Endogenous estrogen mediates vascular reactivity and distensibility in pregnant rat mesenteric arteries

YUNLONG ZHANG, KEN G. STEWART, AND SANDRA T. DAVIDGE
Perinatal Research Centre, Department of Obstetrics and Gynecology, and Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Received 15 June 2000; accepted in final form 15 September 2000

ZHANG, Yunlong, Ken G. Stewart, and Sandra T. Davidge. Endogenous estrogen mediates vascular reactivity and distensibility in pregnant rat mesenteric arteries. *Am J Physiol Heart Circ Physiol* 280: H956–H961, 2001.—The role of estrogen in the maternal systemic cardiovascular adaptations during pregnancy is still controversial. Female Sprague-Dawley rats were implanted at day 14 of pregnancy with either a 50-mg tamoxifen pellet (estrogen receptor blocker, n = 10) or placebo pellet (n = 10). Virgin female rats were a nonpregnant control (n = 7). At days 20–22 of pregnancy, resistance-sized mesenteric arteries were mounted onto a dual-chamber arteriograph system. Pregnancy significantly blunted the pressor response to phenylephrine [measurement of the effective concentration that yielded 50% maximum response (EC50) values were 1.5 ± 0.22 vs. 0.69 ± 0.16 μM (P < 0.05)] and enhanced vasodilation to ACh [EC50 = 1.13 ± 2.53 vs. 3.13 ± 6.04 nM (P < 0.05)] compared with nonpregnant rats. However, tamoxifen treatment during pregnancy reversed these effects. Inhibition of nitric oxide (NO) synthase with Nω-monomethyl-L-arginine (250 μM) shifted only the responses of the placebo-treated pregnant group to both phenylephrine and ACh. Arterial distensibility in the placebo-treated pregnant group was also significantly increased (P < 0.05) compared with nonpregnant and tamoxifen-treated pregnant animals. In summary, endogenous estrogen during pregnancy increases NO-dependent modulation of vessel tone and arterial distensibility.

DURING PREGNANCY, there are a number of well-described changes in the cardiovascular system that include significant increases in cardiac output and blood volume with a profound reduction in peripheral vascular resistance (6, 34). There is a substantial reduction inpressor responsiveness to exogenously administered vasconstrictors (40) during normal pregnancy as well as a remodeling of the vasculature that results in increased distensibility (33). However, the mechanisms that could account for such vascular modifications during normal pregnancy remain poorly understood.

Increased production of vasodilators such as nitric oxide (NO) have been implicated in pregnancy-associated adaptations. Indeed, NO metabolites are elevated during gestation in the rat (8), and endothelial-derived NO-dependent relaxation is augmented in women (7). NO is known to affect vascular remodeling as well (2, 18, 35). Although these data suggest that NO alters the vasculature during pregnancy, the stimuli and precise mechanisms responsible for altered vascular properties in pregnancy are not known.

Altersations in the cardiovascular system during normal pregnancy are likely related to endocrinological changes. Estrogen is proposed to be a potential mediator of pregnancy-induced vascular changes given its established effects on the vasculature and tremendous rise in concentration during pregnancy. 17β-Estradiol has been shown to increase protein levels of endothelial NO synthase (eNOS) and NO production from cultured bovine aortic endothelial cells (19, 23). In addition, eNOS protein expression in the uterine artery endothelium is positively correlated with estrogen levels in cycling and estrogen-replaced ovariectomized ewes (37). Pregnancy has been found to increase eNOS mRNA in the rat aorta (17) and increase eNOS protein expression in sheep uterine and omental artery endothelia (27). Although these data demonstrate that estrogen and pregnancy influence the NOS pathway, the direct effect of endogenous estrogen on NO-dependent modulation of vascular function during pregnancy has not been demonstrated.

Information is limited as to the mechanisms through which estrogen alters vascular function during pregnancy. Studies to date have focused on the exogenous administration of estrogen to nonpregnant animals where estrogen levels approximate those of pregnancy. The results have been conflicting with some reports of attenuation of pressor responsiveness (15, 25, 26, 43) and others showing no effect (9). The conflicting data could be due to methodological differences in steroid doses, acute versus chronic administration, length of ovariectomy before steroid administration, and vascular bed heterogeneity. Therefore it is still unclear whether elevated estrogen levels during pregnancy modulate vascular function. Unlike previous studies of exogenous estrogen administration, our study was designed to inhibit endogenous estrogen during pregnancy. Our hypothesis was that the high estrogenic

**Address for reprint requests and other correspondence:** S. T. Davidge, Perinatal Research Centre, 220 HMRC, Univ. of Alberta, Edmonton, Alberta T6G 2S2, Canada (E-mail: sandra.davidge@ualberta.ca).
state in pregnancy affects cardiovascular adaptations through modulation of both vascular reactivity and remodeling.

