Estrogen, nitric oxide, and hypertension differentially modulate agonist-induced contractile responses in female transgenic (mRen2)27 hypertensive rats

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Brosnihan KB, Li P, Figueroa JP, Ganten D, Ferrario CM. Estrogen, nitric oxide, and hypertension differentially modulate agonist-induced contractile responses in female transgenic (mRen2)27 hypertensive rats. Am J Physiol Heart Circ Physiol 294: H1995–H2001, 2008. First published March 14, 2008; doi:10.1152/ajpheart.01193.2007.—Clinical trials revealed that estrogen may result in cardiovascular risk in patients with coronary heart disease, despite earlier studies demonstrating that estrogen provided cardiovascular protection. It is possible that the preexisting condition of hypertension and the ability of estrogen to activate the renin-angiotensin system could confound its beneficial effects. Our hypothesis is that the attenuation of estrogen to agonist-induced vasoconstrictor responses through the activation of nitric oxide (NO) synthase (NOS) is impaired by hypertension. We investigated the effects of 17β-estradiol (E2) replacement in normotensive Sprague-Dawley (SD) and (mRen2)27 hypertensive transgenic (TG) rats on contractile responses to three vasoconstrictors, angiotensin II (ANG II), serotonin (5-HT), and phenylephrine (PE), and on the modulatory role of vascular NO to these responses. The aorta was isolated from ovariectomized SD and TG rats treated chronically with 5 mg E2 or placebo (P). The isometric tension of the aortic rings was measured in organ chambers, and endothelial NOS (eNOS) in the rat aorta was detected using Western blot analysis. E2 treatment increased eNOS expression in the SD and TG aorta and reduced ANG II- and 5-HT-induced contractions in SD and TG rats. The inhibition of NOS with N-nitro-l-arginine methyl ester enhanced ANG II- and 5-HT- but not PE-induced contractions in SD and TG rats. Only the responses to 5-HT were augmented in hypertensive rats. In conclusion, this study shows that the preexisting condition of hypertension augmented the vascular responsiveness of 5-HT, whereas the attenuation of estrogen by ANG II and 5-HT vascular responses was not impaired by hypertension. The adrenergic agonist was unresponsive to estrogen treatment. The contribution of NO as a factor contributing to the relative refractoriness of the vascular responses is dependent on the nature of the vasoconstrictor and/or the presence of estrogen.

Recent Clinical Trials [Heart and Estrogen/Progestin Replacement Study (HERS), Estrogen Replacement and Atherosclerosis (ERA), and Women’s Health Initiative (WHI)] (10, 11, 34a) showed that estrogen and/or combined estrogen plus progestrone replacement provides no beneficial cardiovascular effects in postmenopausal women. This is despite the fact that observational studies showed that women in their reproductive age have a much lower incidence of atherosclerosis and hypertension than that of the age-matched men and postmenopausal women (28, 29). In both the observational studies and the clinical trials, little attention was paid to the underlying disease at the time of hormone initiation. Specifically, the preexisting condition of hypertension and the ability of estrogen to activate the renin-angiotensin system (RAS) could confound the beneficial effects of estrogen.

Estrogen has both stimulatory and inhibitory effects on components of the RAS, including the downregulation of the angiotensin II (ANG II) type 1 (AT1) receptor (21) and angiotensin I (ANG I)-converting enzyme (ACE) (4) and the upregulation of angiotensinogen (32) and increased circulating levels of ANG II (25). The transgenic (TG) hypertensive rats harboring a single mouse renin (Ren-2) gene [(mRen2)27] provide a genetic model that has many features in common with essential hypertension including low or normal circulating renin (18, 35). The model has an activated tissue RAS (6, 26), which contributes to the fulminant hypertension. This genetic model of hypertension clearly indicates the important role of estrogen in renin-angiotensin-dependent hypertension, since female TG rats have much lower blood pressure than that of age-matched male rats. Studies demonstrated that the in vivo pressor responses to ANG II and phenylephrine (PE) are attenuated in estrogen-treated, ovariectomized TG hypertensive female rats (1). At the same time, chronic estrogen replacement lowers blood pressure, augments the basal contribution of nitric oxide (NO) to vascular tone, and enhances acetylcholine (ACh)-stimulated vascular NO release in TG rats (16). A number of abnormalities in the hypertensive rats were not entirely corrected by estrogen, thus there was a lesser degree of maximal dilation elicited by ACh in TG rats, suggesting the persistence of some degree of endothelial dysfunction (16). Thus the question arose of whether estrogen would...
modify agonist-induced vasoconstriction and NO mediation of these responses under the preexisting condition of endothelial damage in TG hypertensive rats.

