Effect of Estrogen Replacement on Vasoconstrictor Responses in Rat Mesenteric Arteries

Yunlong Zhang, Sandra T. Davidge

Abstract—Recent studies have shown that estrogen can increase endothelial nitric oxide synthase expression and/or activity and that nitric oxide may play a role in attenuating vasoconstrictor responses. Yet there are still controversies in this field. Our hypothesis was that the role of nitric oxide in modulating vasoconstrictor responses in estrogen-replaced animals depends on the agonist. The aim of the study was to determine the effect of long-term estrogen replacement on vascular reactivity of resistance-sized mesenteric arteries in ovariectomized rats with the use of a variety of vasoconstrictors. Female Sprague-Dawley rats were ovariectomized at 11 weeks of age. 17β-estradiol pellets (0.5 mg/pellet) were implanted in the estrogen-replaced group (n=9) for 4 weeks; placebo pellets were used in the ovariectomized group (n=10). Resistance-sized mesenteric arteries were dissected and mounted onto a dual-chamber arteriograph system. Estradiol replacement did not alter the response of mesenteric arteries to either arginine vasopressin or nitroprusside or the thromboxane mimetic U46619. Inhibition of nitric oxide synthase with Nω-monomethyl-L-arginine (100 μmol/L) did not modulate these vasoconstrictor responses in either group of rats. In contrast, the dose-response curve of the adrenergic agonist phenylephrine was significantly attenuated for the estradiol-replaced rats compared with the ovariectomized group (EC50=0.90±0.17 vs 0.44±0.08 μmol/L, P<0.05). After incubation with Nω-monomethyl-L-arginine, the EC50 of phenylephrine significantly decreased in both groups, but a significant difference remained between the 2 groups (EC50=0.41±0.08 vs 0.28±0.02 μmol/L, P<0.05). Importantly, Western immunoblotting demonstrated that the expression of α1-adrenergic receptors was significantly suppressed by estradiol replacement. We conclude that estrogen may have a specific effect on adrenergic vasoconstriction by modulating its receptors. (Hypertension. 1999;34:1117-1122.)

Key Words: steroids ■ nitric oxide ■ receptors, adrenergic, alpha ■ vasopressin ■ thromboxanes

Epidemiological studies have shown that the incidence of cardiovascular disease in premenopausal women is lower than that in men and postmenopausal women.1,2 This is believed to be due to the protective effect of circulating estrogen because estrogen replacement therapy significantly reduces the incidence of cardiovascular disorders in postmenopausal women.3,4 The vascular wall has been shown to contain specific high-affinity receptors for estrogen both in humans5,6 and in animals,7,9 providing strong biological evidence that the cardiovascular system is one of the targets of estrogen. However, the exact mechanisms of the protective effect of estrogen are still unknown. Estrogen treatment in animal models increases systemic and uterine blood flow10–13 and attenuates the pressor response to phenylephrine (PE) in mesenteric14 and aortic arteries.15 Many of these studies have implicated nitric oxide (NO) as the mediator of the altered vascular response resulting from estrogen treatment. This theory is supported by the data showing that estrogen can induce endothelial NO synthase (eNOS) expression and increase eNOS activity.16,17

However, there are still controversies in this field. Although some studies have demonstrated that long-term treatment with estrogen enhances NO-dependent modulation of vascular function,11,13,14 other studies do not concur.18 Further, the specificity of the vasoconstrictor studied may influence the role of NO in modulating vascular response. The aim of the current study was to investigate the effect of long-term estrogen replacement on vascular reactivity of resistance-sized mesenteric arteries in ovariectomized rats with the use of a variety of vasoconstrictors including PE, an α1-adrenergic receptor agonist; arginine vasopressin (AVP), a neurohypophyseal hormone; and U46619, a thromboxane mimetic. The effect of NO to modulate the vasoconstrictor responses was also tested by use of the NOS inhibitor Nω-monomethyl-L-arginine (L-NMMA).

Methods

Animal Model

Female Sprague-Dawley rats were obtained at 10 weeks of age (weight range 200 to 225 g) from Harlan, USA. All animals were allowed to recover from the trip and acclimatize to the university facility for 1 week. Ovariectomy was performed following the university standard procedure. At the time of ovariectomy, one group of rats (n=9) received a 17β-estradiol pellet (0.5 mg/60-day release,
Innovative Research of America) subcutaneously; the control group (n = 10) received a placebo (Innovative Research of America). After 4 weeks, the rats were euthanized under light anesthesia with methohexital sodium (50 mg/kg body wt). Blood samples were collected from the right atrium, allowed to clot, and centrifuged at 3000 rpm for 10 minutes. Sera were stored at −80°C for later assay of serum 17β-estradiol levels. The animal protocols were examined by the University of Alberta Animal Welfare Committee and found to be in compliance with the guideline issued by the Canada Council on Animal Care.