METHODS

Animal model. Female Sprague-Dawley rats were obtained at age 10 wk with a weight range of 200–225 g (Charles River, Quebec, Canada). The animals were bred in our own colony, and each morning vaginal smears were examined microscopically. The presence of sperm was considered day 1 of gestation (term = 22 days). Rats were implanted at day 14 of pregnancy with either a 50-mg pellet of the estrogen receptor blocker tamoxifen (Innovative Research of America; n= 10) or a placebo pellet (n= 10). Tamoxifen was chosen based on previous literature that demonstrated its effectiveness in decreasing plasma estrogen levels and inhibiting pregnancy-related adaptations (oxytocin synthesis) without affecting fetal viability (13). Tamoxifen is also known to be an effective antagonist in the presence of high levels of estrogen such as occurs in pregnancy (21). A group of virgin female rats were used as a nonpregnant control (n= 7). Experiments were performed at days 20–22 of pregnancy. The rats were killed under light anesthesia with methohexital sodium (50 mg/kg body wt). Blood was collected by atrial puncture, and plasma 17β-estradiol levels were measured using a radioimmunounassay kit (Diagnostic Products). The animal protocols were examined by the University of Alberta Animal Welfare Committee and found to be in compliance with the guidelines issued by the Canada Council on Animal Care.

Vessel preparation and equipment. A section of the mesentery 5–10 cm distal to the pylorus was rapidly removed and placed in ice-cold HEPES-buffered physiological saline solution (PSS). The composition of HEPES-PSS was as follows (in mM): 142 NaCl, 4.7 KCl, 1.17 MgSO4, 1.56 CaCl2, 1.18 KH2PO4, 10 HEPES, and 5.5 glucose. Resistance-sized mesenteric arteries were dissected from the fat tissue and transferred to a dual-chamber arteriograph (Living Systems Instrumentation, Burlington, VT). The proximal end of the artery was tied to a glass cannula of the arteriograph, and using a servo pump the artery was 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 mmHg 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 min at an intraluminal pressure of 75 mmHg. Prestretching of the arteries was achieved by increasing the intraluminal pressure from 75–100 mmHg and immediately returning it to 75 mmHg. This pressure was maintained throughout the experiment period. The arteries were equilibrated for 30 min after the prestretch.

Four experiment protocols were used: 1) phenylephrine (PE)-induced vasoconstriction, 2) endothelium-dependent vasodilation to ACh, 3) endothelium-independent vasodilation to sodium nitroprusside (SNP), and 4) distensibility measurements performed using Ca2+-free HEPES-PSS and papaverine (100 μM).

Cumulative doses of PE (0.1–10 μM) were conducted in the absence or presence of the NOS inhibitor Nω-nitro-L-arginine (l-NMMA, 250 μM). To verify the pharmacological inhibition of the NOS pathway, dose-response curves to l-NMMA (3 μM–30 mM) were performed in arteries preconstricted to 10% of maximum response to PE. Sequential doses of l-NMMA were then applied to the vessels and the level of constriction in response to each dose of inhibitor was recorded. Relative to the l-NMMA dose-response curve, the greatest vessel constriction was reached at concentrations of l-NMMA below that used experimentally; this suggests that NOS activity was optimally inhibited. Vasorelaxation responses were conducted on arteries that were preconstricted with the effective concentration of PE that produced 50% of the maximum response (EC50) in the absence or presence of l-NMMA. Cumulative doses of ACh (1 nM–1 μM) or SNP (1 nM–1 μM) were then applied. The reproducibility of repeating curves was determined in preliminary experiments.

Passive mechanics. To compare the distensibility of arteries, active contractile activity must be eliminated. Vascular smooth muscle activity was deactivated by papaverine (0.1 mM), and studies were conducted in Ca2+-free buffer containing 0.1 mM EGTA to remove the effect of extracellular Ca2+. Inactivation of smooth muscle was confirmed by the lack of contraction to potassium chloride (124 mM). Lumen diameter and wall thickness were measured at 11 pressures ranging from 0 to 150 mmHg. Passive pressure-diameter and wall thickness relationships were determined for the arteries. Distensibility was defined as the relative change in diameter per unit change in pressure (24). To obtain the relative change in diameter, the internal diameter of a vessel at each pressure was normalized to an initial diameter observed at 3 mmHg. This reference diameter of 3 mmHg was used because it was not possible to reliably measure arterial diameter at 0 mmHg.

