Gender differences in small intestinal endothelial function: inhibitory role of androgens

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Ba, Zheng F., Yukihiro Yokoyama, Balazs Toth, Loring W. Rue III, Kirby I. Bland, and Irshad H. Chaudry. Gender differences in small intestinal endothelial function: inhibitory role of androgens. Am J Physiol Gastrointest Liver Physiol 286: G452–G457, 2004. First published October 16, 2003; 10.1152/ajpgi.00357.2003.—Although gender differences exist in cardiovascular endothelial function, it remains unclear whether such differences are also seen in small intestinal endothelial function. To determine this, untreated male, age-matched proestrus female, castrated male, and 17β-estradiol (E2)-treated noncastrated male rats were studied. Dose response curves to ACh and nitroglycerin (NTG) were determined by measuring changes in perfusion pressure by using an isolated small intestinal perfusion model. Endothelium-derived nitric oxide (NO) production/release was indirectly determined by the ability of intact endothelium to suppress serotonin (10−5 M)-induced perfusion pressure changes. Intestinal tissue levels of NO were also measured. Moreover, plasma levels of androgen and E2 were determined and correlated with ACh (10−8 M)-induced perfusion pressure reductions. ACh-induced intestinal perfusion pressure reductions in proestrus females, castrated males, and E2-treated noncastrated males were significantly higher than in untreated males. NTG-induced perfusion pressure reductions were not significantly different among groups. Perfusion pressures after administration of serotonin (10−5 M) and intestinal tissue levels of NO in proestrus females, castrated males, and E2-treated noncastrated males were also significantly higher than in untreated males. Plasma androgen levels in proestrus females, castrated males, and in E2-treated noncastrated males were significantly lower compared with untreated males. There was a positive correlation between plasma androgen and ACh-reduced perfusion pressure; however, E2 levels did not show a similar relationship. Thus androgens appear to play an inhibitory role in small intestinal endothelial function. These properties in male vessels can be modulated by decreasing the level of circulating androgens or by E2 treatment.

male; female; endothelium; acetylcholine; serotonin; nitroglycerin

THE SMALL INTESTINAL MICROVASCULATURE is one of the regions most susceptible to injury induced by hemorrhagic shock. Impaired restoration of intestinal perfusion during resuscitation results in persistent mucosal ischemia and subsequent loss of mucosal integrity. This loss of gut mucosal integrity has been implicated in the pathogenesis of multiple organ dysfunction syndrome (4, 8, 11, 34). Moreover, maintenance of intestinal blood flow is essential to supply blood to the portal system that directly affects the hepatic blood flow under such conditions (38). It is well established that gender directly influences the cardiovascular system (28, 31). Clinical studies have shown that the incidence of coronary artery disease and systemic hypertension are higher in men than in age-matched premenopausal women (26). Furthermore, Kauser et al. (24) indicated that sex steroid hormones can influence endothelial function in isolated rat aorta, which may contribute to gender differences observed in the pathogenesis of cardiovascular diseases. Experimental data indicate that vascular endothelial function is gender dimorphic (5). In this regard, it has been shown that testosterone receptor blockade after trauma-hemorrhage improves the depressed cardiac, hepatic, and adrenal functions in male rats (7, 32). Estradiol has been demonstrated to directly relax blood vessels or facilitate the relaxation in response to other vasoconstrictive agents (28). We have previously shown that there are gender differences in the intestinal perfusion pressure reduction after trauma and hemorrhagic shock. However, little is known about the possible effects of sex steroids on small intestinal vascular endothelial function. In light of these previous findings, we hypothesized that differences exist in endothelial function of male and proestrus female animals. To test this, small intestinal vascular endothelial function was determined in male, age-matched proestrus female, castrated male, and noncastrated male rats treated with 17β-estradiol (E2) by using an isolated intestinal perfusion system. In addition, plasma levels of androgen and estrogen were measured and correlated with the observed parameters.

MATERIALS AND METHODS

Animals and experimental groups. All experiments were performed in compliance with the National Institutes of Health guidelines for the use of experimental animals and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Age-matched adult male and female Sprague-Dawley rats (Charles River), weighing 275–325 g and 200–250 g, respectively, were studied. Female rats at the proestrus stage of the estrus cycle (as defined by vaginal smears) made up one group, and male rats were randomly assigned to the following three groups: 1) untreated male rats, 2) castrated male rats, and 3) noncastrated male rats treated by subcutaneous implantation of an E2 pellet (0.5 mg). In castrated animals, the operation was performed 14–20 days before the experiments. In the E2-treatment group, pellets were implanted 10–14 days before the experiments and were set to continuously release E2. Male rats were fasted 16 h before the experiment but were allowed water ad libitum.

