Gender differences in small intestinal perfusion following trauma hemorrhage: the role of endothelin-1


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Ba, Zheng F., Tomoharu Shimizu, Laszlo Szalay, Kirby I. Bland, and Irshad H. Chaudry. Gender differences in small intestinal perfusion following trauma hemorrhage: the role of endothelin-1. Am J Physiol Gastrointest Liver Physiol 288: G860–G865, 2005. First published November 18, 2004; doi:10.1152/ajpgi.00437.2004.—Although gender differences in intestinal perfusion exist following trauma-hemorrhage (T-H), it remains unknown whether endothelin-1 (ET-1) plays any role in these dimorphic responses. To study this, male, proestrus female (female), and 17β-estradiol (E2)-treated male rats underwent midline laparotomy, hemorrhagic shock (blood pressure 40 mmHg, 90 min), and resuscitation (Ringer lactate, 4× shed blood volume, 1 h). Two hours thereafter, intestinal perfusion flow (IPF) was measured using isolated intestinal perfusion. The IPF in sham-operated males was significantly lower than those in other groups and decreased markedly following T-H. In contrast, no significant decrease in IPF was observed in females and E2 males following T-H. The lower IPF in sham-operated males was significantly elevated by ETα receptor antagonist (BQ-123) administration and was similar to that seen in sham-operated females. The decreased IPF in males after T-H was also attenuated by BQ-123 administration. The intestinal ET-1 levels in sham-operated males were significantly higher than in other groups. Although plasma and intestinal ET-1 levels increased significantly after T-H in all groups, they were highest in males. Plasma E2 levels in females and E2 males were significantly higher than in males; however, they were not affected by T-H. There was a negative correlation between plasma ET-1 and E2 following T-H. Thus ET-1 appears to play an important role in intestinal perfusion following T-H in males. The lower IPF in sham-operated males was significantly lower than those in other groups and decreased markedly following T-H. In contrast, no significant decrease in IPF was observed in females and E2 males following T-H. The lower IPF in sham-operated males was significantly elevated by ETα receptor antagonist (BQ-123) administration and was similar to that seen in sham-operated females. The decreased IPF in males after T-H was also attenuated by BQ-123 administration. The intestinal ET-1 levels in sham-operated males were significantly higher than in other groups. Although plasma and intestinal ET-1 levels increased significantly after T-H in all groups, they were highest in males. Plasma E2 levels in females and E2 males were significantly higher than in males; however, they were not affected by T-H. There was a negative correlation between plasma ET-1 and E2 following T-H. Thus ET-1 appears to play an important role in intestinal perfusion following T-H in males. The lower IPF in sham-operated males was significantly lower than those in other groups and decreased markedly following T-H. In contrast, no significant decrease in IPF was observed in females and E2 males following T-H. The lower IPF in sham-operated males was significantly elevated by ETα receptor antagonist (BQ-123) administration and was similar to that seen in sham-operated females. The decreased IPF in males after T-H was also attenuated by BQ-123 administration. The intestinal ET-1 levels in sham-operated males were significantly higher than in other groups. Although plasma and intestinal ET-1 levels increased significantly after T-H in all groups, they were highest in males. Plasma E2 levels in females and E2 males were significantly higher than in males; however, they were not affected by T-H. There was a negative correlation between plasma ET-1 and E2 following T-H. Thus ET-1 appears to play an important role in intestinal perfusion following T-H in males.