Vessel Preparation and Equipment
A section of the mesentery 5 to 10 cm distal to the pylorus was rapidly removed and placed in ice-cold N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid]–buffered physiological saline solution (HEPES-PSS). The composition of HEPES-PSS was as follows (in mM/mL): NaCl 142, KCl 4.7, CaCl 1.56, KH2PO4 1.18, HEPES 10, and glucose 5.5. Resistance-sized mesenteric arteries were dissected from the fat tissue transferred to a dual-chamber arteriograph (Living Systems Instrumentation). The proximal end of an artery was tied to the glass cannula of the arteriograph, and with the use of a Servo pump, arteries were gently flushed with HEPES-PSS buffer to remove residual blood. The distal end of the artery was then mounted to the second glass cannula. Intraluminal pressure was gradually increased to 75 mm Hg to approximate the in vivo pressure of the arteries. All arterial measurements, including inner diameter and wall thickness, were collected by a video camera mounted on the microscope, a dimension analyzer (Living Systems Instrumentation), and a monitor.

Experimental Protocol
The mesenteric arteries were equilibrated in warm (37°C) HEPES-PSS buffer for 30 minutes at the intraluminal pressure of 75 mm Hg. Prestretching of the arteries was achieved by increasing the intraluminal pressure from 75 to 100 mm Hg and immediately returning it to 75 mm Hg. This pressure was maintained throughout the experiment. The arteries were equilibrated for another 30 minutes. All the experiments were started with the dose-response of 1 of 3 vasoconstrictors in the absence of the NO inhibitor. Cumulative doses of AVP (0.1 nmol/L to 100 nmol/L), U46619 (1 nmol/L to 100 μmol/L), or PE (0.1 to 10 μmol/L) were conducted. Only one type of vasoconstrictor was applied to any one mesenteric preparation. With a 30-minute interval and 3 washes, the arteries were then incubated with L-NMMA (100 μmol/L) for 15 minutes. This concentration of L-NMMA has been shown to be an effective inhibitor of eNOS.19 In addition, we tested our mesenteric arteries with a dose-dependent vasoconstriction to L-NMMA (3 to 300 μmol/L). Maximal constriction response occurred with 100 μmol/L, with no further effect with higher doses of L-NMMA. The arterial diameter was taken immediately before and at the end of the L-NMMA incubation period to determine the effect of L-NMMA on vasoactivity at basal condition. Vasoconstrictor dose responses were then repeated in the presence of L-NMMA. The reproducibility of repeating curves had been determined in preliminary experiments.

The vessel diameter changes in response to the different doses of vasoconstrictors were normalized to the maximal constriction of each vasoconstrictor (AVP, U46619, and PE) in each experiment.

Western Immunoblot
Mesenteric arteries were dissected free of surrounding adipose tissue and homogenized. Protein concentrations were determined with the use of the method of Bradford.20 Western immunoblot procedures were conducted as previously described in detail.21 Samples containing 4.6 μg of protein were loaded on 10% polyacrylamide gels, and α-adrenergic receptor expression was evaluated. Because the α1-adrenergic receptor subtype is the predominant type in mesenteric arteries,22 we used polyclonal antibodies to α1-adrenergic receptors (1:1000; Santa Cruz Biotechnology, Inc).

Radioimmunoassay
Rat serum 17β-estradiol was assayed with the materials and methods provided by Diagnostic Products Corp. The limit of detection for the assay was 1.4 pg/mL. In this experimental period, the interassay variability and intra-assay variability were 2% and 2.7%, respectively, for the 2 sets of radioimmunoassays.

Data Analysis and Statistics
The data from 3 vasoconstrictor dose-response curves were fitted to the Hill equation, from which a straight line was generated by linear least regression analysis. The effective concentration that produced a 50% response (EC50) was determined from this line and expressed as the geometric mean±SE. ANOVA with the post hoc Tukey’s test was used to determine statistical difference among the groups. Data were considered significantly different at values of P<0.05.

Results
Animal Model
As anticipated, there was a significant increase in plasma 17β-estradiol levels in the estradiol-replaced rats that were in the physiological range of cycling proestrus and pregnancy levels. Uterine weight, which provides a biological marker of estrogen replacement, was also significantly elevated in the estradiol-replaced group (Table).

