Hypothesis: The female sex steroid 17β-estradiol improves immune functions following trauma-hemorrhage in rodent models. Therefore, we hypothesized that 17β-estradiol administration following trauma-hemorrhage would also improve cardiac output, splanchnic perfusion, and oxygen utilization, even after the induction of subsequent sepsis.

Setting: A university laboratory.

Intervention: Male rats underwent midline laparotomy (ie, soft tissue injury). They were bled to a mean arterial pressure of 35 to 40 mm Hg for 90 minutes and resuscitated over 60 minutes with lactated Ringer solution. At the beginning of resuscitation, 17β-estradiol (1 mg/kg) or a vehicle was administered. At 20 hours after resuscitation, polymicrobial sepsis was induced by cecal ligation and puncture (CLP).

Main Outcome Measures: At 5 hours after CLP, cardiac performance (via a left ventricular catheter), cardiac output, and organ blood flow were determined using strontium 85 microspheres. Blood samples were collected from the femoral artery and jugular, portal, and renal veins to determine systemic and regional oxygen delivery and consumption. Moreover, circulating levels of 17β-estradiol, its adrenal precursor dehydroepiandrosterone (DHEA), and corticosterone were assessed by enzyme-linked immunosorbent assay.

Results: Hemorrhage and subsequent sepsis significantly depressed cardiac performance, cardiac output, organ perfusion, and oxygen consumption. Estrogen did not restore cardiac output or systemic oxygen consumption; nonetheless, it restored the depressed intestinal perfusion. Rats treated with estrogen had significantly elevated levels of plasma 17β-estradiol, but the levels of DHEA or corticosterone were not affected.

Conclusions: The increase in gut perfusion could represent a potential mechanism for the salutary effects of 17β-estradiol following trauma-hemorrhage. Because 17β-estradiol improves systemic and intestinal perfusion after trauma-hemorrhage and induction of subsequent sepsis, this agent appears to be a promising adjunct for the treatment of trauma victims.

Arch Surg. 2002;137:74-79

Traumatic injury is the leading cause of death among young people in the United States. Patients who survive the immediate consequences of their injuries and the accompanying loss of blood are susceptible to subsequent sepsis and multiple organ dysfunction. The mechanisms responsible for the development of multiple organ dysfunction have not yet been established; nonetheless, it has been shown that macrocirculatory and microcirculatory disturbances are important factors in the pathogenesis of impaired organ and immune functions observed in multiple organ dysfunction. It appears that after an initial insult such as hemorrhagic shock, the capacity of the cardiovascular and immune systems to respond to a septic challenge is reduced, contributing to increased susceptibility to sepsis in trauma victims. Under such conditions, the perfusion of the splanchnic vessel bed appears to be particularly sensitive to hypoperfusion. Hypoxia of the gut is considered to be a driving force of multiple organ failure, and hypoperfusion of intestinal mucosa can occur even when the mean arterial pressure fails to indicate any sign of shock. Thus, therapies to augment systemic and, in particular, intestinal perfusion could prevent hypoxia and improve the outcome in critically ill patients.

The gonadal steroid 17β-estradiol has been shown to protect the cardiovascular system against ischemic, inflammatory, and metabolic injury. Moreover, recent studies from our laboratory have shown that administration of 17β-estradiol in male or ovariectomized female animals following trauma-hemorrhage restores depressed cardiac, hepatocellular, and immune functions. However, it is unknown whether administration of 17β-estradiol after trauma-hemorrhage improves circulation and organ perfusion after subsequent sepsis is induced. The aim of our study was to investigate whether administration of a single dose of 17β-estradiol...
MATERIALS AND METHODS

EXPERIMENTAL PROTOCOL

Rats were divided into 3 groups: the sham group, the hemorrhage-cecal ligation and puncture (HEM-CLP) group, and the estradiol group. Animals in the sham group underwent sham trauma-hemorrhage and cecal ligation and puncture (CLP). Animals in the HEM-CLP group were subjected to trauma-hemorrhage then CLP and received a vehicle during resuscitation. Animals in the estradiol group were subjected to trauma-hemorrhage and CLP and were then treated with a single dose of 17β-estradiol (Sigma-Aldrich Corp, St Louis, Mo) during resuscitation.