hemorrhagic shock; BQ-123; tissue trauma; 17β-estradiol

THE SMALL INTESTINAL MICROVASCULATURE is one of the most susceptible regions to injury induced by hemorrhagic shock. Impaired restoration of intestinal perfusion during resuscitation results in persistent mucosal hypoxia and subsequent loss of mucosal integrity. This loss of gut mucosal integrity has been implicated in the pathogenesis of multiple organ dysfunction syndrome (4, 10, 12, 33, 38). Moreover, maintenance of intestinal blood flow is essential for supplying blood to the portal system, which directly affects the hepatic blood flow under such conditions (41-43, 48). Thus better understanding of the vasoregulatory mechanisms of the intestinal circulation may offer a more appropriate therapeutic approach. It is well established that gender directly influences the cardiovascular system (23, 27, 29) and immune functions (19, 20, 22). In this regard, it has been shown that testosterone receptor blockade following trauma-hemorrhage (T-H) improves the depressed cardiac, hepatic, adrenal, and immune functions in male rats (7, 30, 34). Conversely, estradiol has been demonstrated to relax blood vessels and facilitate the relaxation in response to other vasoconstrictive agents (23, 27, 46). Our previous studies have shown that estrogen administration following T-H improves cardiovascular and hepatocellular functions and restores immune functions (2, 19, 21, 26). We have previously shown that there are gender differences in the reduction of intestinal perfusion pressure following trauma and hemorrhagic shock. We also found that androgens appear to play an inhibitory role in small intestinal endothelial function (8). However, the precise mechanism by which sex hormones influence vascular tone regulation following hemorrhage still remains unclear.

Endothelin-1 (ET-1), a 21-amino acid peptide produced by vascular endothelium, is a potent vasoconstrictor and a component of local regulation of vascular tone through its paracrine effect on underlying vascular smooth muscle (17, 45). Studies have demonstrated that there are significant sex differences at rest and stress-induced ET-1 release (40). In our primary experiments, we also found a significantly higher ET-1 level in intestinal tissue in males than females. Furthermore, our recent studies have shown that estradiol treatment prevents hepatic damage and alters portal response to ET-1 following T-H (47). Thus we hypothesized that the levels of ET-1 in regional tissues such as the intestine may be higher in males than in females or 17β-estradiol (E2)-treated males following hemorrhagic shock. To test this hypothesis, the relationship between plasma levels of ET-1 and estrogen was examined. Moreover, the intestinal gene expression and peptide levels of ET-1 were also determined in males, age-matched proestrus females, and E2-treated male rats. To determine the influence of ETs on the intestinal perfusion, an antagonist to the predominant ETα receptor (BQ-123) was administered in an isolated intestinal perfusion system.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study. All experiments were performed in adherence with the National Institutes of Health (NIH) guidelines for the use of experimental animals and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Animal model of T-H. Harlan Sprague-Dawley male rats (275–325 g) were used in this study. All experiments were performed in adherence with the National Institutes of Health (NIH) guidelines for the use of experimental animals and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

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sota, FL) and fasted 16 h before the experiment but were allowed water ad libitum (5, 8). The animals were anesthetized with 1.5% isoflurane with air inhalation and underwent a 5-cm ventral midline laparotomy to induce tissue trauma before the onset of hemorrhage. Both femoral arteries and one femoral vein were cannulated with PE-50 tubing for bleeding, monitoring of mean arterial pressure, and fluid resuscitation. The animals were then restrained in a supine position, and the areas of incision were bathed with 1% lidocaine (Eliks-Sinn, Cherry Hill, NJ) to minimize postoperative pain. After the procedure, anesthesia was removed, and when the mean arterial pressure reached ~120 mmHg, the animals were bled to a pressure of 40 mmHg (i.e., severe hypotension) within 10 min. The blood pressure of 40 mmHg was maintained by removing more blood until the animal was no longer able to maintain blood pressure (i.e., maximum bleed out). The blood pressure was then maintained at that level by infusing Ringer lactate (RL) intravenously until 40% of the shed blood volume was returned. The animals were resuscitated with four times the volume of maximum bleed out with RL over a period of 60 min at a constant rate. After resuscitation, the catheters were removed, the vessels were ligated, and skin incisions were closed with sutures. The animals were maintained conscious and without heparin injection throughout the hemorrhage and resuscitation procedure. Blood pressure was monitored with a blood pressure analyzer (Digi-Med, Louisville, KY), and the resuscitation perfusion pump was a Pump 11 (Harvard Apparatus, Holliston, MA). Sham-operated animals underwent the same surgical procedure but were neither bled nor resuscitated. The time required for maximum bleed out was ~45 min; the volume of maximum bleed out was ~60% of the calculated circulating blood volume.

