Mechanism of salutary effects of androstenediol on hepatic function after trauma-hemorrhage: role of endothelial and inducible nitric oxide synthase


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Shimizu, Tomoharu, Laszlo Szalay, Mashkoor A. Choudhry, Martin G. Schwacha, Loring W. Rue, III, Kirby I. Bland, and Irshad H. Chaudry. Mechanism of salutary effects of androstenediol on hepatic function after trauma-hemorrhage: role of endothelial and inducible nitric oxide synthase. Am J Physiol Gastrointest Liver Physiol 288: G244–G250, 2005. First published September 23, 2004; doi:10.1152/ajpgi.00387.2004.—Recent studies have shown that administration of dehydroepiandrosterone (DHEA) after trauma-hemorrhage (T-H) improves cardiovascular and hepatic function in male animals. Although androstenediol, one of the DHEA metabolites, has been recently reported to produce salutary effects on cardiac function and splanchnic perfusion after T-H, it remains unknown whether androstenediol per se has any salutary effects on hepatic function under those conditions. To study this, male Sprague-Dawley rats underwent laparotomy and 90 min of hemorrhagic shock (35–40 mmHg), followed by resuscitation with four times the shed blood volume in the form of Ringer lactate. Androstenediol (1 mg/kg body wt iv) was administered at the end of resuscitation, and the animals were killed 24 h later. T-H significantly reduced portal blood flow, bile production, and serum albumin levels. Portal pressure, serum alanine aminotransferase, hepatic nitrate/nitrite, inducible nitric oxide synthase (iNOS), and endothelin-1 markedly increased after T-H. The alterations in these parameters induced by T-H were significantly attenuated in rats treated with androstenediol. Endothelial NOS (eNOS) expression, which was not different between T-H and sham, was found to be significantly elevated in T-H and androstenediol-treated rats. These data suggest that improvement in hepatic perfusion by androstenediol after T-H is likely due to a decrease in endothelin-1 and induction of eNOS. Moreover, the decrease in hepatic damage after androstenediol administration is likely related to liver iNOS downregulation. Thus androstenediol appears to be a novel and useful adjunct for restoring hepatic function in male animals after adverse circulatory conditions.

Hepatic blood flow remains depressed after severe hemorrhagic shock despite adequate fluid resuscitation (43). This splanchnic hypoperfusion appears to contribute to hepatic injury and inflammatory response under such conditions. Several studies (39, 48) have supported the concept that maintenance of hepatic circulation after hemorrhagic shock is essential for preventing hepatocellular dysfunction. Furthermore, previous studies (2, 20, 28) have also shown that endothelin and nitric oxide (NO) play a role in maintaining hepatic circulation after hemorrhagic shock. In addition, recent studies from our laboratory (49) have shown that 17β-estradiol treatment prevents hepatic damage and alters portal response to endothelin-1 after trauma-hemorrhage. Additional findings suggested that endogenous endothelial-derived NO, which is produced by endothelial NO synthase (eNOS), modulates hepatic vascular tone in the normal rat liver (34). L-arginine, which is the precursor of NO and the substrate for NOS, restored the depressed hepatic blood flow and ameliorated organ dysfunction after trauma-hemorrhage (2). Furthermore, a recent study performed by Kawachi et al. (18) in eNOS knockout mice concluded that eNOS and not inducible isoform (iNOS)-derived NO plays an important role in limiting liver injury after ischemia-reperfusion.

On the other hand, iNOS is significantly upregulated after hemorrhagic shock in liver and is thought to be one of the major contributors of hepatic injury caused by severe hemorrhagic shock or sepsis (8, 9, 37, 38). Furthermore, experimental studies using iNOS inhibitor or iNOS knockout mice have shown that iNOS-derived NO participates in liver injury and in the inflammatory cascade produced by hemorrhagic shock (15, 22, 23).

Dehydroepiandrosterone (DHEA) is the most abundant steroid hormone in plasma and is an intermediary in the pathway for the synthesis of testosterone and estrogen. Our previous studies (7, 16) have shown that DHEA treatment after trauma-hemorrhage improves organ functions and normalizes cytokine production after circulatory stress. Androstenediol (adiol or 5-androstene-3β, 17β-diol), one of the metabolites of DHEA, has also been reported to produce protective effects after ionizing radiation in mice (21). Furthermore, androstenediol has been reported to have greater protective effects than DHEA against lethal bacterial infections and endotoxin shock (5). Additionally, it is reported that the conversion of DHEA leads to an increase of downstream effector hormones in macrophages, which may play an important role in local immunomodulation (33). Recently, we have shown that androstenediol administration after trauma-hemorrhage improves cardiovascular function and splanchnic perfusion in male animals. Our studies (35) suggested that these effects of androstenediol are mediated via modulation of nitrate/nitrite and IL-6 levels after trauma-hemorrhage. It is likely that an increase in NOS activity in androstenediol-treated animals may contribute to the improvement of cardiac output and splanchnic perfusion after trauma-hemorrhage (35).