Isolated small intestine and measurement of ACh or NTG-induced small intestinal vessel relaxation. Animals underwent an isolated small intestinal perfusion model as described previously by Ba et al. (5). Briefly, the right femoral vein was cannulated under isoflurane
Table 1. Plasma androgen (sum of testosterone and dihydrotestosterone) and estrogen levels in males, age-matched proestrous females, castrated males, and 17β-estradiol-treated noncastrated male rats

<table>
<thead>
<tr>
<th>Androgen, pg/ml</th>
<th>Estrogen, pg/ml</th>
<th>Androgen-to-Estradiol Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>971±77</td>
<td>7.6±0.2</td>
<td>127.76</td>
</tr>
<tr>
<td>Females</td>
<td>62±7.7*</td>
<td>108±8.4*</td>
</tr>
<tr>
<td>Castrated males</td>
<td>26±0.7*</td>
<td>12±2</td>
</tr>
<tr>
<td>17β-estradiol-treated noncastrated males</td>
<td>98±22*</td>
<td>182±7.8*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE and compared by one-way ANOVA and Tukey’s test; n = 6 rats in each group. *P < 0.05 vs. males.

RESULTS

Plasma androgen and estrogen levels. Plasma androgen levels in females, castrated males, and E2-treated noncastrated males were significantly lower than in untreated males (Table 1). Plasma E2 concentrations in females and E2-treated noncastrated males were significantly greater than in castrated males and untreated males. Although the plasma levels of estrogen in castrated males were higher than in untreated males, the difference was not statistically significant.

ACh and NTG-induced perfusion pressure reduction in isolated intestine. ACh-induced intestinal perfusion pressure reduction was significantly greater in females, castrated males, and E2-treated males than in untreated males at the examined concentrations of ACh (Fig. 1A). However, the difference in NTG-induced perfusion pressure reduction was not significant among these groups at the tested NTG concentrations (Fig. 1B).
Pressure responses to serotonin in the isolated intestine. Serotonin induced a significantly diminished intestinal perfusion pressure increase in females, castrated males, and E2-treated noncastrated males than in untreated males (Fig. 2A). However, these differences could not be observed when the NO synthase inhibitor L-NNA was used in the perfusate in addition to serotonin (Fig. 2B). Differences in serotonin-induced and L-NNA-induced intestinal perfusion pressure reduction revealed that the suppression in perfusion pressures with intact endothelium was significantly lower in untreated males than among females, castrated males, and E2-treated noncastrated males. It indirectly indicates that the NO release in intact endothelium is decreased in untreated males compared with females, castrated males, and E2-treated noncastrated males (Fig. 2C).

Intestinal tissue NO levels. The observed intestinal tissue NO levels were significantly higher in females, castrated males, and E2-treated noncastrated males than in untreated males (Fig. 3).

Correlation between the plasma sex steroid levels and ACh-induced perfusion pressure reduction. There was a positive correlation between plasma androgen levels and ACh \(10^{-8}\) M-induced intestinal perfusion pressure reductions \(R^2 = 0.698\) (Fig. 4A). However, we did not observe a similar
correlation between plasma E2 levels and ACh-induced intestinal perfusion pressure reductions (Fig. 4B).

**DISCUSSION**

Gender influences and modulates the function and responsiveness of the cardiovascular system under normal and pathological conditions (14, 15, 30, 33, 35). Direct receptor-mediated effects of sex steroids on blood vessels and indirect effects, such as modulation of vascular reactivity to different vasoactive substances, have been well described (17). Demonstration of estrogen receptors in endothelial cell culture supports the hypothesis that estrogens may have a direct effect on vascular endothelium (9). In addition to this, previous data from our laboratory have shown that females in the proestrus stage of the estrus cycle and males treated with female sex hormones are protected from the deleterious effects of trauma and hemorrhagic shock (3). The depressed cardiac and hepatocellular function, as observed in males, was not observed in proestrus females under similar conditions (21). For instance, cardiac output is maintained in proestrus females, whereas it is significantly decreased in males after trauma-hemorrhage and resuscitation (21). Similarly, hepatocellular function, as measured by the clearance of indocyanine green, was maintained in proestrus females but was markedly depressed in males after trauma-hemorrhage (21). Experimental evidence also showed that E2 significantly augmented ACh-induced endothelium-dependent relaxation in coronary arteries of cholesterol-fed ovariectomized cynomolgus monkeys (41). Furthermore, chronic treatment with E2 enhanced endothelium-dependent relaxation in the rabbit aorta (22). Although gender has been shown to influence vascular function in large vessels, it is not clear whether similar effects are present in the microcirculation. In the present study, we examined the potential gender differences and the effects of sex steroids on the small intestinal endothelial function under normal conditions. To study this, we investigated the indirect effects of gender and E2 by using an isolated intestinal perfusion system to obtain local circulatory responses from the small intestine and thus prevent the potential effects of other organs on the small intestinal circulation. Studies (21) have shown that the gender-related differences are most pronounced between males and females in the proestrus stage of their estrus cycle. Therefore, we used cycle-matched proestrus female and male rats of similar age.

