, the precise mechanisms of estrogen’s effect in the attenuation of IL-6 following trauma-hemorrhage remain to be clarified.

In summary, the present results suggest that cardiac function was significantly depressed at 2 h after trauma-hemorrhage and resuscitation in E, ME, DE, and OVX female rats; however, it was maintained in PE females under those conditions. Furthermore, cardiomyocyte IL-6 expression and plasma IL-6 levels significantly increased after trauma-hemorrhage in E, ME, DE, and OVX female rats. Nonetheless, the increases in cardiomyocyte IL-6 expression as well as systemic IL-6 were not apparent in PE females. Moreover, the cardiomyocyte NF-κB/IκB-α system was activated in E, ME, DE, and OVX females following trauma-hemorrhage; however, it was inhibited in PE females. These data collectively suggest that the estrus cycle plays an important role in cardiac function following trauma-hemorrhage. The salutary effect seen in PE females following trauma-hemorrhage is likely due to a decrease in NF-κB-dependent cardiac IL-6 pathway.

GRANT

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