Although androstenediol has been reported to produce the above-mentioned salutary effects, it remains unknown whether...
DHEA metabolite has any salutary effects on hepatic function after trauma-hemorrhage. We hypothesized that androstenediol is a useful adjunct for improving the depressed hepatic function after trauma-hemorrhage. In this study, we examined the effects of androstenediol administration on hepatic function after trauma-hemorrhage. Furthermore, we examined whether androstenediol-induced changes in hepatic function are mediated via alterations in nitrate/nitrite and endothelin-1 levels. In addition, we also determined whether androstenediol treatment influences eNOS or iNOS in the liver after trauma-hemorrhage.

MATERIALS AND METHODS

Animals. Adult male (275–325 g) 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 guidelines for the use of experimental animals and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Experimental procedures. A nonheparinized rat model of trauma-hemorrhage, as previously described, was used in this study (39). Briefly, male Sprague-Dawley rats were fasted overnight before the experiment but allowed water ad libitum. Rats were anesthetized using isoflurane (Attane; Minrad, Bethlehem, PA) inhalation. A 5-cm midline laparotomy was then performed to induce soft tissue trauma. After this, the abdomen was closed in layers, and both femoral arteries and the right femoral vein were cannulated with [polyethylene (PE)-50 tubing; Becton-Dickinson, Franklin Lakes, NJ]. The animals were then restrained in a supine position, and the areas of incision were bathed with 1% lidocaine (Elkins-Sinn, Cherry Hill, NJ) to minimize postoperative pain. The rats were then allowed to awaken, and were rapidly bled to a MAP of 35–40 mmHg within 10 min. The time at which the animals could no longer maintain a MAP of 35–40 mmHg without infusing some fluid was defined as maximum bleed-out volume. The rats were maintained at this MAP until 40% of the shed blood was returned in the form of Ringer lactate. The animals were then resuscitated with 4 times the volume of shed blood. After resuscitation, catheters were removed, vessels were ligated, and skin incisions were closed with sutures. Sham-operated animals underwent the same groin dissection, which included the ligation of the femoral arteries and cannulation of the femoral vein, but the animals were neither subjected to trauma-hemorrhage nor resuscitated. The animals were returned to their cages and were allowed food and water ad libitum and were killed at 24 h after the end of resuscitation.

In the treatment group, androstenediol (1 mg/kg body wt) (Steraloids, Newport, RI) was administered intravenously at the end of the resuscitation. In the vehicle-treated group (control group), rats received the same volume of vehicle (Intralipid, 1 ml/kg body wt; Sigma, St. Louis, MO).

Measurement of portal pressure and bile flow. Portal pressure and bile flow measurements were performed as described previously (39). At 24 h after the end of resuscitation or sham operation, a laparotomy was performed, and the intestines were covered with wet gauze to minimize evaporative loss during measurements. The portal vein was performed, and the intestines were covered with wet gauze to minimize evaporative loss during measurements. The portal vein was identified and exposed. The common bile duct was cannulated with a PE-10 catheter, and bile flow was measured in preweighed tubes for 10 min. A PE-10 catheter filled with saline was inserted into the portal vein without compromising the flow. The catheter was then connected to a low pressure analyzer (Digi-Med, Louisville, KY).

Measurement of hepatic function and injury. Blood samples were obtained at 24 h after the end of resuscitation or sham operation. Hemoglobin and hematocrit levels were analyzed using a blood analysis machine (model ABL715; Radiometer America, Westlake, OH). After centrifugation, serum samples were separated, immediately frozen, and stored at −80°C until assayed. Hepatic injury was determined by measuring alanine aminotransferase (ALT) using a commercially available colorimetric reaction kit according to the manufacturer’s instructions (Sigma). To evaluate hepatic function, serum albumin levels were measured using a rat albumin ELISA quantitation kit (Bethyl, Montgomery, TX) according to the manufacturer’s instructions.