ANIMAL MODEL OF TRAUMA-HEMORRHAGE

A nonheparinized model of trauma-hemorrhage and resuscitation in the rat, as previously described, was used in this study.11 Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, Mass) weighing 275 g to 325 g were fasted earlier.11 The animals were anesthetized with methoxyflurane inhalation and underwent a 5-cm ventral midline laparotomy (ie, induction of soft tissue trauma). Both femoral arteries and 1 femoral vein were then cannulated with polyethylene-50 tubing. All incisions were bathed with 1% lidocaine, which provided analgesia throughout the experiment. The animals were allowed to wake and then were rapidly bled to a mean arterial pressure of 35 mm Hg (ie, severe hypotension) within 10 minutes. The rapid bleeding on awakening puts animals in a state of depressed sensibility, thus minimizing distress. Blood pressure was kept at 35 mm Hg by removing more blood in small increments until animals were no longer able to maintain blood pressure at that level (ie, maximum bleedout). After that point, blood pressure was maintained by infusing lactated Ringer solution intravenously in small bolus increments until it replaced 40% of the shed blood volume. Over a period of 60 minutes, the animals were resuscitated with lactated Ringer solution 4 times the volume of the shed blood. Over a period of 60 minutes, the animals were resuscitated with lactated Ringer solution 4 times the volume of the shed blood. Sham animals underwent the same surgical procedure, but they were neither bled nor resuscitated. The time required for maximum bleedout was about 45 minutes, the volume of maximum bleedout was about 60% of the calculated circulating blood volume, and the total hemorrhage time was about 90 minutes. At the beginning of resuscitation, 17β-estradiol (1 mg/kg of body weight of 17β-estradiol sulfate) or a vehicle (isotonic sodium chloride solution) was administered intravenously.

This study adhered to the National Institutes of Health guidelines for the use of experimental animals. The Institutional Animal Care and Use Committee of the University of Alabama at Birmingham approved this project.

ANIMAL MODEL OF CLP

After fluid resuscitation, the animals were returned to their cages and were allowed water and food ad libitum. At 20 hours after resuscitation, polymicrobial sepsis was induced by CLP according to the method described by Chaudry et al.11 The animals were anesthetized with methoxyflurane inhalation and the 5-cm midline laparotomy was reopened. The cecum was then exposed, ligated just distal to the ileocecal valve to avoid intestinal obstruction, punctured twice with an 18-gauge needle, and returned to the abdominal cavity. The abdominal incision was then closed in 2 layers, and the animals received normal saline subcutaneously (3 mL/100 g body weight). The area of incision was bathed with 1% lidocaine to provide analgesia. With this model, blood cultures were positive for Escherichia coli, Streptococcus bovis, Proteus mirabilis, Enterococcus species, and Bacteroides fragilis within 1 hour.13

MEASUREMENT OF CARDIAC OUTPUT, CARDIAC PERFORMANCE, AND BLOOD FLOW

Cardiac output, performance, and organ blood flow were determined with a radioactive microsphere technique, as previously described.11 Briefly, at 5 hours after CLP in the hemorrhaged rats, animals were anesthetized again with methoxyflurane. The right femoral artery and vein were re-catheterized, and an additional polyethylene-50 catheter was inserted into the left ventricle via the right carotid artery, as previously described. The position of the catheter tip in the left ventricle was confirmed using the left ventricular pressure curve, and its exact position in the left ventricle was verified at the postmortem examination. The maximal rates of the left ventricular pressure rise (+dp/dt max) and fall (−dp/dt max) were determined with a heart performance analyzer. The measurement lasted for about 20 minutes, and the data from the last 10 measurements were averaged and analyzed in this study. Over 20 seconds, strontium 85-labeled microspheres (DuPont/NEN, Boston, Mass) were injected into the left ventricle via the left ventricular catheter at a constant rate. The reference blood sample was withdrawn from the femoral arterial catheter. Rats were then killed with an overdose of lidocaine, and the small intestine, kidneys, adrenal glands, liver, triceps femoris muscle, and dorsal skin were harvested, weighed, and placed in test tubes. Their specific radioactivity was measured with an automatic γ counter (1470 Wizard; Wallac, Gaithersburg, Md). The reference blood sample was transferred to a test tube for radioactivity measurement. The remaining microspheres, which were left in the syringe and the ventricular catheter after injection, were also counted. Cardiac output and organ blood flow were calculated as described earlier.11

