17β-Estradiol modulates vasoconstriction induced by endothelin-1 following trauma-hemorrhage


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Ba ZF, Lu A, Shimizu T, Szalay I, Schwacha MG, Rue LW, III, Bland KI, Chaudry IH. 17β-Estradiol modulates vasoconstriction induced by endothelin-1 following trauma-hemorrhage. Am J Physiol Heart Circ Physiol 292: H245–H250, 2007; doi:10.1152/ajpheart.00809.2006.—Although endothelin-1 (ET-1) induces vasoconstriction, it remains unknown whether 17β-estradiol (E2) treatment following trauma-hemorrhage alters these ET-1-induced vasoconstrictive effects. In addition, the role of the specific estrogen receptor (ER) subtypes (ER-α and ER-β) and the endothelium-localized downstream mechanisms of actions of E2 remain unclear. We hypothesized that E2 attenuates increased ET-1-induced vasoconstriction following trauma-hemorrhage via an ER-β-mediated pathway. To study this, aortic rings were isolated from male Sprague-Dawley rats following trauma-hemorrhage with or without E2 treatment, and alterations in tension were determined in vitro. Dose-response curves to ET-1 were determined, and the vasoactive properties of E2, propylpyrazole triol (PPT, ER-α agonist), and diarylpropionitrile (DPN, ER-β agonist) were determined. The results showed that trauma-hemorrhage significantly increased ET-1-induced vasoconstriction; however, administration of E2 normalized ET-1-induced vasoconstriction in trauma-hemorrhage vessels to the sham-operated control level. The ER-β agonist DPN counteracted ET-1-induced vasoconstriction, whereas the ER-α agonist PPT was ineffective. Moreover, the vasorelaxing effects of E2 were not observed in endothelium-denuded aortic rings or by pretreatment of the rings with a nitric oxide (NO) synthase inhibitor. Cyclooxygenase inhibition with indomethacin had no effect on the action of E2. Thus, E2 administration attenuates ET-1-induced vasoconstriction following trauma-hemorrhage via an ER-β-mediated pathway that is dependent on endothelium-derived NO synthesis.

Estrogen receptor; nitric oxide; endothelium; aortic ring

IT IS WELL ESTABLISHED THAT SEX difference influences cardiovascular and immunological responsiveness via sex hormones, which have potent vasoactive properties (3, 20, 24, 27, 32, 34). In this regard, the beneficial effects of 17β-oestradiol (E2) administration have been demonstrated by improvement in organ perfusion and function under a range of conditions (3–5, 25). These studies and others (3, 9, 11) have led to the development of the concept that treatment of male trauma victims with estrogenic compounds can alleviate or attenuate the physiological and immunological derangements associated with such injury. Estrogen can improve circulation via vasodilatation induced by either prostacyclin or nitric oxide (NO) synthesis and/or by decreasing the production of vasoconstrictor agents, such as endothelin or angiotensin II (4, 27, 30). ET-1 is a potent vasoactive peptide produced by vascular endothelial cells and exerts its vasoconstrictor effects locally on the underlying vascular smooth muscle cells (31). The effects of E2 on ET-1-mediated vasoconstriction are mediated through downstream mechanisms that are induced by estrogen receptor (ER) activation. In this regard, two major subtypes of ERs have been identified, ER-α and ER-β (21, 35). Although studies have demonstrated that vascular endothelial cells and myocytes express both the ER-α and ER-β subtypes, their expression has been shown to be species, sex, and organ specific. Moreover, studies (15, 17, 19) have reported both endothelium-dependent and-independent vasodilator responses to E2, thereby further complicating the identification of downstream mechanisms responsible for E2 action. Our recent study (37) has shown that E2 can reduce ET-1-induced vasoconstriction. Nonetheless, the relative contributions of ER subtypes and downstream mechanisms remain to be elucidated. The present study was directed at examining the contribution of ER subtypes and the effector mechanisms involved in E2-mediated modulation of ET-1-induced vasoconstriction following trauma-hemorrhage.