In the present study, we hypothesized that the attenuation of estrogen to agonist-induced vasoconstrictor responses through the activation of NO synthase (NOS) is impaired by hypertension. We investigated the effects of chronic 17β-estradiol (E2) treatment on the vascular contractile responses to the vasoconstrictors ANG II and its precursor ANG I, serotonin (5-HT), and PE and the modulatory role of endothelial NO on each of these vasoconstrictor responses. The variety of agonists selected may uncover whether the amplification of estrogen on the release of NO in endothelial cells is sensitive to modulation under conditions of endothelial damage associated with hypertension (16). Comparisons were conducted between normotensive and TG hypertensive rats to determine whether the effects of estrogen on vascular responsiveness are altered in the animals with angiotensin-dependent hypertension.

MATERIALS AND METHODS

Animal preparations. Following approval by the Institutional Animal Care and Use Committee, 56 12-wk-old, female heterozygous hypertensive (TG) and Hannover Sprague-Dawley (SD) rats (220–250 g body wt) were obtained from the Hypertension Center Transgenic Animal Research Colony of Wake Forest University School of Medicine. The rats were anesthetized with ketamine (30 mg/kg im) and xylazine (5 mg/kg im) and bilaterally ovariec tomized. The animals were allowed free access to water and normal powder chow providing 17 meq of Na+ and 28 meq of K+ per 100 g of solid weight (Rodent Laboratory Chow 5001; Purina Mills, Richmond, IN). After a 2-wk recovery period, the rats were implanted subcutaneously with pellets containing either E2 (5 mg/rat, for 19–21 days release; Innovative Research of America, Sarasota, FL) or placebo (P) for control as previously reported (8, 16). The animals were randomized into four groups: 1) ovariec tomized TG/P, 2) ovariec tomized TG/E2, 3) ovariec tomized SD/P, and 4) ovariec tomized SD/E2. The animals were housed individually in a room maintained at 22°C on a 12-h:12-h light-dark cycle. At the end of 3 wk of E2 treatment, the systemic blood pressure of conscious rats was measured by using the tail-cuff method under conditions of endothelial damage associated with hypertension (16). Comparisons were conducted between normotensive and TG hypertensive rats to determine whether the effects of estrogen on vascular responsiveness are altered in the animals with angiotensin-dependent hypertension.

Vascular ring reactivity. On the next day following the last blood pressure measurements, vascular reactivity was evaluated using isolated aortic rings mounted in organ chambers as previously reported (16). The rats were euthanized by decapitation, and blood was collected may uncover whether the relaxation induced by ACh was <60%. The aortic rings were stimulated with 80 mM of potassium chloride (KCl) twice to generate the maximal contraction. Cumulative concentration-dependent response curves to PE (10−8–10−4 M) and 5-HT (10−8–10−4 M) were produced in quiescent rings. To evaluate the contribution of vascular NO production to the vasoconstriction induced by different vasoconstrictor agonists, the aortic rings were pretreated with the NOS inhibitor N-nitro-l-arginine methyl ester (l-NNAME; 10−4 M) for 30 min, and the contractile responses to PE and 5-HT were then repeated. To prevent tachyphylaxis induced by ANG II in rat vascular rings in vitro (2), each aortic ring was only exposed to one dose of ANG II (10−8–10−6 M) or ANG I (1 µM); separate vessels were studied in the absence or presence of 100 µM l-NNAME or 1 µM lisinopril. The inhibition of vascular NO production was confirmed by the absence of ACh-induced relaxation. Pretreatment with l-NNAME had no effect on the basal tension of resting aortic rings except a minimal contraction in a few rings. A 60-min equilibration period was allowed between each response curve, and the rings were washed with warm Krebs buffer every 15 min.

