Gender dimorphic tissue perfusion response after acute hemorrhage and resuscitation: role of vascular endothelial cell function

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Gender dimorphic tissue perfusion response after acute hemorrhage and resuscitation: role of vascular endothelial cell function. Am J Physiol Heart Circ Physiol 284: H2162–H2169, 2003. First published February 6, 2003; 10.1152/ajpheart.00724.2002.—Proestrous female rodents are protected from the deleterious effects of trauma-hemorrhage that are observed in males. We hypothesized that the gender dimorphic outcome after trauma-hemorrhage might be related to gender differences in endothelial function and organ perfusion under such conditions. Male and cycle-matched proestrous female Sprague-Dawley rats underwent a midline laparotomy, hemorrhagic shock (40 mmHg for ~90 min), and resuscitation (Ringer lactate, 4× shed blood volume over 60 min). Various parameters were measured 2 h after completion of resuscitation. In the first set of animals, the left ventricle was cannulated and heart performance (maximal rate of left ventricular pressure increase) as well as cardiac output and organ perfusion rates were determined with $^{85}$Sr microspheres. In the second set of animals, aortic vessel rings were harvested and relaxation in response to acetylcholine and nitroglycerin was measured. In the third set of animals, in situ isolated small intestine was perfused to measure the response of the splanchnic vessel bed to acetylcholine and nitroglycerin. After trauma-hemorrhage and resuscitation, females maintained cardiac output and demonstrated increased splanchnic and cardiac perfusion compared with males. Moreover, female intestines did not manifest the endothelial dysfunction that was observed in male intestines after hemorrhagic shock. We conclude that proestrous females show improved endothelial function and tissue perfusion patterns after hemorrhagic shock and that this gender-specific response might be a potential mechanism contributing to the beneficial effects of the proestrous stage under such conditions.

cardiac output; endothelial function; coronary artery; gut; liver

Several studies have demonstrated a gender dimorphic response to tissue trauma and hemorrhagic shock (1, 11). Female rodents, especially during the proestrus stage, appear to be protected from the deleterious effects of trauma-hemorrhage and resuscitation, i.e., they show no signs of the cardiovascular and immune suppression that is observed in males. Additional studies using ovariectomized and castrated rodents and hormone replacement therapies demonstrated that differences in the hormonal milieu are crucial for the gender-specific response to trauma-hemorrhage (12, 13, 19). Despite the vast amount of data implicating a divergent response associated with gender and sex steroids, the underlying mechanisms remain unclear. In this regard, it is well known that male and female sex steroids directly affect the cardiovascular system (16, 18). For example, numerous studies on isolated vessel rings have shown that estradiol in various concentrations can either directly relax blood vessels or facilitate the relaxation in response to other agents (16). Administration of a single high dose of estradiol in vivo has been shown to dampen the subsequent pressure response to norepinephrine (NE) (20). Studies have also shown that continuous treatment of ovariectomized rats with estradiol significantly lowers mean arterial pressure (8). In contrast, androgen in physiological concentrations has been shown to inhibit vasorelaxation (4), and the continuous treatment of rats with testosterone significantly increased mean arterial blood pressure (5). Moreover, a recent study by Kuebler et al. (14) demonstrated that treatment of male rats with estradiol in a “double-hit” model of trauma-hemorrhage and induction of subsequent sepsis specifically increased the splanchic circulation under those conditions. In this regard, it is well known that endothelial cell function is impaired early during hemorrhagic shock that persists after resuscitation (27). This is associated with an impaired blood flow to organs such as the gut and the liver after resuscitation and appears to be a determining factor in the subsequent development of organ dysfunction (15).

In light of these findings, we hypothesized that the improved outcome in proestrus female rats in our model of soft tissue trauma and hemorrhagic shock could be related to differences in organ perfusion in male and proestrus female rats under such conditions. We therefore determined tissue perfusion in male and cycle-matched proestrus female rats with

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radioactive microspheres. Because gender-dependent differences were observed, we also investigated the mechanism of gender dimorphism by using isolated aortic vessel rings and an in situ intestinal perfusion model to determine endothelial function in male and proestrous female rats after trauma-hemorrhage and resuscitation.