Preparation of isolated small intestine and measurement of intestinal perfusion flow. At 2 h postresuscitation, the animals were anesthetized again with isoflurane (1.5% with air) and the anesthesia was maintained by injection of pentobarbital sodium (30 mg/kg body wt) via the femoral vein. Animals underwent an isolated small intestinal perfusion model as described previously with minor modification (5). Briefly, the small intestine was isolated and perfused without removal from the abdominal cavity. The branches of blood vessels to and from the cecum, ascending colon, and transverse colon were then ligated. Five minutes after intravenous injection of 0.3 ml heparin solution (500 U), the superior mesenteric artery and portal vein were cannulated with a PE-50 and PE-90 catheter, respectively. Although the rat was still alive, the isolated intestine was perfused with 95%O2-5%CO2 oxygenated Krebs-Ringer-HCO3 buffer (in mM: NaCl 118, KCl 4.7, CaCl2-2H2O 2.5, MgSO4 1.2, KH2PO4 2.0, NaHCO3 0.26 Ca-EDTA, and 11.1 D-glucose, with 0.22 g/l NaCl, 4.7 KCl, 2.5 CaCl2/H2O for 15 min at 4°C to separate plasma. Plasma was immediately frozen (-80°C) until assayed. The assay for ET-1 and estradiol was performed by using a commercially available Endothelin-1 enzyme immunoassay kit (Assay Designs, Ann Arbor, MI) and estradiol EIA kit (Cayman Chemical, Ann Arbor, MI).

Intestinal gene expression and peptide levels of ET-1. The mRNA levels of ET-1 in intestinal tissue were determined by real-time PCR. Total RNA was isolated from total intestinal tissue using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. cDNA was generated from the total RNA samples by using a transcription kit (TaqMan Reverse Transcription Reagents; Applied Biosystems, Foster City, CA). Each real-time PCR reaction was performed in a mix of 10-μl reaction mixture containing 20 ng of cDNA, 2× PCR Master Mix (Applied Biosystems), and each probe and primer set. TaqMan Gene Expression assays (Applied Biosystems) for ET-1 were purchased as probe and primer sets. Reaction mixture was denatured for 1 cycle of 2 min at 50°C, 10 min at 95°C, and incubated for 40 cycles (denaturing for 15 s at 95°C and annealing and extending for 1 min at 60°C) using ABI Prism 7900HT (Applied Biosystems). All samples were tested in triplicate, and average values were used for quantification. 18S RNA was used as an endogenous control. Analysis was performed using SDS 2.1 software (Applied Biosystems) according to the manufacturer’s instruction. The comparative CT method (ΔΔCT) was used for quantification of gene expression. The calibrator sample was designed as the most highly expressed time point for each gene and, therefore, has set an expression of one. Thus all samples are expressed as the fold expression of this value. To decide the calibrator sample, preliminary experiments were performed to ensure that amplification efficiencies for the target genes and 18S were equivalent.

Peptide levels of ET-1 in intestinal tissue were also examined. The intestinal tissue was sampled, immediately placed in liquid nitrogen, and stored at -80°C until assayed. Tissue samples (100 mg wet wt) were homogenized in 1 ml PBS (pH 7.4) containing aprotnin (Sigma, St. Louis, MO) in an ice bath and centrifuged at 12,000 g for 20 min at 4°C. The supernatant was analyzed by using a commercially available endothelin-1 enzyme immunoassay kit (Assay Designs).

Statistical analysis. All data are presented as means ± SE. One-way ANOVA and Tukey’s test were employed for the comparison between each group. Correlation between plasma ET-1 and estradiol was evaluated by Pearson’s correlation analysis. The differences were considered significant at P < 0.05.