MATERIALS AND METHODS

Animals and model of trauma-hemorrhage. Adult male Sprague-Dawley rats (Charles River, Wilmington, MA), weighing 275–325 g, were studied. Animals were fasted 16 h before the experiment but were allowed water ad libitum. The Institutional Animal Care and Use Committee of the University of Alabama at Birmingham approved this project. 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 polyethylene (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 bathed with 1% lidocaine (Elkins-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). Blood pressure was then maintained 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. Following resuscitation, the catheters were removed, the vessels were ligated, and skin incisions were closed with sutures. Animals were maintained conscious and without heparin injection throughout the hemorrhage and resuscitation procedure. Blood pressure was

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monitored with a Blood Pressure Analyzer (Digi-Med, Louisville, KY), and the resuscitation perfusion pump was a Harvard Apparatus Pump 11 (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. 17β-Estradiol water soluble (E2, 1.0 mg/kg; Sigma, St. Louis) or the same volume of vehicle was applied intravenously at the beginning of resuscitation.

Aortic ring preparation. At 2 h after resuscitation, rats were anesthetized by 1.5% isoflurane inhalant before surgery. The chests were opened, and thoracic aortas were isolated and rapidly removed. The isolated thoracic aorta was immediately immersed in Krebs-Ringer HCO₃ solution containing (in mM) 118.3 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.0 NaHCO₃, 0.026 Ca-EDTA, and 11.1 glucose, which was aerated with 95% O₂-5% CO₂ (pH 7.4; PO₂ = 580 mmHg). The thoracic aorta was trimmed carefully to prevent damage to the endothelial cells and cut into rings of ~2.5 mm in length. The aortic ring was 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 was connected to an isometric force displacement transducer (model FT03, Grass Instruments, Quincy, MA) coupled to a calibrated polygraph (model 7D, Grass Instruments).

Determination of ET-1-induced vessel constriction. The aortic rings were incubated for 60 min at a tension of 1.0 g, during which time the aortic vessel was rapidly removed from the chest and fixed in 4% paraformaldehyde and embedded in paraffin. Paraflin-embedded tissues were cross-sectioned at 4 µm thickness, and the sections were deparaffinized through xylene and rehydrated through a series of alcohols. Treatments with 0.3% hydrogen peroxide for 30 min inhibited endogenous peroxidase activity. Primary antibodies ER-α (MC-20), rabbit polyclonal antibody, or ER-β (Y-19), goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), were both applied at 1 µg/ml and incubated at 4°C overnight. Incubation of ER-α was followed by an addition of a secondary antibody, biotinylated anti-rabbit IgG, whereas ER-β was followed by incubation with biotinylated anti-goat IgG secondary antibody for 1 h at room temperature. Horseradish peroxidase-conjugated streptavidin (Vectastain kits, Vector) was applied for 30 min at room temperature. All dilutions were based on the manufacturer’s instructions. Peroxidase was visualized using 3,3′-diaminobenzidine hydrochloride (Vector). The nuclei was visualized by staining with hematoxylin. For control sections, primary antibodies were omitted and replaced by buffer.

Statistical analysis. All data are presented as means ± SE. Statistical differences among groups were determined by one-way ANOVA, followed by Tukey’s test. Differences were considered significant if P < 0.05.

RESULTS

The influence of E2 on ET-1-induced vasocostriction. As shown in Fig. 1A, the aortic vasoconstrictions induced by ET-1 (1.0–100 ng/ml) were significantly increased (P < 0.05) following trauma-hemorrhage compared with the sham-operated group; however, this increase was not observed in the E2-treated trauma-hemorrhage group. Moreover, as shown in Fig. 1B, the aortic vasorelaxation induced by E2 (10⁻⁷–10⁻⁵ M) was significantly attenuated (P < 0.05) following trauma-hemorrhage compared with the sham-operated group. E2 treatment prevented this attenuation in vasorelaxation in the traumahemorrhage group.

The role of ER subtypes in E2-induced vasorelaxation. To determine the contribution of the α- and β-subtypes of ER in the E2-mediated vasorelaxation, the vessel relaxing capacities of DPN (ER-β agonist) or PPT (ER-α agonist) were investigated following ET-1-induced vasocostriction. The results in Fig. 2A show that between the concentration ranges of 10⁻⁸ and 10⁻⁶ M, DPN (the ER-β agonist) attenuated vascular tension 82.5 ± 9.3% in ET-1-preconstricted aortic rings from sham-operated rats. DPN vasorelaxing properties were significantly less in the trauma-hemorrhage group compared with those in the sham-operated group. However, DPN attenuated vascular tensions to a comparable degree in the sham-operated and trauma-hemorrhage E2 groups at a concentration of 10⁻⁶ M. Within the concentration range of 10⁻⁸–10⁻⁵ M, PPT (the ER-α agonist) failed to result in signifi-
cant vasorelaxation of ET-1-preconstricted aortic ring from all of three groups (Fig. 2).