MATERIALS AND METHODS

Animals. Age-matched adult male and female Harlan Sprague-Dawley rats weighing 275–325 and 200–250 g, respectively, were studied. All female rats were studied at the proestrus stage of the reproductive cycle, defined by the presence of both leukocytes and nucleated epithelial cells in approximately equal numbers on vaginal smears. Animals were fasted for 16 h before the experiment but were allowed water ad libitum. This project was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Trauma-hemorrhage model. The animals were anesthetized with 1.5% isoflurane and air inhalation; they underwent a 5-cm ventral midline laparotomy to induce tissue trauma before the onset of hemorrhage. The abdominal incision was then closed in layers. Both femoral arteries were cannulated with polyethylene (PE)-50 tubing for bleeding or monitoring of mean arterial pressure. All incisions were then closed and bathed with 1% lidocaine hydrochloride to provide analgesia throughout the experiment. The animals were then bled to a mean arterial pressure of 40 mmHg (i.e., severe analgesia throughout the experiment. The animals were then resuscitated with RL solution at four times the blood volume was returned in that form. After this, the lactate (RL) solution intravenously until 40% of the shed blood was no longer able to maintain its blood pressure at that level was no longer able to maintain its blood pressure at that level of severe hypotension). After injection, the animals were monitored of heart performance with a heart performance analyzer. The measurement of cardiac output and organ blood flow. At 2 h after resuscitation or sham operation, organ blood flow and cardiac output were determined by using a radioactive microsphere technique previously described by us (25).

Briefly, strontium 85-labeled microspheres (DuPont NEN, Boston, MA) were suspended in 10% dextran containing 0.01% polysorbate 80 (Tween 80; Sigma-Aldrich) to prevent aggregation. The microspheres were 15 μm in diameter. They were dispersed with a vortex shaker for 3 min before infusion. A 0.2- to 0.25-ml suspension of microspheres with an activity of −2–2.5 μCi (~500,000 cpm) was injected manually into the left ventricle in each rat via the left ventricular catheter for 20 s at a constant rate. The reference blood sample was withdrawn from the femoral arterial catheter into a 3-ml syringe, beginning 20 s before microsphere infusion and continuing for an additional 60 s at a rate of 0.7 ml/min, with a pump (Harvard Apparatus, Holliston, MA). Isotonic sodium chloride solution was infused manually at the rate of 0.7 ml/min immediately after microsphere infusion to replace the volume of blood lost. The rat was then killed with an overdose of inhaled isoflurane. Various organs were removed, weighed, and harvested, washed thoroughly with test tubes, and organ radioactivity was counted with an automatic gamma counter (1470 Wizard; Wallac, Gaithersburg, MD). The reference blood sample was transferred from a syringe into a test tube for radioactivity measurement. The remaining microspheres, which were left in the syringe after injection, were also counted. Cardiac output and organ blood flow were calculated according to the following equations: cardiac output = \([\text{RBF} \times C_r]/C_t\) × 1/100 and organ blood flow = \([\text{RBF} \times C_r]/C_t\) × 1/100, where RBF is the reference blood sample withdrawal rate (0.7 ml/min), \(C_r\) is counts per minute of total injected dose, \(C_t\) is counts per minute per gram of tissue, and \(C_r\) is counts per minute in the reference blood sample.