Measurement of nitrate/nitrite and endothelin-1 in the liver. Production of NO in the liver was evaluated by measuring tissue levels of nitrate/nitrite using a commercially available colorimetric assay kit (Cayman Chemical, Ann Arbor, MI). Tissue levels of endothelin-1 were detected using enzyme immunoassay kits (Assay Design, Ann Arbor, MI). Liver samples were obtained at 24 h after the end of resuscitation or sham operation and were immediately placed in liquid nitrogen and stored at −80°C until assayed. Tissue samples (100 mg wet wt) were homogenized in 1 ml of PBS (pH 7.4) containing aprotinin (Sigma) in an ice bath and centrifuged at 12,000 g for 20 min at 4°C. The supernatant was analyzed according to the manufacturer’s description for measuring endothelin-1. For measuring nitrate/nitrite, supernatant (250 μl) was further centrifuged by using a 30-kDa molecular mass cut-off filter (Fisher Scientific, Pittsburgh, PA). The outflow of cut-off filters was analyzed according to the manufacturer’s description.

Quantitative real-time PCR. mRNA levels of endothelin-1 and iNOS in liver were determined by real-time PCR. Total RNA was isolated from total liver 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 endothelin-1 and iNOS were purchased as probe and primer sets. The 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 rRNA was used as an endogenous control. Analysis was performed using SDS version 2.1 software (Applied Biosystems) according to the manufacturer’s instructions. The comparative cycle threshold method (ΔΔCT) was used for quantification of gene expression.

Western blot analysis. Liver samples were homogenized in 10 times volume of cell lysis buffer (PBS, pH 7.2) containing (in mM) 50 sodium fluoride, 10 tetrasodium pyrophosphate, 2.5 sodium orthovanadate, 50 PMSF, and 1 M of dithiothreitol plus protease inhibitors (Sigma). The lysate was clarified by centrifugation at 12,000 g for 30 min at 4°C, and the clear supernatant was collected. The total soluble protein concentration was determined (Bio-Rad Laboratories, Hercules, CA). Samples were stored at −80°C until analysis. A total of 50 μg of protein was used for each lane. The protein aliquots were mixed with 4× LDS sample buffer.

Samples were analyzed using electrophoresis, which was performed on precasted gels (Nupage, Bis-Tris; Invitrogen) with running MOPS SDS running buffer (Invitrogen) for 50 min at 200 V. Proteins from the gel were transferred to nitrocellulose membranes at 30 V for 60 min. The membrane was incubated first with 5% nonfat dry milk to block remaining binding sites and then with monoclonal mouse anti-rat primary antibody (iNOS, 1:250 or eNOS, 1:250 in 5% nonfat dry milk, Stress Gen) overnight at 4°C. Membranes were washed and later incubated with a secondary antibody (horseradish peroxidase-conjugated goat anti-mouse antibody, 1:1,500 for iNOS in 5% and 1:7,500 for eNOS in 3% nonfat dry milk) for 1 h at room temperature. The detection of the conjugate was performed with an enhanced chemiluminescent reaction. Membranes were reblotted with antibodies to β-actin (Abcam, Cambridge, MA) to confirm equal protein loading in each lane. The degree of induction of proteins was measured using an enhanced chemiluminescent reaction. Membranes were reblotted with antibodies to β-actin.
Table 1. Hepatic responses following trauma-hemorrhage

<table>
<thead>
<tr>
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<th>Sham Vehicle</th>
<th>Sham Androstenediol</th>
<th>T-H Vehicle</th>
<th>T-H Androstenediol</th>
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<tr>
<td>Portal pressure, mmHg</td>
<td>5.03±0.44</td>
<td>5.03±0.42</td>
<td>7.45±0.18*</td>
<td>6.35±0.42†</td>
</tr>
<tr>
<td>Bile flow, mg·min⁻¹·100 g⁻¹ body wt</td>
<td>3.88±0.53</td>
<td>3.95±0.31</td>
<td>2.71±0.39</td>
<td>4.14±0.28</td>
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<tr>
<td>Portal blood flow, ml·min⁻¹·100 g·body wt⁻¹</td>
<td>120±6</td>
<td>114±7</td>
<td>74±6*</td>
<td>112±12</td>
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Values are means ± SE for 6–7 animals per group. Measurements were performed at 24 h after sham operation or trauma-hemorrhage (T-H). *P < 0.05 vs. other groups, †P < 0.05 vs. sham.