DETERMINATION OF OXYGEN DELIVERY AND CONSUMPTION

Oxygen delivery and consumption were assessed as described by Ba et al.14 After the reference blood sample for determination of cardiac output was taken, a 1-mL heparinized syringe with a 22-gauge needle was inserted into the portal vein, secured with superglue to prevent blood leakage, and used for portal venous blood sampling. The same technique was used for renal venous blood sampling. Blood samples (about 0.15 mL each) were collected immediately after microsphere infusion from the femoral artery, femoral vein, hepatic vein, portal vein, and renal vein. PO2 and PCO2 were measured with a blood gas machine (ABL5; Radiometer, Copenhagen, Denmark). Total oxygen content was measured with an OSM hemoximeter (Radiometer). The oxygen delivery of the whole body, Continued on next page
after trauma-hemorrhage had any salutary effects on cardiovascular response and gut perfusion following the induction of polymicrobial sepsis.

RESULTS

HEART PERFORMANCE, HEART RATE, AND CARDIAC OUTPUT

The maximal rates of the left ventricular pressure increase and decrease in rats following trauma-hemorrhage and CLP were similar to values in sham-operated controls (Figure 1A). There was a significant increase of the +dP/dt_{max} in 17β-estradiol–treated animals, in comparison with the other groups; however, the difference in −dP/dt_{max} was not significant. There was no significant difference in cardiac output (Figure 1B) or heart rate (Figure 1C) among the control, HEM-CLP, and estradiol groups.

ALTERATIONS IN ORGAN BLOOD FLOW

A significant decrease in blood flow was observed in the intestine, colon, adrenal glands, liver, kidneys, muscle, and spleen of the HEM-CLP group compared with control animals (Table, Figure 2). There was no signifi-

DETERMINATION OF PLASMA STEROIDS

Plasma testosterone was measured with a coated-tube radioimmunoassay (RIA) kit (Diagnostic Systems, Webster, Tex). Cross-reactivity of the RIA was as follows: 100% for testosterone; 3.4% for 5α-dihydrotestosterone; 2.2% for 5α-androstane-3β, 17β-diol; 2% for 11-oxytestosterone; and less than 1% for all other steroids. Plasma 17β-estradiol concentration was determined with an RIA kit specifically designed for rats and mice (ICN Biomedicals, Costa Mesa, Calif). Cross-reactivity of the RIA was 100% for 17β-estradiol and 20% or 1.51% for estrone or estril, respectively. For all other steroids, the cross-reactivity was less than 0.01%. Plasma levels of dehydroepiandrosterone (DHEA) were determined with an RIA kit (ICN Biomedicals). Cross-reactivity of the RIA was 100% for DHEA and less than 1% for all other steroids. Plasma corticosterone concentration was determined with an RIA kit specifically designed for rats and mice (ICN Biomedicals). Cross-reactivity of the RIA was 100% for corticosterone. For all other steroids, the cross-reactivity was less than 0.01%.

STATISTICAL ANALYSIS

All data are presented as mean ± SEM. Analysis of variance and Student-Newman-Keul tests were used; differences were considered significant at P < .05.

Figure 1. Cardiac performance (A) (maximal rate of left ventricular pressure increase and decrease), cardiac output (B), and heart rate (C) in sham-operated animals, animals subjected to trauma-hemorrhage and cecal ligation and puncture (HEM-CLP), and animals subjected to HEM-CLP that were treated with 17β-estradiol sulfate (1 mg/kg body weight) (n = 7 for each group). Error bars indicate SEM; and asterisk, P < .05 vs sham.