The role of NO and eicosinoids in E2-mediated vasorelaxation. The data in Fig. 3 show that E2 attenuated ET-1-induced constriction of aortic rings with intact endothelium; however, ET-1-induced vasoconstriction was not altered by E2 pretreatment in denuded aortic rings. Following administration of ET-1 (0–50 nM), the elevation in tension was significantly greater in the vascular rings denuded of endothelium compared with intact vascular rings (Fig. 3). To evaluate the relative contribution of NO and cyclooxygenase derivatives in the E2-induced vasorelaxation, the NO synthase inhibitor L-NNA or the cyclooxygenase inhibitor indomethacin were added to the organ baths before E2 administration. Administration of L-NNA prevented the E2-induced relaxation of ET-1-constricted aortic rings, whereas preincubation with the cyclooxygenase inhibitor indomethacin had no influence on E2-induced vasorelaxation (Fig. 4).

ER-α and ER-β localization to vascular endothelial cells. The results in Fig. 5 show the immunohistochemical staining of ER-α and ER-β in representative aortic vessels. The brown color is indicative of specific staining of ER-α or ER-β. It can be seen that the dark brown stain is localized to the endothe-

**Fig. 1.** The effect of trauma-hemorrhage (T-H) and T-H-17β-estradiol-treated animals (T-H-E2) on endothelin-1 (ET-1)-induced vasoconstriction (A) and E2 vasorelaxation in ET-1 (50 nM)-induced preconstricted aortic rings (B). Data are presented as means ± SE (n = 6 to 7) and compared by one-way ANOVA and Tukey’s test. *P < 0.05 vs. the corresponding equimolar concentration of sham-operated group.

**Fig. 2.** Vasorelaxation induced by diarylpropionitrile (DPN, ER-β agonist; A) and propylpyrazole triol (PPT, ER-α agonist; B) in sham-operated, T-H, and T-H-E2 rats on aortic rings following ET-1 (50 nM) preconstriction. Data are presented as means ± SE (n = 6 to 7) and compared by one-way ANOVA and Tukey’s test. *P < 0.05 vs. the corresponding equimolar concentration of sham-operated group. +P < 0.05 vs. the corresponding equimolar concentration of T-H group.

**Fig. 3.** Effects of E2 on ET-1-induced vasoconstriction in aortic rings with intact or denuded endothelium. Data are presented as means ± SE (n = 6 to 7) and compared by one-way ANOVA and Tukey’s test. *P < 0.05 vs. the corresponding equimolar concentration of vehicle administration.
lrium as opposed to the smooth muscle (Fig. 5A) or ER-β (Fig. 5B). Brown color is absent in sections used for control staining for ER-α (Fig. 5C) or ER-β (Fig. 5D).

**DISCUSSION**

There is a growing body of evidence that indicates that E2 administration improves organ perfusion and function following low-flow conditions or an ischemic insult (13, 37). Among the mechanisms, the attenuation of endothelin release has been implicated as a significant contributor to the E2-mediated salutary effects on organ function (4, 37). The finding of the present study provides further evidence that estradiol not only reduces ET-1 production but also inhibits its vasoconstrictive action on the vasculature. Although a recent study from our laboratory has reported a reduced response to exogenous ET-1 following E2 treatment, it should be noted that those studies were carried out on portal circulation (37), a vascular bed that differs markedly from the aortic vasculature.

Previous studies (18, 25, 37) from our laboratory have also shown salutary effects of E2 and its derivatives in restoring organ functions following trauma-hemorrhage. Furthermore, it has been demonstrated that trauma-hemorrhage-induced ET-1 release in proestrus female rats, which have elevated estrogen levels, remained at significantly lower levels than in their male counterparts (4). This sex dimorphism was also reflected in the inverse correlation of endogenous plasma ET-1 and E2 levels. Estrogens mediate their biological effects via different receptor subtypes. To date, three ER subunits have been identified: ER-α, ER-β, and ER-γ. The biological relevance for only the ER-α and ER-β subtypes has been described (16, 22, 28), whereas very little is known about ER-γ. Vascular endothelium and myocytes express both ER-α and ER-β, and their local distribution is dependent on both sex difference and anatomical location. Previous studies have shown both ER-α and ER-β expression in the rat aorta (2, 23, 29). In the present

Fig. 4. Effects of E2 on ET-1-induced vasoconstriction in aortic rings with N\(^\bullet\)-nitro-L-arginine (L-NNA, nitric oxide synthase inhibitor) or indomethacin (cyclooxygenase-2 inhibitor) pretreatment. Data are presented as means ± SE (n = 6 to 7) and compared by one-way ANOVA and Tukey’s test. *p < 0.05 vs. the corresponding equimolar concentration of vehicle administration.