Western blot analysis. Endothelial NOS (eNOS) isoform protein expression of the rat aorta was analyzed according to a previously published method (36). Protein concentration was measured with the bicinchoninic acid method using BSA as the standard. The lysate of cultured primary bovine endothelial cells was used as the standard of the eNOS isoform.

RT-PCR assay. The AT1 receptor mRNA was measured by RT-PCR assay as previously published (12). Briefly, 1 µg of RQ1 DNase-treated, total RNA isolated from the aorta with the TRIzol reagent (GIBCO) was quantified by ultraviolet spectroscopy and RT-PCR assay. Amplification conditions and primers for the AT1 receptor and the control elongation factor-1α are as previously published (12). Amplified products were separated on a 6% polyacrylamide gel, visualized using a PhosphorImager, and quantified by computerized densitometry.

Chemicals and drugs. Antibodies for the eNOS isoform were purchased from Transduction Laboratories (Lexington, KY). ANG II and ANG I were obtained from Bachem (Torrance, CA). All other drugs were obtained from Sigma Chemical (St. Louis, MO). Indomethacin was freshly dissolved in 0.2 mol/L Na2CO3 solution in stock and diluted in Krebs buffer. Other agents were prepared in distilled water and stored at −20°C as stock solution. The concentration of drugs reported was expressed as the final molar concentration in the organ chamber.

Data analysis and statistics. Vascular constriction was normalized as a percentage of 80 mmol/L KCl-induced maximal constriction (17). The concentration of drugs inducing 50% (EC50) of the maximal constriction was calculated by using a nonlinear regression method (Sigmoid curve fitting program; GraphPad, San Diego, CA). All values are means ± SE. One-way ANOVA and Student’s t-test for unpaired observation were used for statistical analysis. A value of P < 0.05 was considered statistically significant.

RESULTS

Chronic estrogen replacement reduced the systolic blood pressure of TG female hypertensive rats (189 ± 8 vs. 161 ± 7 mmHg, each group contains 10–12 rats; P < 0.05; P vs. E2). Estrogen did not significantly decrease the systolic blood pressure of SD rats (123 ± 6 vs. 113 ± 5 mmHg, each group includes 8–10 rats; P > 0.05; P vs. E2). The serum levels of E2 were 1,255 ± 104 and 1,231 ± 91 pg/ml for estrogen-treated SD and TG rats, respectively. Both P-treated SD and TG groups had a serum level of E2 <15 pg/ml.

Effect of estrogen treatment on the expression of eNOS in aorta. Figure 1, top, shows a representative expression of eNOS in P- and E2-treated SD and TG rat aortic tissue. Chronic E2 treatment increased vascular eNOS expression by twofold and 1.8-fold in the female ovariec tomized normotensive SD and TG hypertensive rat aorta, respectively, compared with the
P-treated SD and TG aorta (Fig. 1, bottom;  𝑃 < 0.05; each group contains 5–7 rats). There was no significant difference of eNOS expression between the E2-treated SD and TG rat aorta.