RESULTS

Intestinal perfusion flow following T-H. As shown in Fig. 1A, the intestinal perfusion flow in sham-operated males was significantly lower than proestrus females and E2-treated males and was significantly decreased after T-H; however, there were no significant changes in proestrus females and E2-treated males under such conditions. The lower sham level of intestinal perfusion flow in males was significantly elevated by treatment with the blockade of ETA receptor, BQ-123. This increase was similar to sham levels in proestrus females but still lower than in E2-treated males. In male rats treated with E2 in the T-H group, the use of BQ-123 significantly increased the perfusion flow in males after T-H. Moreover, the decreased intestinal perfusion flow in males after T-H was also attenuated by treatment with BQ-123 (Fig. 1B).

Alteration and correlation of plasma levels of ET-1 and estradiol. As shown in Fig. 2A, the plasma levels of ET-1 significantly increased following T-H among three experimental groups. The increase of ET-1 following T-H in males was significantly higher than that in females or E2-treated males. Furthermore, Fig. 2B shows that the plasma levels of E2 in males were significantly lower than in females and E2-treated
males. T-H had no influence in plasma level of E2 among three experimental groups (Fig. 2).

When the data from all groups (male, proestrus female, and E2-treated male rats following T-H) were included, plasma levels of ET-1 were inversely correlated to plasma levels of E2 according to the Pearson’s correlation analysis ($r^2 = 0.604$, $P < 0.05$; Fig. 3).

Intestinal tissue ET-1 gene expression and peptide levels of ET-1. The intestinal ET-1 gene expression determined by real-time PCR is shown in Fig. 4A. The results show that the ET-1 gene expression in sham-operated males was significantly higher than female shams and sham-operated E2-treated males. Although ET-1 gene expressions were slightly increased compared with sham by T-H, there was no significant difference among all experimental groups following T-H.

Although intestinal peptide levels of ET-1 were significantly elevated by T-H in all experimental groups, the increased intestinal peptide level of ET-1 was significantly higher in males than that in females and E2-treated males following T-H (Fig. 4B).

DISCUSSION

Gender influences the function and responsiveness of the cardiovascular system under normal and pathophysiological conditions (14, 15, 28, 31, 36). Previous data from our laboratory have shown that females in the proestrus stage of the estrus cycle maintain cardiovascular function and immune responses compared with male rats following T-H (3, 5, 26, 27). Moreover, endothelial dysfunction did not develop in the intestine of proestrus females but was observed in the male intestine after T-H. These results collectively suggest that maintenance of vascular endothelial function in proestrus females following T-H might be a potential mechanism respon-
ANOVA and Tukey’s-test: *P < 0.05 vs. sham in the same group; #P < 0.05 vs. corresponding male sham-operated group. RQ, relative quantity.

Fig. 4. The intestinal gene expressions (A) and peptide levels (B) of ET-1 in male, proestrus female, and E2-treated male rats at 2 h following sham operation and TH. Data are presented as means ± SE and compared by 1-way ANOVA and Tukey’s-test. *P < 0.05 vs. sham in the same group; #P < 0.05 vs. corresponding male sham-operated group. RQ, relative quantity.

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significantly lower intestinal peptide levels of ET-1 following T-H in proestrus females and in E2-treated males, it strongly indicates that estradiol reduces ET-1 peptide production following T-H. We therefore propose that the sustained vasoconstriction induced by T-H is related to the increased ET-1 production, which, in turn, could be counteracted by estradiol-mediated mechanisms.

In conclusion, these results indicate that the endogenous vasoconstrictor peptide ET-1 appears to play an important role on intestinal perfusion failure following T-H shock in males. Because a high level of E2 can modulate this vasoconstrictor effect of ET-1, these findings may partially explain the previously observed salutary effect of estrogen in improving intestinal perfusion following T-H shock in males. Because estradiol has the potential ability to restore impaired tissue perfusion following T-H, this hormone may be a useful therapeutic adjunct for reducing elevated endothelin production following low-flow conditions.

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REFERENCES