Fig. 5. A and B: immunohistochemistry staining for estrogen receptor (ER)-α (A) and ER-β (B) in normal rat aortic vessel. Specific staining ER-α or ER-β is evident by brown peroxidase positive staining. C and D: control staining for ER-α (C) and ER-β (D). EC, endothelial cell; SM, smooth muscle.
study, using immunohistochemistry, we have demonstrated that both ER-α and ER-β are present in the rat aorta and are localized to the endothelial cells. However, in mesenteric arteries, PPT, the ER-α agonist, was found to be more effective in inducing acute relaxation than DPN, the ER-β agonist (26), suggesting a preponderance of ER-α in that vascular bed.

Estrogen has been reported to induce NO production through phosphorylation-dependent processes; however, the precise pathways responsible for endothelial NO synthase activation have not been fully elucidated (14, 33). Some studies (10, 12) suggest that the acute effects of E2 on NO synthase activity and NO production are mediated via ER-α activation. In contrast, Chambliss et al. (8) reported that overexpression of ER-β enhanced endothelial NO synthase activation by E2. In contrast, mice deficient in ER-β display multiple functional abnormalities, and Zhu et al. (38) have suggested an essential role for ER-β in the regulation of vascular function and blood pressure. NO can also counteract ET-1-mediated vasoconstriction via guanylate-cyclase activation and reduced endothelin receptor affinity (36). Our present study also suggests that ER-β plays an important role in E2-mediated vasorelaxation. The present study indicates that while both ER-α and ER-β are present on the aortic endothelium, the ability of the ER-β, but not the ER-α, agonist to induce vasorelaxation indicates that the acute effects of E2 on NO synthase activity and NO production are mediated via ER-β, and not ER-α, activation. Findings by Al Zubair et al. (1) are consistent with our findings here. They observed that in rat aortic rings contracted with KCl, the ER-β agonist DPN produced significantly greater relaxations than the ER-α agonist PPT. In contrast, Bolego et al. (6) reported that aortic rings isolated from E2-treated ovariec-tomized rats responded with higher potency to an ER-α agonist than an ER-β agonist. The apparent discrepancy between the results of Bolego et al. (6) and our findings may be related to the use of different sexes, treatment regimen, and differences in ER-subtype predominance and/or connecting downstream mechanisms that can modify responses to ER agonists.

In our experiments, E2, the ER-β agonist DPN, but not the ER-α agonist PPT, attenuated the ET-1-induced vasoconstriction. The lack of effectiveness of PPT suggests that the role of ER-β subtype is predominant in our experimental settings. The abolishment of E2-induced vasodilatation by endothelial cell denudation underlines the endothelial source as key elements participating in this process. Furthermore, the elimination of E2-induced effects with NO synthase, but not cyclooxygenase, inhibition indicates that NO, but not prostacyclin, plays a crucial role in E2-mediated vasorelaxation. Since it has been reported that indomethacin inhibition results in increased NO production, it is possible that indomethacin treatment eliminates the negative feedback of prostacyclin on NO production, and thus the increased NO release can compensate for the lack of prostacyclin-induced vasodilator effect (7). It should, however, be emphasized that the current study examined only the acute effects of E2 on vascular reactivity. It is plausible that receptor expression and other components of signal transduction and cellular activation are altered by chronic administration of E2.

The interest in the effects of estrogen of vascular reactivity in males is related to the development of the concept that treatment of males in hemorrhagic shock estrogens or related compounds can prevent the deleterious consequences of this form of injury (3, 9, 11). Experimental studies have shown that the use of estrogens and/or androgen antagonists as salutary adjuncts, without any adverse effects on gastrointestinal, hepatic, and renal functions, can normalize immunological and physiological responses after trauma-hemorrhage (3, 9, 11) and, therefore, would appear to be advantageous for the treatment of such derangements after severe blood loss in male trauma victims. In conclusion, our findings provide additional insights into the underlying mechanisms responsible for E2-induced maintenance of organ function under pathophysiological conditions.

GRANTS

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