Marked differences were observed in the reactivity of the intestinal vessel bed to ACh, which is an endothelium-dependent vasodilator (5, 6, 39). Male rats showed significant pressure reductions compared with proestrus females, castrated males, and normal intact males with E2 treatment. However, there was no difference among the responses of the proestrus female, castrated males, and noncastrated males treated with E2. The pressure reduction generated by NTG (an endothelium-independent vasodilator) was not significantly different among the groups. Moreover, the perfusion pressure by intact endothelium after serotonin treatment was significantly greater in proestrus females than in males. Additionally, intestinal tissue NO levels in proestrus females were significantly higher than in males. However, not only did the proestrus female animals show significantly greater ACh-induced isolated intestinal perfusion pressure reduction, but similar differences were observed in castrated males and E2-treated males as well. Furthermore, there was a correlation between the plasma levels of androgen and ACh-induced intestinal perfusion pressure reduction. We therefore postulate that the observed decreased pressure reduction in males, compared with proestrus females, castrated males, and noncastrated males with E2 treatment, are at least partly mediated by differences in the endothelial function. Because there was no difference among the responses of the proestrus female, castrated males, and noncastrated males with E2 treatment after NTG treatment, it appears that such gender differences are due to sex hormone levels. In this regard, our data support the hypothesis that not only high E2 levels but also the ratio of androgen/estrogen contributes to the observed changes in the endothelial function. In males, the androgen/estrogen ratio is extremely high compared with the castrated groups and the proestrus females. This high ratio is accompanied by a decreased pressure reduction. In proestrus females, castrated males, and E2-treated noncastrated males, the androgen levels were low, and those were associated with a higher endothelial response. It therefore appears that not only the high E2 levels but also the low androgen-to-estrogen ratio plays an important role in the female-like endothelial responses as well. Thus our results show gender-specific differences in the function of the intestinal vasculature. However, to determine the potential therapeutic applications of these findings, the underlying mechanisms must be elucidated.

The observed beneficial effects of E2 treatment on endothelial function can be explained as the consequence of direct or indirect actions of this sex steroid. E2 can increase the release of prolactin and thus exert indirect actions (42). The direct effects can be generated via two different types of E2 receptors: estradiol receptor-α and estradiol receptor-β. The contribution of these receptor subtypes should be determined. Another possibility could be the use of an androgen receptor antagonist in males. In this regard, flutamide has been shown to imitate the immunomodulatory effects of castration (40) and to improve cardiac and hepatocellular functions after trauma and hemorrhagic shock (32). Whether or not flutamide has any effects on endothelial response remains to be determined.

Whereas it is widely recognized that men are generally more susceptible to coronary artery diseases compared with women, studies have paid little attention to the effects of androgens on the vasculature. In this regard, Herman et al. (18) reported that androgen deprivation in adult men enhanced endothelium-dependent relaxation. Furthermore, administration of testosterone in hypercholesterolemic rabbits and monkeys impaired endothelium-dependent relaxation and promoted the progression of atherosclerosis (1, 19). Previous studies (2, 16, 23, 25, 27, 43) have shown that there are gender differences in vascular reactivity. Moreover, studies (10, 12, 22, 29, 36) have shown that sex hormones can alter the sensitivity of blood vessels to different agonists. However, these studies were focused on large vessels. A possible influence of sex steroids on the cardiovascular system has also been shown in the incidence of atherosclerosis or the risk of cardiovascular diseases (20). However, these studies were focused on the gender differences in endothelial function in large vessels, such as aorta. The functional differences between large and small vessels can be significant. To our best knowledge, the present study is the first to report that there are significant gender differences in small intestinal endothelial function under normal physiological conditions.
It has been previously documented that the release of endothelium-derived NO from endothelial cells contributes to vascular dilatation and that the tissue level of NO directly correlates to endothelial functions (13). Furthermore, prior studies (9, 16, 22, 37, 41) suggest that the gender differences in NO production may be due to the effect of estrogens on endothelial function. In this regard, it has been shown that the basal release of NO is significantly augmented by estrogen in the aorta of ovariectomized rabbits, although circulating -arginine concentrations are not different (16). Moreover, intra-arterial infusion of estrogen into the uterine artery of sheep produced vasoconstriction and increased uterine blood flow, which could be inhibited by an -arginine analog, suggesting that NO mediated the effect (37). Proestrus females, castrated males, and noncastrated E2-treated male animals had significantly higher NO levels measured by nitrate/nitrite levels in our experiments. On the basis of these results and our previous data, it could be speculated that one possible factor contributing to the enhanced relaxation compared with males might be the increased NO production. However, further experiments are required to determine the exact underlying mechanisms.

In conclusion, our results indicate that the endothelium-dependent (ACh-induced) intestinal perfusion pressure reduction in males was significantly lower than in proestrus females. This effect can be reversed by gonadectomy or estrogen treatment. Furthermore, the observed correlation between androgen plasma levels and ACh-induced perfusion pressure suggest that androgens can influence intestinal endothelial function. The increased endothelial pressure reduction might be at least partly due to the increased NO production in proestrus females, castrated males, and noncastrated E2-treated males. The observed changes in the small intestinal vasculature could thus represent a potential mechanism for the circulatory gender differences between males and females.

GRANTS

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REFERENCES