Aortic ring preparation and determination of endothelial function. In an additional group of animals, aortic endothelial function was determined with isolated aortic vessel rings. Male and female rats were killed with an overdose of isoflurane 2 h after trauma-hemorrhage and resuscitation or sham operation, the chest was opened, and the thoracic aorta was rapidly removed. The isolated thoracic aorta was immediately immersed in Krebs-Ringer HCO₃ solution (composition in mM: 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, 0.028 Ca-EDTA, and 11.1 glucose), which was aerated with 95% O₂-5% CO₂ (pH = 7.4; \(P_O_2\) = 580 mmHg). The thoracic aorta was trimmed with care to prevent any damage to vascular endothelial cells and was cut into rings ~2.5 mm in length. The aortic ring was carefully mounted on two specimen holders and placed in a glass organ chamber containing 20 ml of aerated Krebs-Ringer HCO₃ solution at 37°C. One holder was stationary, whereas the other holder was connected to an isometric force-displacement transducer (model FT03; Grass Instruments, Quincy, MA) coupled to a polygraph (model 7D; Grass Instruments). The aortic ring was incubated for 60 min at a tension of 1,000 mg, during which time the organ chamber was rinsed every 15 min with the aerated Krebs-Ringer HCO₃ buffer. Although a length-tension curve was not performed in the aortic rings, our current and previous studies (15, 16) have suggested that a 1-g resting tension is required to generate adequate additional tension induced by NE. When basal tension was stable, a submaximal contraction (~75% of maximal contraction) was induced by 2 × 10⁻⁷ M NE (Sigma, St. Louis, MO). An endothelium-dependent vasodilator, Ach (Sigma; concentration range from 10⁻⁸ to 10⁻⁵ M), or an endothelium-independent vasodilator, nitroglycerin (NTG)
(American Regent Laboratories, Shirley, NY; concentration range from $10^{-9}$ to $10^{-6}$ M), was applied cumulatively thereafter. Response to NTG was also determined in endothelium-denuded aortic rings. Endothelium denuding was carried out by using the rough side of forceps, with care, to gently remove endothelial cells and to prevent any damage to vascular smooth muscles. After each series of agent additions, the ring preparations were washed with aerated Krebs-Ringer HCO$_3$ solution and allowed to reequilibrate for at least 30 min. At the end of the experiment the contractile responses of the vessel ring preparations were checked by using 5 x $10^{-6}$ M NE, and no significant decrease in vascular tension induced by such an agent was found.

**Isolated small intestine and determination of ACh- and NTG-induced small vessel relaxation.** In additional animals, the right femoral vein was cannulated under isoflurane (1.5% balanced with air) anesthesia and the anesthesia was maintained by injection of pentobarbital sodium (30 mg/kg body wt) via the femoral catheter. Immediately after intravenous injection of 0.5 ml of heparin solution (500 units), the small intestine was isolated without removal from the abdominal cavity. The superior mesenteric artery and portal vein were cannulated with PE-50 and PE-90 tubing, respectively. The branches of blood vessels to and from the cecum and the ascending and transverse portions of the colon were then ligated. While the rat was still alive, the isolated intestine was perfused with aerated Krebs-Ringer HCO$_3$ buffer (95% O$_2$-5% CO$_2$, 37°C) through the superior mesenteric artery catheter (PE-50). Dextran 70 (6%; Sigma) was added to Krebs-Ringer HCO$_3$ buffer to prevent intestinal edema. After 30-min equilibration at a constant perfusion rate of 5 ml/min, perfusion pressure was 28.5 ± 1.5 mmHg. Blood vessels in the isolated intestine were then contracted with 5 x $10^{-6}$ M NE, which increased perfusion pressure to 113.7 ± 6.1 mmHg. The vascular responses to ACh or NTG were measured thereafter by determining the changes in perfusion pressure in the isolated small intestine. The intestinal preparations were viable throughout the study period as determined by the similar rise in the NE-induced increase in perfusion pressure at the end of the experiments versus that at the beginning of the experiments.

**Statistical analysis.** Data are presented as means ± SE. Vascular responses are expressed as percent reduction of the initial vascular tension (i.e., $2 \times 10^{-7}$ M NE-induced contraction in isolated thoracic aortic ring study and $5 \times 10^{-6}$ M NE-induced contraction in isolated small intestine). Two aortic rings from each animal were studied, and the values were averaged. One-way ANOVA and Tukey’s test were used for comparisons between groups, and the differences were considered significant at $P < 0.05$.

**RESULTS**

**Heart performance and heart rate.** As shown in Fig. 1A, the values of +dP/dt$_{max}$ were 11,481 ± 902 and 10,874 ± 1,796 mmHg/s for male and female animals, respectively, under sham operation conditions. After trauma-hemorrhage, the values of +dP/dt$_{max}$ were significantly reduced to 5,851 ± 600 mmHg/s in male rats. In contrast, heart performance in proestrous female rats remained unchanged after hemorrhagic shock (10,791 ± 741 mmHg/s) and was significantly higher than in males. There was no significant difference in heart rate between the different groups under sham operation conditions or after trauma-hemorrhage (Table 1).