Alteration in hepatic nitrate/nitrite levels and protein levels of eNOS and iNOS. The levels of nitrate/nitrite in the liver increased significantly after trauma-hemorrhage. Although androstenediol treatment significantly decreased the levels of nitrate/nitrite, they remained significantly higher than sham levels (Fig. 3). No significant difference was observed in levels of nitrate/nitrite (Fig. 3), protein levels of iNOS (Fig. 4) and eNOS (Fig. 5) in the liver between androstenediol- and vehicle-treated sham animals. The iNOS gene expression and protein levels were markedly upregulated after trauma-hemorrhage. The iNOS induction was significantly reduced after androstenediol treatment (Fig. 4). There was no difference in eNOS protein levels in livers between sham and vehicle-treated trauma-hemorrhaged animals. However, liver eNOS protein levels were markedly upregulated in the androstenediol-treated trauma-hemorrhage rats (Fig. 5). In this study, we were unable to determine eNOS gene expression, because eNOS primers for rats are not available.

Alteration in hepatic endothelin-1 levels. As shown in Fig. 6, endothelin-1 mRNA and protein levels were significantly increased in the liver after trauma-hemorrhage. Androstenediol treatment significantly reduced trauma-hemorrhage-mediated increase in endothelin-1 mRNA and protein levels in the liver; however, protein levels of endothelin-1 were still significantly higher than for sham animals. No difference in endothelin-1 gene expression and protein levels was observed in the liver from androstenediol-treated and the corresponding sham groups.

Alterations in portal pressure, bile flow, and portal blood flow. There was no significant difference in the portal pressure, bile flow, and portal blood flow between androstenediol-treated and -nontreated sham animals. Trauma-hemorrhage significantly increased portal pressure in vehicle-treated rats. Furthermore, trauma-hemorrhage induced a significant decrease in the portal blood flow and bile production compared with sham animals. In androstenediol-treated animals, the increase in portal pressure was significantly attenuated but remained higher than vehicle-treated shams. Androstenediol treatment restored bile flow and portal blood flow to sham levels (Table 1).

Alteration in levels of ALT, albumin, and hematocrit. Trauma-hemorrhage significantly increased serum ALT levels (Fig. 1). Androstenediol treatment attenuated the trauma-hemorrhage-induced increase in serum ALT, but the levels remained higher than for sham animals. In contrast, trauma-hemorrhage significantly decreased serum albumin levels that were attenuated by androstenediol treatment (Fig. 2A). Hemoglobin and hematocrit levels decreased significantly after trauma-hemorrhage regardless of androstenediol treatment (Fig. 2, B and C). Because androstenediol treatment did not alter the trauma-hemorrhage-induced decrease in hemoglobin and hematocrit, the degree of hemodilution was similar, indicating that the increase in albumin levels are due to improved hepatic function. There was no difference in hemoglobin and hematocrit levels between androstenediol-treated and the corresponding sham groups.

Figure 1. Effect of androstenediol on serum alanine aminotransferase (ALT) at 24 h after sham operation or trauma-hemorrhage. Data are presented as means ± SE (n = 6 animals/group). *P < 0.05 vs. sham, #P < 0.05 vs. other groups. S, sham; T-H, trauma-hemorrhage; A, androstenediol.

Quantified by scanning densitometry of the autoradiographs and was corrected according to the corresponding β-actin density (Chemilmager 5500; Alpha Inotech, San Leandro, CA). Densitometric values obtained from 3–4 rats/group were pooled and presented as means ± SE.

Measurement of portal blood flow. In an additional set of animals, portal blood flow was determined by using a radioactive microsphere technique as previously described (44). Briefly, strontium85-labeled microspheres (~500,000 counts/min; DuPont New England Nuclear, Boston, MA) were injected manually into the left ventricle. The reference blood sample was withdrawn from the femoral arterial catheter for 60 s at a rate of 0.7 ml/min. Isotonic saline solution was infused at the same rate to replace the volume of blood lost. The animals were then killed, and abdominal organs were harvested and weighed. The radioactivity in the organs and a reference blood sample were counted with an automatic gamma counter (1470 Wizard; Wallac, Gaithersburg, MD). Organ blood flow = ([RBF × Ct]/Cr) × 100, where RBF is the reference blood sample withdrawal rate (0.7 ml/min); Cr is counts/min in the reference blood sample; and Ct is counts·min⁻¹·g⁻¹ of tissue.