Estrogen treatment reduces ANG II- and ANG I-induced vasoconstriction in rat aorta and AT1 receptor mRNA. Figure 2 shows that chronic E2 treatment reduces AT1 receptor mRNA in the aorta of SD and TG rats. ANG II caused concentration-dependent vasoconstriction in the aortic rings exposed to a single dose of ANG II. There was no difference of ANG II-induced constriction between SD and TG rats. Estrogen reduced ANG II-induced vasoconstriction in both SD and TG rats compared with P-treated control groups (Fig. 3). Pretreatment with L-NAME enhanced the contractile responses to ANG II at doses of 10^{-7} M in P-treated SD rats and at doses of 10^{-7} and 10^{-6} M in P-treated TG rats. With estrogen treatment in SD and TG rats, pretreatment with L-NAME significantly enhanced the vasoconstrictor response to ANG II at the concentration of 10^{-7} and 10^{-6} M, reaching levels that were essentially the same as the those of P-treated control groups. However, in the presence of L-NAME, the ANG II-induced contractile response remained significantly attenuated with E2 treatment in both the SD and TG groups compared with the P plus L-NAME groups. ANG I, at 1 μM concentration, caused both SD and TG aortic ring constriction, and estrogen significantly inhibited the ANG I-induced vascular contraction (Fig. 4;  𝑃 < 0.05). Pretreatment with lisinopril (1 μM) for 10 min nearly abolished the ANG I-induced vascular constriction in both SD and TG rats, indicating that the local vascular tissue ACE converts ANG I to ANG II.

Estrogen replacement attenuates 5-HT-mediated vascular constriction. The maximal vascular contractile responses to 5-HT were significantly augmented in the P- and E2-treated TG groups compared with the P- and E2-treated SD groups (Table 1). The EC50 of 5-HT in both the P- and E2-treated TG group was significantly lower compared with that of the P- and E2-treated SD groups. The EC50 of 5-HT in the presence of L-NAME in...
both the P and E2 of the TG group was significantly lower compared with that of the SD group, even though there was no difference between their maximal contractile responses. E2 treatment attenuated 5-HT-induced maximal responses at higher concentrations in both treated SD and TG groups with and without L-NAME treatment (Fig. 5). L-NAME pretreatment enhanced 5-HT-induced maximal and lowered EC50 contractile responses in both P-treated SD and TG groups. L-NAME pretreatment did not augment the 5-HT-maximal or lower the EC50-induced responses from E2-treated SD and TG rats.

**Effects of estrogen on the PE-induced vasoconstriction.** PE-induced contractile responses of aortic rings were not significantly different between SD and TG rats compared with those groups with EC50 and maximal contraction (Fig. 6 and Table 2). Chronic E2 treatment did not alter PE-induced vasoconstriction in both the SD and TG groups (EC50, 91.76 ± 5.74 vs. 83.23 ± 5.75 nM and maximal contraction, 111 ± 13.1% vs. 106 ± 8.7% in E2- and P-treated SD rats; and EC50, 91.27 ± 6.06 vs. 75.56 ± 4.76 nM and maximal contraction, 101 ± 4.8% vs. 113 ± 5.1% in E2- and P-treated TG rats; *P > 0.05). Pretreatment with l-NAME significantly augmented the contractile response to PE in both the E2- and P-treated SD and TG rats.

**DISCUSSION**

Our studies suggest that estrogen differentially influences both the vascular responses to three vasoconstrictors and the contribution of NO as a modulating influence to each of the agonists in normotensive and hypertensive rats. Although an increase in NOS expression with E2 treatment was found in the aorta of both normotensive and hypertensive animals, the actual participation of NO as a modulator in opposing the vascular responses was observed with ANG II and PE. In the case of 5-HT, there was NO modulation only in the P groups but not the estrogen-treated groups. The persistence of the attenuation of the ANG II response after NOS inhibition with estrogen treatment is consistent with our and others’ demonstration of estrogen downregulation of the AT1 receptor. The reduction of ANG I-induced vascular constriction is consistent with the downregulation of the AT1 receptor and inhibition of NO.