DETERMINATION OF PLASMA STEROIDS

Plasma testosterone was measured with a coated-tube radioimmunoassay (RIA) kit (Diagnostic Systems, Webster, Tex). Cross-reactivity of the RIA was as follows: 100% for testosterone; 3.4% for 5α-dihydrotestosterone; 2.2% for 5α-androstane-3β, 17β-diol; 2% for 11-oxytestosterone; and less than 1% for all other steroids. Plasma 17β-estradiol concentration was determined with an RIA kit specifically designed for rats and mice (ICN Biomedicals, Costa Mesa, Calif). Cross-reactivity of the RIA was 100% for 17β-estradiol and 20% or 1.51% for estrone or estril, respectively. For all other steroids, the cross-reactivity was less than 0.01%. Plasma levels of dehydroepiandrosterone (DHEA) were determined with an RIA kit (ICN Biomedicals). Cross-reactivity of the RIA was 100% for DHEA and less than 1% for all other steroids. Plasma corticosterone concentration was determined with an RIA kit specifically designed for rats and mice (ICN Biomedicals). Cross-reactivity of the RIA was 100% for corticosterone. For all other steroids, the cross-reactivity was less than 0.01%.

STATISTICAL ANALYSIS

All data are presented as mean ± SEM. Analysis of variance and Student-Newman-Keul tests were used; differences were considered significant at P < .05.
PLASMA LEVELS OF SEX STEROIDS

There was no change in the circulating plasma levels of 17β-estradiol between sham-operated male animals and vehicle-treated animals subjected to trauma-hemorrhage and subsequent CLP (Figure 4A). However, rats treated with a single dose of 17β-estradiol showed significantly elevated estrogen levels. There was no significant difference in the circulating levels of corticosterone or DHEA among the different groups (Figure 4B).

Several factors contribute to the progression of multiple organ failure. Control mechanisms regulating cell and organ functions fail to produce an adequate response to tissue damage and blood loss. As a consequence, the ability of the body to defend itself against an infectious insult is impaired, leading to increased susceptibility to sepsis and increased incidence of septic shock. The ability of the cardiovascular system to provide adequate nutritive perfusion is essential for the function of vital organs. Microcirculatory impairment and the loss of fine feedback mechanisms controlling local blood flow are characteristic features of patients who have sustained severe blood loss and subsequent sepsis. The clinical observation that multiple organ failure most likely occurs in patients following multiple, sequential insults, such as the development of pneumonia following a traumatic injury, has led to the development of different “multiple hit” animal models that more closely approximate the clinical situation. Although the effects of trauma-hemorrhage and sepsis on circulation have been extensively studied, little is known about the organ blood flow and tissue perfusion rate following a combined injury such as trauma-hemorrhage and CLP. To study organ perfusion under such conditions, we used a multiple hit model of trauma-hemorrhage and induction of subsequent polymicrobial sepsis. Radioactive-labeled microspheres with a diameter of approximately 15 µm were used to measure blood flow through terminal arterioles to minimize the effect of the septic arteriovenous shunting. Previous studies have shown that at 5 hours after CLP, animals not subjected to prior trauma-hemorrhage have increased cardiac output and gastrointestinal blood flow compared with increased oxygen consumption, thus mimicking the clinical picture of the septic patient. However, in our model of multiple injuries, cardiac output and systemic oxygen consumption were significantly depressed. Although our data suggest that the stress related to hemorrhagic shock and resuscitation reduces the cardiovascular capacity of the rats to respond to a subsequent septic challenge, the exact mechanisms, such as decreased adrenergic responsiveness or decreased circulating blood volume, remain to be determined. The reduction of organ blood flow was most pronounced in the small intestine, colon, and mesentery, where oxygen consumption decreased to approximately 26% of the controls. These findings concur with previous studies by Garrison et al and Turnbull et al, who observed that cardiac output as well as the response of the splanchnic vessel bed (ie, gut perfusion) were significantly impaired after initial hemorrhage and subsequent infusion of live E coli or endotoxin.