Statistical analysis. Data are presented as means ± SE. Statistical differences among groups were determined by one-way ANOVA followed by Fisher’s least significance difference as a post hoc test. Differences were considered significant if P was < 0.05.
DISCUSSION

The present study indicates that administration of androstenediol after trauma-hemorrhage improved portal vascular tone, blood flow, and hepatic function. This improvement in hepatic perfusion was associated with attenuation of trauma-hemorrhage-mediated alterations in nitrate/nitrite and endothelin-1 levels in the liver. The failure in hepatic microcirculation appears to be one of the major determinants in the development of liver injury after hemorrhagic shock (39, 48). Our previous studies (43) have shown that sustained impairment of hepatic perfusion occurs after trauma-hemorrhage despite adequate fluid resuscitation. The decrease in hepatic blood flow appears to be related to endothelial cell dysfunction occurring early after the onset of hemorrhagic shock (45). L-arginine (the precursor of NO and the substrate for NOS) restored the depressed organ blood flow in liver and hepatic function after trauma-hemorrhage (2). Recent studies (40) have shown that androstenediol increases NOS activity in the vagina of ovariectomized rabbits. Our study has shown that androstenediol administration after trauma-hemorrhage improves cardiovascular function. Moreover, our findings suggest that the increase in NOS activity in androstenediol-treated animals may have played a role in improving cardiac output and splanchnic perfusion after trauma-hemorrhage (35). In the present study, hepatic nitrate/nitrite levels were found to be significantly reduced by androstenediol administration compared with trauma-hemorrhage vehicle-treated animals; however, these levels remained significantly higher than for sham animals. Androstenediol treatment also reduced trauma-hemorrhage-mediated increase in iNOS levels in the liver. In addition, hepatic eNOS expression increased significantly after andro-
stenediol treatment compared with sham and trauma-hemorrhage vehicle treated rats. The difference in hepatic nitrate/nitrite levels between shams and androstenediol-treated animals is likely due to an upregulation of eNOS protein. Thus hepatic eNOS upregulation and endothelial-derived NO after androstenediol treatment are likely beneficial in maintaining hepatic blood flow and organ function after trauma-hemorrhage. However, additional studies using specific inhibitor for iNOS and eNOS are needed to determine the relative contribution of the two systems in nitrate/nitrite production after trauma-hemorrhage.

The induction of iNOS after severe hemorrhage and resuscitation is thought to contribute to the hepatic injury (8). This notion is supported by studies that indicate salutary effects of iNOS inhibition in the reduction of liver injury after hemorrhagic shock (15, 22, 23). In agreement with those studies, our present study also shows that there is a positive correlation between hepatic injury and iNOS expression. In addition, under substrate starvation or stressful conditions, NOS can directly produce superoxide radicals (41, 42). It is therefore possible that iNOS in the liver produces both NO and superoxide concurrently. Thus the downregulation of hepatic iNOS after androstenediol treatment may be responsible for the decreased hepatic damage under those conditions.

In addition to modulation of iNOS/eNOS levels, we found that androstenediol treatment also prevented the increase in endothelin-1 after trauma-hemorrhage. We also noted that androstenediol administration after trauma-hemorrhage reduced portal vascular tone and improved blood flow in trauma-hemorrhaged animals. These findings corroborate previous studies (28, 29) supporting the role of endothelin in controlling hepatic portal circulation after adverse circulatory conditions. Previous studies (30) have also shown that blockade of endothelin receptors attenuates the increase in portal resistance and prevented sinusoidal perfusion failure in shocked animals.

Thus the improvement in portal blood flow after androstenediol treatment may be associated with attenuation of the trauma-hemorrhage-induced endothelin-1 production. However, the precise mechanism by which androstenediol produces its salutary effects on eNOS, iNOS, and endothelin-1 after trauma-hemorrhage remains unknown.