**Table 1. Effects of chronic estrogen treatment on the serotonin-induced vasoconstriction in SD and TG rat aorta**

<table>
<thead>
<tr>
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<th>EC50, µM</th>
<th>Maximal Contraction, %KCl</th>
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<tr>
<td>SD</td>
<td>4.1 ± 0.17</td>
<td>146 ± 9.2</td>
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<tr>
<td>SD + P</td>
<td>2.28 ± 0.12*</td>
<td>189 ± 12.35*</td>
</tr>
<tr>
<td>SD + E2</td>
<td>2.52 ± 0.07</td>
<td>116 ± 9.45‡</td>
</tr>
<tr>
<td>SD + E2 + l-NAME</td>
<td>2.56 ± 0.14</td>
<td>144 ± 10.23‡</td>
</tr>
<tr>
<td>TG</td>
<td>1.07 ± 0.06†</td>
<td>169 ± 7.65†</td>
</tr>
<tr>
<td>TG + P</td>
<td>0.81 ± 0.04‡</td>
<td>185 ± 14.25*</td>
</tr>
<tr>
<td>TG + E2</td>
<td>1.48 ± 0.10†</td>
<td>155 ± 9.55‡</td>
</tr>
<tr>
<td>TG + E2 + l-NAME</td>
<td>1.19 ± 0.04†</td>
<td>154 ± 11.45‡</td>
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Values are means ± SE; each group has 10-15 aortic rings. SD, Sprague-Dawley; TG, transgenic; P, placebo; l-NAME, N-nitro-l-arginine methyl ester; E2, 17β-estradiol. *P < 0.05 for effects of l-NAME treatment (SD + P vs. SD + P + l-NAME or TG + P vs. TG + P + l-NAME groups); †P < 0.05 for effects of hypertensin (SD + P vs. TG + P, SD + P + l-NAME vs. TG + P + l-NAME, SD + E2 vs. TG + E2, and SD + E2 + l-NAME vs. TG + E2 + l-NAME); ‡P < 0.05 for effects of estrogen (SD + P vs. SD + E2, TG + P vs. TG + E2, SD + P + l-NAME vs. SD + E2 + l-NAME, and TG + P + l-NAME vs. TG + E2 + l-NAME).
ACE activity (1, 4) by estrogen. The augmentation of the PE and ANG II responses after NOS inhibition suggests that NO is a primary modulator of adrenergic and ANG II-dependent vasoactivity responses regardless of the presence or absence of estrogen. On the other hand, only 5-HT elicited an increased responsiveness in hypertensive animals with E2 treatment, attenuating the responses in both normotensive and hypertensive animals and NO playing little or no role with E2 treatment. These studies illustrate the complexities of the regulation of vascular tone when animals possess enhanced cardiovascular risk with hypertension and are undergoing estrogen replacement treatment. The significance of these findings for hypertensive menopausal women receiving hormone replacement therapy (HRT) is threefold. First, there are beneficial effects from HRT increasing NO, which opposes the vasoconstrictor action of specific agonists, namely ANG II and PE. Second, in the case of 5-HT, there was a HRT beneficial effect, but it was not mediated through NO but most likely through the downregulation of the 5-HT receptor. Finally, the preexisting condition of hypertension specifically is detrimental for 5-HT vasoconstrictor effects.

The inhibition of ANG II-induced contraction following chronic estrogen treatment is consistent with the reports in ovarietomized rats (2) and sheep (22) treated with estrogen. These findings are consistent with our finding of a downregulation of the AT1 receptor gene expression by estrogen in the aorta from SD and TG rats and similar findings reported by Nickenig et al. (21). Cheng and Gruetter (2) studied the ANG II response in rat aortic vessels with and without the endothelium and showed that in the presence or the absence of the endothelium there were markedly attenuated ANG II responses with the magnitude of difference being greater in the endothelium-denuded vessels. In our study, we used NOS inhibition to remove the NO modulation. Similar to the studies of Cheng and Gruetter (2), we found that chronic E2 treatment reduced the ANG II responses, and we were able to show that this occurred with or without the blockade of NOS. The increase of the ANG II response after NO inhibition reveals the contribution of NO as a counterbalancing modulator. In the ovariecotomized, P-treated SD rats, the modulation by NO of the ANG II response was not observed (at ANG II concentrations of $10^{-8}$ and $10^{-6}$ M), suggesting little or no role for NO modulation in ANG II-mediated responses when estrogen is absent. These studies reveal that there are at least two components contributing to the depression of the ANG II response with E2 treatment, i.e., the downregulation of the AT1 receptor and increased contribution of NO. It is likely that the increase in NO originated from the upregulation of eNOS by estrogen; however, our studies do not eliminate a possible contribution of inducible NOS, which has been shown to be present in endothelial and vascular smooth muscle cells (13) and would be inhibited by L-NAME and regulated by estrogen (13, 20). The contribution of other endothelial factors that may be upregulated and contribute to the attenuated ANG II response was not tested in these studies. In our study, the presence of hypertension did not significantly alter the pattern or magnitude of ANG II responses. Previous studies from our group (4) and others (24) reported that chronic estrogen treatment decreases serum and tissue ACE activity and downregulates tissue ACE mRNA. In the present study, we showed that E2 treatment suppressed ANG I-induced vascular constriction. Because the ANG I response is mediated by the AT1 receptor after its conversion to ANG II, both the downregulation of the AT1 receptor and the NO modulation of this response are components of this response. ACE inhibition of ANG I, however, acts more proximally to the ANG II response and illustrates that the downregulation of ACE by estrogen may contribute to blood pressure reduction.