Basal Conditions
The inner diameters of unpressurized (0 mm Hg) arteries were not significantly different between ovarietomized control (n = 10) and estradiol-replaced (n = 9) rats (205 ± 16.4 and 182 ± 7.95 μm, respectively). In addition, the diameters of the arteries at 75 mm Hg in fully relaxed arteries (ie, inactivation of vascular smooth muscle with papaverine and removal of extracellular calcium from the buffer) were not different in ovarietomized rats compared with estradiol-replaced animals (306 ± 24.8 and 337 ± 13.2 μm, respectively). For the experimental basal condition with HEPES-PSS buffer, there was also no difference in vessel diameter at 75 mm Hg between the 2 groups (307 ± 12.8 and 316 ± 15.8 μm, respectively). However, after L-NMMA incubation, arteries from ovarietomized rats constricted significantly (307 ± 12.8 vs 294 ± 18.2 μm, NS). These data suggest that there is a higher basal level of NO in the ovarietomized control animals that may reflect a compensatory mechanism to ovarietomy.

Vessel Responses to AVP and U46619
There was no difference in either the sensitivity or the maximum constriction to AVP (Figure 1) or U46619 (Figure 2) between the estradiol-replaced and ovarietomized rats. L-NMMA did not modulate the vasoconstrictor responses of the arteries from either group.
Arterial Response to PE
In contrast to the vasoconstriction responses of AVP and U46619, the vasoconstrictor response to PE was significantly altered by long-term replacement of estrogen in ovariectomized rats. The EC$_{50}$ for PE was significantly greater in the arteries from estradiol-replaced animals than that from ovariectomized animals (EC$_{50}$ = 0.90 ± 0.17 vs 0.44 ± 0.08 μM, P < 0.05, Figure 3A); however, the maximal constriction did not differ between the 2 groups (Figure 3B). After incubation with L-NMMA, the maximal constriction was enhanced and the EC$_{50}$ of PE was significantly decreased in both groups (Figure 3A). The extent of the shift of EC$_{50}$, however, was significantly greater (P < 0.05) in the estradiol-replaced animals compared with that from ovariectomized control animals. This observation indicated that the NO pathway was partly involved in the decreased sensitivity to PE in estradiol-replaced animals. Nevertheless, a significant difference remained between the 2 groups after NOS inhibition (EC$_{50}$ = 0.41 ± 0.08 vs 0.28 ± 0.02 μmol/L, P < 0.05).

Western Immunoblot of α$_{1}$-Adrenergic Receptor
Because a significant difference between ovariectomized and estradiol-replaced rats occurred only with adrenergic vasoconstriction, α$_{1}$-adrenergic receptor density was evaluated with the use of Western blot analysis. Figure 3C is a representative immunoblot for α$_{1}$D-adrenergic receptors in the mesenteric arteries of ovariectomized (lanes 1 to 3) and estrogen-replaced (lanes 4 to 8) rats. A major immunoreactive protein band was observed at ~60 kDa from arteries of the ovariectomized rats. The expression of the α$_{1}$-receptors was suppressed below detectable limits in the estradiol-replaced group.

Discussion
Our study focused on the vascular responses to different receptor-mediated vasoconstrictors and the possible involvement of the NO pathway in modulating the response in estradiol-replaced ovariectomized rats. Physiological levels of estradiol attenuated vasoconstriction only in response to an adrenergic agonist (PE). Two other vasoconstrictors, AVP and U46619, were not affected by estradiol replacement in the rat. This difference may occur at the receptor level. Indeed, in this study, we show that α$_{1}$-adrenergic receptor
expression is decreased in the estradiol-replaced rats compared with the ovariectomized animals.

In our study, the 3 vasoconstrictors (AVP, U46619, and PE) were chosen on the basis of their physiological roles in vivo. AVP is a neurohypophyseal hormone that induces vasoconstriction through V1 receptors. In contrast to our initial hypothesis, estradiol replacement in ovariectomized rats did not alter the vascular response to AVP, nor was the NO pathway involved. Information regarding this issue is controversial. One study demonstrated that long-term estrogen replacement in ovariectomized rats attenuated the pressor response to AVP in vivo. In contrast, another study showed that a short-term (1-day treatment) estradiol replacement increased the AVP pressor response in ovariectomized rats in vivo, and this was accompanied by an increase in the density of AVP binding sites.

However, Martinez et al. showed that denuded endothelium of human omental arteries had no effect on vessel contraction to AVP. In our study, NOS inhibition did not alter mesenteric arterial response in the ovariectomized or estradiol-replaced rats. It appears that data vary, depending on the animal model and vascular bed studied, with the mechanisms of all these complexities remaining unknown.