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**Fig. 1.** Cardiac performance (rate of left ventricular pressure increase (+dP/dt)) and cardiac output in male and cycle-matched proestrous female rats under sham operation conditions and at 2 h after trauma-hemorrhage (Hem). There were 7 animals in each group. Data are means ± SE (compared by 1-way ANOVA and Tukey’s test). A: maximal +dP/dt. *P < 0.05 vs. male sham operated. B: cardiac output in milliliters per minute per 100 g of body weight. *P < 0.05 vs. male sham operated.

**Alterations in cardiac output and organ blood flow.** Male rats showed a significant decrease of cardiac output after trauma-hemorrhage compared with sham-operated animals (sham operation: 26.9 ± 2.4 ml/min $100 g^{-1}$; trauma-hemorrhage: 15.6 ± 2.0 ml/min $100 g^{-1}$). In contrast, the values of cardiac output in proestrous female rats under sham operation conditions and after hemorrhagic shock showed no significant difference (sham operation: 22.4 ± 1.9 ml/min $100 g^{-1}$; trauma-hemorrhage: 18.9 ± 1.4 ml/min $100 g^{-1}$) (Fig. 1B).

Figure 2 shows organ perfusion rates for male and proestrous female rats under sham operation conditions and after trauma-hemorrhage. As seen in Fig. 2A, coronary blood flow remained unchanged in male rats (sham operation: 463 ± 64 ml/min $10^{-1} g^{-1}$; trauma-hemorrhage: 457 ± 106 ml/min $10^{-1} g^{-1}$). In proestrous female rats, however, coronary blood flow increased significantly after trauma-hemorrhage (sham operation: 318 ± 33 ml/min $10^{-1} g^{-1}$; trauma-hemorrhage: 673 ± 76.6 ml/min $10^{-1} g^{-1}$). The small intestine blood flow shown in Fig. 2B demonstrates a significant decrease after trauma-hemorrhage in male rats (sham operation: 129 ± 19 ml/min $10^{-1} g^{-1}$; trauma-hemorrhage: 66 ± 10 ml/min $10^{-1} g^{-1}$), but proestrous female rats had maintained intestinal perfusion after trauma-hemorrhage with values similar to those in sham-
operated animals (sham operation: 100 ± 13 ml·min⁻¹·g⁻¹; trauma-hemorrhage: 97 ± 12 ml·min⁻¹·g⁻¹). A similar pattern was observed in total hepatic blood flow (Fig. 2C). Males showed significantly reduced values after trauma-hemorrhage (sham operation: 137 ± 8 ml·min⁻¹·g⁻¹; trauma-hemorrhage: 77 ± 13 ml·min⁻¹·g⁻¹), whereas proestrous females showed maintained hepatic perfusion rates under those conditions (sham operation: 135 ± 17 ml·min⁻¹·g⁻¹; trauma-hemorrhage: 114 ± 15 ml·min⁻¹·g⁻¹). However, as seen in Fig. 2D, both male and proestrous female rats showed a significant reduction in renal perfusion rates after trauma-hemorrhage (male sham operation: 470 ± 20 ml·min⁻¹·g⁻¹; male trauma-hemorrhage: 117 ± 26 ml·min⁻¹·g⁻¹; female sham operation: 338 ± 37 ml·min⁻¹·g⁻¹; female trauma-hemorrhage: 151 ± 60 ml·min⁻¹·g⁻¹). As shown in Table 2, the perfusion rates in muscles were significantly lower in females under sham operation conditions as well as after trauma-hemorrhage compared with males. The other organs showed no significant difference between males and females.

Alterations in reactivity of aortic vessel ring and isolated perfused intestine. As indicated in Fig. 3A, the response to ACh-induced relaxation of aortic vessel rings harvested 2 h after trauma-hemorrhage was impaired in male animals compared with male sham-operated animals. The values of ACh-induced vascular relaxation was also lower in females after hemorrhagic shock compared with sham-operated females, but the differences were not significant. The response to NTG was not affected by either gender or the trauma-hemorrhage procedure (Fig. 4A). In mechanical endothelium-denuded vessels, the relaxation to ACh was eliminated; however, a significant relaxation to NTG was observed. The relaxation to NTG was similar to that seen in intact vessels.

As shown in Fig. 3B, in contrast to the modest differences observed in aortic vessel rings, the reactivity of the intestinal vessel bed to all doses of ACh was significantly impaired in male rats after trauma-hemorrhage. However, the perfused intestine of proestrous female rats showed no change after trauma-hemorrhage and the values were similar to those of male and female sham-operated rats. Furthermore, the values to all doses were significantly higher than those in males after trauma-hemorrhage. The response to NTG in intestinal perfusion was not significantly affected by either gender or the trauma-hemorrhage procedure (Fig. 4B).