![Graph](image)

**Fig. 6.** Effects of E2 and L-NAME treatment on phenylephrine-induced vascular reactivity in SD (top) and TG (bottom) rats. See Table 2 for statistical comparisons. Each group includes 10–16 aortic rings.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50, nM</th>
<th>Maximal Contraction, %KCl</th>
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<tbody>
<tr>
<td>SD + P</td>
<td>91.76 ± 5.74</td>
<td>111 ± 13.1</td>
</tr>
<tr>
<td>SD + P + L-NAME</td>
<td>62.29 ± 5.14</td>
<td>169 ± 7.5*</td>
</tr>
<tr>
<td>SD + E2</td>
<td>83.23 ± 5.75</td>
<td>106 ± 8.7</td>
</tr>
<tr>
<td>SD + E2 + L-NAME</td>
<td>22.81 ± 5.14</td>
<td>167 ± 13.7*</td>
</tr>
<tr>
<td>TG + P</td>
<td>91.27 ± 6.06</td>
<td>101 ± 4.8</td>
</tr>
<tr>
<td>TG + P + L-NAME</td>
<td>55.61 ± 4.14</td>
<td>142 ± 10.5*</td>
</tr>
<tr>
<td>TG + E2</td>
<td>75.56 ± 4.75</td>
<td>113 ± 5.1</td>
</tr>
<tr>
<td>TG + E2 + L-NAME</td>
<td>37.20 ± 3.04</td>
<td>161 ± 5.3*</td>
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Values are means ± SE; each group includes 10–16 aortic rings. *P < 0.05 for L-NAME treatment (SD + P vs. SD + P + L-NAME, SD + E2 vs. SD + E2 + L-NAME, TG + P vs. TG + P + L-NAME, and TG + E2 vs. TG + E2 + L-NAME).
Reports on estrogen effects on the responses to PE or norepinephrine have been somewhat controversial. Estrogen has been reported to downregulate, upregulate, and cause no change of adrenergic receptor-mediated responses (2, 7, 30, 37). Whether estrogen attenuates or potentiates the pressor/vasoconstrictor response to PE/norepinephrine may be dependent on the vascular beds studied and the duration and magnitude of estrogen treatment. In our study, we observed that the in vitro response to PE in rat aortic vessels was not influenced by the E2 treatment in SD or TG hypertensive rats. Similar findings to PE were reported in spontaneously hypertensive rats (33). The blockade of NOS revealed that NO played a major role in depressing the PE response since the maximal responses were enhanced in both SD and TG rats. Zhang and Davidge (37) found that N\textsuperscript{\textalpha}-monomethyl-L-arginine significantly increased the sensitivity to PE in mesenteric vessels from both ovariectomized and estrogen-replaced SD rats. Because there was no difference in the L-NAME-treated vessels whether estrogen was present or not, our data would suggest that the \alpha-adrenergic responses in aortic vessels comprised a strong opposing modulatory influence by NO, regardless of the presence or absence of estrogen.