Previous studies have shown an estrogen-like effect of androstenediol at physiological concentrations in breast cancer cells. Additionally, androstenediol causes an increase in estrogen receptor-dependent β-galactosidase activity in yeast (25). It is therefore possible that the effects of androstenediol are mediated via estrogen receptors. Although the present study did not evaluate the relationship between androstenediol and estrogen receptors, previous studies (27) have shown that estrogen administration promotes vasodilation in both humans and experimental models, in part, by stimulating prostacyclin and NO synthesis, as well as by decreasing the production of vasoconstrictor agents such as cyclooxygenase-derived products, reactive oxygen species, angiotensin II, and endothelin-1. Harada et al. (13) have reported that the protective effects of estrogen administration in male animals after hepatic ischemia-reperfusion are due to the estradiol/ER-α-mediated activation of hepatic eNOS. Sakamoto et al. (31) have reported that estrogen upregulates eNOS expression in cultured rat hepatic sinusoidal endothelial cells. These findings support the concept that 

![Fig. 5. Effect of androstenediol on protein levels of endothelial NOS (eNOS) in the liver at 24 h after sham operation or trauma-hemorrhage. Data are presented as means ± SE (n = 3–4 animals/group). #P < 0.05 vs. other groups.](http://ajpgi.physiology.org/)

![Fig. 6. Effect of androstenediol on gene expression (A) and protein levels (B) of endothelin-1 in the liver at 24 h after sham operation or trauma-hemorrhage. Data are presented as means ± SE (n = 3–4 animals/group for gene expression and n = 6 animals/group for protein expression). *P < 0.05 vs. sham, #P < 0.05 vs. other groups.](http://ajpgi.physiology.org/)
that eNOS upregulation by estrogen may be involved in the regulation of hepatic sinusoidal microcirculation. Estrogen has also been reported to regulate endothelin-1 levels both under clinical and experimental conditions. For instance, short-term intracoronary 17β-estradiol administration in humans decreases coronary endothelin-1 levels (47). Furthermore, studies (12) have shown that 17β-estradiol administration inhibits hypoxic induction of endothelin-1 gene expression in the lung by interfering with hypoxia-inducible transcription factor activity. It has also been reported that estradiol reduces mRNA expression and protein release of endothelin-1 in endothelial cells in vitro (1, 6). Although several studies have shown that expression and protein release of endothelin-1 in endothelial cells in vitro (1, 6). 17β-estradiol decreases mRNA and enzyme activity in rat vascular smooth muscle cells (17); however, 17β-estradiol was also found to increase iNOS expression in the ovine coronary artery (24). In the myocardium, both endothelial and inducible forms of NOS activity are upregulated after estradiol administration (26). These results therefore indicate that effects of 17β-estradiol on iNOS induction are highly tissue specific.

Another possibility is that the effects of androstenediol are mediated via DHEA specific receptors, because androstenediol is one of the metabolites of DHEA. Liu et al. (19) have demonstrated the possibility that DHEA receptors are expressed on endothelial cell plasma membranes. Simoncini et al. (36) have reported that DHEA modulates eNOS activation and induction on the vascular wall and that these genomic and nongenomic effects were not blocked by antagonists of estrogen, progesterone, glucocorticoid, or androgen receptors. These findings support that DHEA acts through a specific receptor (19, 36). Reduction of iNOS expression after DHEA treatment has also been reported in some studies. Aragno et al. (3) have shown that DHEA pretreatment improves ischemia-reperfusion injury in the kidney with decrease of iNOS induction. It has also been reported (4) that DHEA inhibited LPS-induced production of nitrite and induction of iNOS protein in the microglia. However, whether or not androstenediol utilizes DHEA-specific receptors still remains unclear.

Androstenediol is also shown to mediate its effect through peroxisome proliferator-activated receptor activators (PPARs). Although we have not examined whether androstenediol upregulates PPARs, Waxman (46) has reviewed studies in a recent article suggesting that androstenediol effects the activity of peroxisome proliferator when administered in vivo. Furthermore, studies (11, 32) have shown that ligands of PPARs suppress endothelin-1 secretion from vascular endothelial cells. Another study (10) has shown that a ligand of the PPAR-γ reduces carrageen-induced iNOS expression in the rat lung. Thus it is likely that androstenediol may work at multiple sites; however, more studies are needed to delineate the precise underlying mechanism of action of this metabolite after trauma-hemorrhage.

In summary, our study indicates that androstenediol administration ameliorates alterations in hepatic function and portal blood flow after trauma-hemorrhage. The improvement in hepatic perfusion after androstenediol treatment is likely due to a reduction of endothelin-1 levels and the induction of eNOS in the liver by this agent. Furthermore, the decrease in hepatic damage may be due to downregulation of iNOS in the liver after trauma-hemorrhage and androstenediol treatment. Because androstenediol administration after trauma-hemorrhage improves hepatic perfusion and organ function, this agent appears to be a novel and useful adjunct for restoring the depressed hepatic function in male animals after adverse circulatory conditions.

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