**DISCUSSION**

Gender and gender-related differences in the hormonal milieu modulate the responsiveness of the cardiovascular system. The direct effects of sex steroids on the blood vessel, mediated via androgen and estrogen receptors on endothelial cells and/or vascular smooth muscle cells, have been studied extensively (7). Moreover, indirect effects such as modulation of reactivity to catecholamines and other vasoactive mediators have been described (9). In previous studies from our laboratory (1, 11) and others, it was observed that female gender and female sex hormones are protective factors in rodents after trauma-hemorrhage. In the present study, we examined whether the improved outcome of proestrous female rats after trauma-hemorrhage is associated with differences in organ perfusion. To further elucidate potential mechanisms of any differences ob-

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**Table 1. Body weight, blood pressure, heart rate, hematocrit, and maximal bleed-out in male and cycle-matched proestrous female rats**

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>322 ± 9.4</td>
<td>330 ± 11.9</td>
</tr>
<tr>
<td>BP, mmHg</td>
<td>113.4 ± 2.4</td>
<td>73.8 ± 3.4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>369 ± 10.98</td>
<td>298 ± 29*</td>
</tr>
<tr>
<td>Hct, %</td>
<td>42.7 ± 1.2</td>
<td>18.5 ± 0.48</td>
</tr>
<tr>
<td>BOmax, ml/100 g</td>
<td>3.55 ± 0.067</td>
<td>3.33 ± 0.08†</td>
</tr>
</tbody>
</table>

Data are means ± SE for sham operation conditions (Sham) and at 2 h after trauma-hemorrhage (Hem). BP, blood pressure; HR, heart rate; Hct, hematocrit; BOmax, maximal bleedout. *P < 0.05 vs. sham operated, †P < 0.05 vs. males (1-way ANOVA and Tukey’s test).
served, we studied in vitro effects of gender after trauma-hemorrhage by using an isolated intestinal perfusion system and vessel rings. Previous studies demonstrated that gender-related differences in the response to shock are most pronounced between male rodents and female rodents during the proestrus stage of the menstrual cycle (11, 21). To maximize the potential differences, we used cycle-matched proestrous female and male rats of similar age. We observed that at 2 h after completion of trauma-hemorrhage and resuscitation, male rats showed significantly decreased cardiac output and cardiac performance compared with sham-operated rats. In contrast, proestrus female rats did not show a depression in cardiac parameters. Moreover, male rats under such conditions had the same coronary flow as sham-operated rats, whereas in proestrous females coronary blood flow significantly increased after trauma-hemorrhage and resuscitation. Although there was no difference in the extent of renal blood flow reduction observed in male and female rats after trauma-hemorrhage, proestrous female rats maintained total hepatic and small intestine perfusion whereas male rats showed a significant reduction in the perfusion rate of both organs. As measured in the isolated perfused intestine, the decreased intestinal

### Table 2. Organ perfusion rates in male and cycle-matched proestrous female rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>Male</th>
<th>Female</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Hem</td>
</tr>
<tr>
<td>Liver</td>
<td>136.8 ± 8.1</td>
<td>76.0 ± 13.1*</td>
</tr>
<tr>
<td>Portal</td>
<td>121 ± 10.5</td>
<td>74.2 ± 8.2*</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>17.6 ± 2.9</td>
<td>12.9 ± 2.1</td>
</tr>
<tr>
<td>Brain</td>
<td>38.9 ± 2.4</td>
<td>46.3 ± 3.8</td>
</tr>
<tr>
<td>Lung</td>
<td>63.6 ± 18.1</td>
<td>45.8 ± 3.8</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>169.7 ± 93.2</td>
<td>185.0 ± 40.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>149.4 ± 29.4</td>
<td>24.7 ± 5.6*</td>
</tr>
<tr>
<td>Skin</td>
<td>9.1 ± 0.7</td>
<td>2.6 ± 0.9*</td>
</tr>
<tr>
<td>Muscle</td>
<td>9.9 ± 1.6</td>
<td>5.2 ± 1.1*</td>
</tr>
</tbody>
</table>

Data (in ml·min⁻¹·100 g⁻¹) are means ± SE. *P < 0.05 vs. sham operated, †P < 0.05 vs. males (1-way ANOVA and Tukey's test).