Previous studies have suggested that abnormal hyperresponsive vascular function can be detected relatively easily with the use of 5-HT under conditions of atherosclerosis, hypertension, and endothelial damage (9, 15). Further confirmation of this statement is observed in our study, where TG rats had an augmented maximal vasoconstrictor response to 5-HT compared with that of normotensive rats. The findings between the SD and TG groups with P and E2 treatment were confirmed when the EC\textsubscript{50} was used, but its use additionally showed that TG animals treated with L-NAME had reduced EC\textsubscript{50} compared with that of the SD animals. Overall, our results when comparing the maximal responses and the EC\textsubscript{50} are similar; however, it is known that the EC\textsubscript{50} value is more sensitive in demonstrating effects. An endothelial component to the 5-HT response has been suggested by studies that demonstrate an enhanced contraction to 5-HT in denuded vessels (23) and in carotid arteries from female eNOS knockout mice compared with wild-type mice (14). Another possibility is that the changes in vascular reactivity in the (mRen2)27 rat are due to changes in reactive oxygen species production, which have been demonstrated to be elevated in the (mRen2)27 rat (34). In our study in aortic vessels from P-treated SD and TG rats, there was NO modulation of the 5-HT response, consistent with endothelial NO attenuating the 5-HT response. Yet with the estrogen treatment, there was no evidence of NO modulation of the 5-HT response in either the SD or TG rats. This lack of an effect of NO modulation with estrogen treatment was unexpected, especially in light of the increase in NOS expression with E2 treatment and the presence of NO modulation of 5-HT in the P-treated animals. Estrogen treatment, however, attenuated the constrictor response to 5-HT in the presence and absence of NO inhibition. The attenuation of the 5-HT response with estrogen treatment has been previously described in rabbit basilar artery segments (3, 27), human internal mammary arteries (19), and porcine coronary arteries (31). Taken together, these latter findings would be consistent with estrogen downregulating the 5-HT receptor, altering 5-HT signal transduction, or augmenting NO release, although the contribution of other endothelium-derived relaxing factors has not been eliminated.

In these studies, one would have anticipated that since HRT elevated NOS, its effects on all of the responses of the agonists would have been comparable. Yet this was not the case, and this may be due in part to the multiple steps modifying any agonist-mediated response. For example, it appears that HRT can downregulate the receptor of the agonist, which was demonstrated for the AT\textsubscript{1} receptor and is consistent with our 5-HT responses. In the case of ANG II, HRT acts through multiple pathways in both NOS upregulation and downregulation of its receptor to achieve its beneficial effects. It is unclear why the preexisting condition of hypertension, which entails long-term vascular compliance changes and endothelial damage, only augmented the 5-HT responses. Overall, the specific modification of each agonist response indicates why it is difficult to predict whether HRT will be beneficial or detrimental to hypertensive postmenopausal women.

One limitation of the studies conducted is that aortic vessels were used rather than resistance vessels such as the mesenteric and femoral artery. It is entirely possible that the prehypertensive effects of the agonists and their interaction with estrogen and NO may more readily be observed at the level of the resistance vessels. Finally, the dose of estrogen used in these studies is within the range that would be found with pregnancy or hormone replacement rather than the lower levels found during the normal estrous cycle.

In conclusion, these studies demonstrated that only 5-HT vasoreactive responses are markedly augmented by the preexisting condition of hypertension. In addition, although the studies confirmed that NOS is upregulated with estrogen treatment, they revealed that the contribution of NO as a factor contributing to the relative refractoriness of the vascular responses is dependent on the nature of the vasoconstrictor agonists and/or the presence of hypertension. In the case of PE and ANG II, the NO contribution to the attenuated response appeared to be unaffected by estrogen treatment, whereas the NO contribution to 5-HT was only found with P treatment. Estrogen attenuated the 5-HT response without a significant contribution of NO. These studies illustrate the complexities of regulation of vascular tone when animals possess enhanced cardiovascular risk with hypertension and are undergoing estrogen replacement treatment.

GRANTS

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