U46619 is a vasoconstrictor that acts through the thromboxane/PGH2 receptor. In our study, the vascular response to U46619 in the estradiol-treated group did not differ from the ovariectomized rats. These data are consistent with a report from Miller and Vanhoucke showing no effect of estrogen on modifying vascular responses to U46619 in rabbit aorta. However, previous studies have demonstrated that the effect of estrogen to modify U46619 is very complex. The maximal response of coronary and renal arteries to U46619 was higher in male dogs than in female dogs. Tamoxifen, an antagonist of estrogen, increased the pressor response to U46619 in the female dogs, and estrogen treatment in the male dogs decreased the pressor response. However, in the perfused rat lung, ovariectomy decreased the pulmonary pressor responsiveness to U46619, whereas 17β-estradiol treatment induced an increased pressor responsiveness in a dose-dependent manner to U46619. Similarly, estrogen also potentiated the responsiveness of mesenteric arteries from male rats to U46619. Overall, the data indicate that U46619 is a vasoconstrictor for the coronary, renal, mesenteric, and pulmonary vasculature. However, whether estrogen attenuates or potentiates the pressor response to U46619 may be dependent on the gender and vascular bed studied. In these previous studies, however, the role of NO was not tested. Very recently, a study demonstrated that estrogen replacement decreases guinea pig coronary artery contractility to U46619 through NO. However, this effect only occurred with the lowest dose of estrogen (0.25 mg/pellet), but not in the doses of 0.5, 1.5, and 7.5 mg/pellet. These data are contradictory to our study, but the different species and vascular beds studied may explain the different observation. In addition, the doses of estrogen replaced may be a factor in the difference, as demonstrated by the lack of effect at the higher doses of estrogen in their study. Serum estradiol levels were not reported, nor was the physiological relevance of the estradiol dose in the guinea pig discussed. In our study, estradiol replacement achieved a physiological range of pro-estrus to pregnant levels for the rat, yet there was no significant effect on the NO pathway for modulating the response to U46619.

PE is an α1-adrenergic receptor agonist, and adrenergic receptors serve a primary role in the maintenance of arterial resistance. A previous study has shown that the maximal pressor response to PE is greatly attenuated in aortas from female rats as compared with that from male rats. In addition, 17β-estradiol has been shown to significantly decrease the contractile response to PE in aortic rings of male rats. Although our results do not demonstrate an effect of estrogen to blunt the maximal response to PE, our data as well as that of Meyer et al. show that long-term replacement of estrogen significantly blunted the adrenergic sensitivity of mesenteric arteries.
The role of NO in modulating the blunted sensitivity to adrenergic vasoconstriction has been evaluated in estradiol-replaced rats. NOS inhibition with Nω-nitro-l-arginine (L-NNA) significantly enhanced the vasoconstrictor response in only the estradiol-replaced rats in the study of Meyer and coworkers. In our present study, L-NMMA significantly increased the sensitivity in both the ovariectomized and estradiol-replaced groups. Although the extent of increased sensitivity to PE was greater in estradiol-replaced animals, there remained a significant difference between the groups. These data suggest that the NO pathway was only partly involved in the mechanisms of blunting the adrenergic response in our model of estradiol-replaced rats.

We next tested whether the expression of α1-receptors was reduced because of estrogen. A previous study had shown that estrogen decreased α1α-adrenergic receptor expression in the central nervous system; however, little is known about the modulation of adrenergic receptors by estrogen in the resistance vascular system. Our data clearly indicate a suppression of α1α-adrenergic receptors in the mesenteric arteries of the estradiol-replaced rats compared with placebo-controlled ovariectomized rats. Because the α1α-adrenoceptor is a major regulator of vascular function, these data represent an important mechanism for vascular control. Indeed, increased levels of cardiac α1-receptors have been reported in spontaneously hypertensive rats. Further, the maximal binding of α1α-adrenoceptors in cardiac ventricles has been found to be greater in male than in female Dahl rats. Interestingly, these authors observed that there was a greater variability in maximal binding in the female rats that may have been related to the estrous cycle. Our data demonstrate a novel role of estradiol on α1α-adrenergic expression. Further studies specifically to address the effect of estrogen on binding capacity and molecular regulation are necessary.

In summary, estrogen modulation of vasoconstriction only occurred with an adrenergic vasoconstrictor, which was, in part, mediated by NO. Further, α1α-adrenergic receptor expression was reduced in the mesenteric arteries of the estradiol-replaced rats. AVP and U46619, 2 other receptor-mediated vasoconstrictors, were not affected by estrogen nor by the NO pathway. We conclude that estrogen may have a specific effect to modulate adrenergic vasoconstriction by modulating its receptors.

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References


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