### Fig. 3. Aortic vessel ring relaxation (A) and isolated intestinal perfusion pressure reduction (B) to ACh in male and cycle-matched proestrous female rats under sham operation conditions and at 2 h after trauma-hemorrhage. Data are means ± SE percentile changes (compared by 1-way ANOVA and Tukey's test). *P < 0.05 vs. male sham operated.

### Fig. 4. Aortic vessel ring relaxation (A) and isolated intestinal perfusion pressure reduction (B) to nitroglycerin in male and cycle-matched proestrous female rats under sham operation conditions and at 2 h after trauma-hemorrhage. Data are means ± SE percentile changes (compared by 1-way ANOVA and Tukey's test).
blood flow observed in male rats was associated with a significantly reduced response to the endothelium-dependent vasorelaxant after trauma-hemorrhage. However, no change was observed in the isolated perfused intestine of proestrous female animals under such conditions.

Several studies from our laboratory (12, 17) have shown that cardiac output as well as cardiac performance in male rodents subjected to trauma and hemorrhagic shock is compromised despite crystalloid resuscitation. Furthermore, Jarrar et al. (11) showed that at 24 h after hemorrhagic shock, males demonstrated significantly reduced cardiac output and cardiac function, whereas no depression of cardiac function was observed in proestrous female rats under those conditions. Similar to those results, in the present study we observed that this gender-divergent response was detectable even at 2 h after completion of resuscitation. The difference in cardiac output was associated with a significant reduction in \( +\frac{dP}{dt}_{\text{max}} \) in males, whereas no difference in \( +\frac{dP}{dt}_{\text{max}} \) was observed in proestrous females. In this regard, it is well established that estradiol has direct cardioprotective properties during low-flow conditions and ischemia (30). However, the impaired cardiac function during and after hemorrhagic shock is partially caused by intrinsic cardiac dysfunction and also depends on other factors such as preload and afterload that also could be influenced by gender. Moreover, we found that coronary blood flow at 2 h after resuscitation in proestrous female rats was significantly elevated compared with that in sham-operated animals, whereas coronary perfusion in male rats was unchanged under such conditions. In this regard, it is well known that the coronary reserve is limited and the protective effects of female sex steroids such as estradiol have been attributed to their direct relaxing properties on the coronary arteries, shown in vitro and in vivo (22–24). Moreover, after the stress of trauma-hemorrhage and the subsequent hemodilution that results from crystalloid resuscitation, it could be speculated that differences in coronary blood flow and myocardial \( O_2 \) delivery might contribute to the observed gender differences in cardiac function in our model. Nonetheless, it is interesting that although cardiac output was maintained in proestrous females after trauma-hemorrhage, a significant decrease in renal perfusion rate was observed in females, similar to that in males. In this regard, renal blood flow is tightly regulated and maintained over a wide range of blood pressure. In our studies, both male and female rats showed only a modest although significant reduction of mean arterial pressure after trauma-hemorrhage. Although under physiological conditions it is unlikely that the decrease in mean arterial pressure could cause an impairment of renal perfusion, the marked decrease in blood pressure as observed in our studies could be responsible for the decrease in renal perfusion. Because we used radioactive microspheres (<15 \( \mu \)m) to measure tissue perfusion, i.e., end-arteriolar/capillary perfusion, it could be speculated that the microcirculatory disturbances could also contribute to the decreased blood flow in the kidneys. Blood flow to the small intestine and total hepatic flow were significantly reduced in male rats after trauma-hemorrhage, similar to our previous findings (2). However, in contrast to the significantly decreased renal perfusion, intestinal perfusion and total hepatic blood flow remained essentially unchanged in proestrous females after trauma-hemorrhage. This suggests that perfusion of individual organs is differentially affected after trauma-hemorrhage and resuscitation. Our previous study (14) showed that treatment of male rats after trauma-hemorrhage with estradiol increased splanchnic perfusion even after the induction of subsequent sepsis. Thus it appears that high levels of female sex hormones, as observed during the proestrus stage or after treatment with estradiol, can alter the regulation of the blood flow in different organs after trauma-hemorrhage. It should be noted that the different perfusion patterns were only detectable in trauma-hemorrhaged animals, potentially indicating that the differences were injury or stress related.