Several investigators have reported that for females, the risk of ischemic insults such as myocardial infarction or stroke is significantly reduced, which has been attributed to the effects of estrogen. Estrogen is considered to
increase in the intraventricular pressure. This suggests that 17β-estradiol specifically improved the circulation in the splanchnic vessel bed. This observation is supported by findings from Magness et al,21 who showed that in female sheep, 2 hours of continuous, low-dose estradiol infusion significantly increased perfusion to the small intestine, as well as to other nonreproductive organs in the splanchnic bed. Ma et al22 reported that administration of 17β-estradiol dilated the mesenteric arteries in ovariectomized female rats in vitro and preserved endothelial function following ischemia and reperfusion in vivo. In the context of these findings, our data suggest a direct effect of estradiol on mesenteric vessels and/or gut parenchyma. While the present study did not assess the effects of estrogen administration on survival following trauma-hemorrhage and subsequent sepsis, previous studies have shown that maintenance of intestinal perfusion correlates with reduced mortality.23,24 Thus, it is likely that 17β-estradiol administration would improve survival under such conditions.

It is well known that estrogen, via its receptor, can directly affect endothelial cell function.25 Hisamoto et al26 have shown that 17β-estradiol can rapidly activate endothelial nitric oxide synthase in endothelial cells following the activation of the estrogen receptor α. Therefore, activation of the estrogen receptor α within the endothelial cells of the intestine, with subsequent activation of endothelial nitric oxide synthase and local vasodilatation, could be a mechanism for the salutary effect of 17β-estradiol in our model. Nonetheless, other mechanisms such as the inhibition of endothelin production, inhibition of mitogen-activated protein kinase activity,27 and direct vasodilatation via relaxation of smooth muscle cells28 could contribute to the observed increase in splanchnic perfusion.
Our results indicate that in vehicle-treated rats subjected to trauma and hemorrhagic shock, adrenal perfusion was significantly impaired at 5 hours after CLP. However, rats treated with a single dose of 17β-estradiol showed a significant increase in adrenal perfusion compared with vehicle-treated animals. To examine whether the increased adrenal blood flow could affect the circulating levels of DHEA, we measured the plasma concentrations of 17β-estradiol and DHEA, which is a precursor in the steroid synthesis that has been shown to be protective following hemorrhagic shock29 as well as sepsis.30 Although there was a significant increase in circulating levels of 17β-estradiol in estrogen-treated male rats, we did not detect any difference in the plasma levels of DHEA between the vehicle and estradiol groups. These results suggest that the single dose of 17β-estradiol was sufficient to increase circulating plasma levels of estrogen during our measurements and that the beneficial effects of estrogen were not mediated by DHEA.

In this study, a single dose of estradiol was administered following trauma-hemorrhage and various parameters were measured at 5 hours following the induction of sepsis. Thus, it remains to be determined whether multiple doses or long-term treatment with estradiol would further improve or restore cardiac output and cardiovascular functions. However, long-term treatment with estradiol (1 mg, 3 times per day) can induce reversible gynecomastia and erectile dysfunction in male patients. Moreover, changes in blood coagulation41 and genotoxic changes in the liver32 have been observed with prolonged elevation in circulating estrogen levels. Our findings, along with these observations, suggest that administration of a single dose of estradiol is the most efficacious regimen for the preservation of intestinal perfusion following trauma-hemorrhage and sepsis.

A single dose of 17β-estradiol administered during resuscitation improved depressed splanchic circulation and oxygen consumption in a model of trauma-hemorrhage and CLP. Therefore, our results suggest that 17β-estradiol administration should be considered as a novel approach to increasing intestinal oxygen utilization in critically ill male trauma victims.

This investigation was supported by grant R37 GM 39519 (Dr Chaudry) from the National Institutes of Health, Bethesda, Md. Dr Wang is the recipient of NIH Independent Scientist Award KO2 AI 01461.

We thank David Ornan, BS, and Zheng F. Ba, BA, for their superb technical assistance.

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