The control of organ blood flow is finely regulated to ensure a sufficient tissue perfusion. However, after hemorrhagic shock the maintenance of tissue perfusion is impaired, resulting in focal hypoperfusion and microcirculatory disturbances. Moreover, it has been shown that the impaired tissue perfusion under such conditions is related to endothelial cell dysfunction (26, 27). In the present study, we observed that isolated aortic vessel rings of rats subjected to trauma-hemorrhage and resuscitation, especially in male animals, showed an impaired relaxation. Because the relaxation to NTG, which acts directly on vascular smooth muscle cells, was unchanged, the impaired relaxation in response to \( ACh \) (which acts via stimulation of endothelial cells) suggests that functional capacity of aortic endothelial cells is decreased. However, the differences between the aorta from sham-operated and trauma-hemorrhaged animals amounted only to ~35% in male animals and 15% in female animals. In contrast, with the isolated intestinal perfusion system, marked changes in the reactivity of the intestinal vessel bed to \( ACh \) were observed in male and female rats. Male rats, at all concentrations of \( ACh \), showed significant pressure reductions after trauma-hemorrhage, whereas in proestrous female rats there was no difference in the response of the intestinal vessel bed between sham-operated animals and animals subjected to hemorrhagic shock. Nonetheless, the response to NTG in terms of pressure reduction was not significantly different after trauma-hemorrhage in males and proestrous females. Thus it could be speculated that the changes in organ perfusion in male and proestrous female rats are at least partially mediated by differences in endothelial function.

Organ perfusion is a product of pressure-dependent tone, shear stress, and organ metabolism (3, 6). The current study only examined receptor-mediated dilation (\( ACh \)) to directly assess endothelial function. Because organ perfusion relies more on shear, myogenic and metabolic metabolism, it could be argued that...
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these factors rather than receptor-mediated dilation might play a more important role in the observed effects. The effects of shear stress, myogenic, and metabolic mechanisms were not examined, however, because they were beyond the scope of the present study, and thus the role and contribution of those factors in the observed effects remain unknown. Another issue that can be raised is that because the circulating levels of ACh can affect perfusion of the coronary, intestinal, hepatic, and renal vascular beds, the levels of ACh should have been measured. However, because we did not measure the circulating levels of ACh in our model of trauma-hemorrhage and resuscitation, the contribution of ACh in the observed effects remains unknown. Furthermore, because estrogen has been shown to affect flow-dependent response, i.e., shear stress (10), as well as intrinsic (28) and myogenic (29) tone, it is possible that a portion of our results of organ perfusion in proestrous females may be due to hormonal effects on smooth muscle tone, potentially involving the potassium or calcium channels independent of the endothelium. However, because there was no difference in the extent of renal blood flow reduction in male and female rats after trauma-hemorrhage, it might suggest that the perfusion in all organs after trauma-hemorrhage is not due to hormonal effects on smooth muscle tone.

Previous studies demonstrated that female rodents subjected to hemorrhagic shock have improved/maintained cardiac, hepatic, and immune functions (1, 11). In this regard, it is well established that the inability to provide adequate tissue perfusion under such conditions is a major cause of the delayed organ dysfunction and immune depression observed in experimental settings as well as in trauma patients. Thus we postulate that the selective modulation of the organ perfusion pattern by sex steroids could represent a potential mechanism for the beneficial effects observed in proestrous females after trauma-hemorrhage in this and previous studies. However, further studies are needed to better understand the cellular mechanisms for the area-specific alteration in blood flow in male and proestrous females after trauma-hemorrhage.

In conclusion, cycle-matched proestrous female rats maintain cardiac output and have higher coronary and splanchnic perfusion compared with male rats after trauma-hemorrhage. However, because the improved organ perfusion was not evident in all organs uniformly, we suggest that male and cycle-matched proestrous female rats have different circulatory patterns after trauma-hemorrhage and resuscitation. Moreover, the alterations in organ blood flow were associated with local endothelial reactivity. The changes observed in the circulatory priorities could thus represent a potential mechanism for the improved organ and immune functions in proestrous females after low-flow conditions.

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