Western immunoblot. Mesenteric arteries were dissected free of surrounding adipose and connective tissue and homogenized. Protein concentrations were determined using the method of Bradford (5). Western immunoblot procedures were conducted as previously described in detail (11). Samples containing 4.6 μg of protein were loaded on 8% polyacrylamide gels. Monoclonal antibodies for eNOS (Transduction) were used to evaluate expression levels in mesenteric arteries.

Data analysis and statistics. Data are summarized as the means ± SE. ANOVA was used to determine the statistical differences of the parameters among the three groups of rats shown in Table 1. The data from the dose-response curves were fitted to the Hill equation from which a straight line was generated by linear least-regression analysis. The EC50 was determined from this line and expressed as the geometric mean ± SE. ANOVA with a post hoc Tukey’s test was used to determine the statistical difference among the groups. Data were considered significantly different at values with P < 0.05.

Table 1. Animal model: nonpregnant, placebo-treated pregnant, and tamoxifen-treated pregnant Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Maternal Body Wt, g</th>
<th>Fetus Wt, g</th>
<th>Litter Size, no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>7</td>
<td>280 ± 4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant + placebo</td>
<td>10</td>
<td>379 ± 9.1a</td>
<td>2.94 ± 0.33</td>
<td>14.1 ± 1.16</td>
</tr>
<tr>
<td>Pregnant + tamoxifen</td>
<td>10</td>
<td>365 ± 7.2a</td>
<td>3.27 ± 0.27</td>
<td>15.0 ± 0.51</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. *P < 0.05 vs. nonpregnant rats.
RESULTS

Animal model. Administration of tamoxifen did not affect maternal body weight, fetus weight, or fetus number (Table 1). In agreement with Fang and colleagues (13), tamoxifen treatment in pregnant rats suppressed the plasma concentration of 17β-estradiol to levels similar to nonpregnant rats, whereas estrogen levels were much higher in the placebo-treated pregnant rats (median values of 2.3, 6.8, and 122.4 pg/ml, respectively).

Arterial response to PE. Pregnancy significantly attenuated the sensitivity of mesenteric arteries to PE, but this effect was reversed by the estrogen receptor antagonist tamoxifen (Fig. 1). After incubation with l-NMMA, the EC50 of PE was significantly decreased in the placebo-treated pregnant group (Fig. 2) eliminating any difference among the three groups. This observation indicates that the NO pathway contributes to the decreased sensitivity to PE in pregnant rats in an estrogen-dependent manner.

Arterial response to ACh. Pregnancy significantly enhanced the sensitivity of mesenteric arteries to ACh; however, this effect was blunted by the estrogen receptor antagonist tamoxifen (Fig. 3). Incubation with l-NMMA significantly increased the EC50 for ACh in the placebo-treated pregnant group (Fig. 3), which implies that estrogen and the NO pathway were involved in the increased sensitivity to ACh during pregnancy. Similar to the findings of McCulloch and Randall (28), NOS inhibition did not affect endothelium-dependent relaxation in mesenteric arteries from nonpregnant female rats.

Arterial response to SNP. There were no differences in artery sensitivity to SNP among the nonpregnant, placebo-treated pregnant, and tamoxifen-treated pregnant rats (EC50 values were 52.5 ± 15.2, 47.8 ± 3.1, and 42.0 ± 4.7 nM, respectively).

Passive mechanics. The inner diameters of unpressurized (3 mmHg) arteries were not significantly different among nonpregnant, placebo-treated, and tamoxifen-treated rats (162.5 ± 13.1, 165.4 ± 6.50, and 166.9 ± 6.57 μm, respectively; P > 0.05). At 75 mmHg
in HEPES-PSS (where myogenic tone contributes to vessel diameter), vessels from the placebo-treated pregnant, tamoxifen-treated pregnant, and nonpregnant rats displayed similar diameters (mean values were 343.9, 312.9, and 308.6 μm, respectively). However, when myogenic tone was eliminated by Ca\(^{2+}\)-free buffer, distensibility measurements were significantly greater \((P < 0.05)\) in the arteries of placebo-treated pregnant rats compared with both the nonpregnant and tamoxifen-treated pregnant animals (Fig. 4). There was no difference in vascular distensibility between the nonpregnant and tamoxifen-treated pregnant animals despite the fact that vessel wall thickness in the tamoxifen-treated group changed less in response to increasing pressure than in the nonpregnant group (Fig. 5). The decrease in vessel wall thickness in response to increasing pressure was greater in the placebo-treated pregnant group than in both the nonpregnant and tamoxifen-treated groups (Fig. 5); thus estrogen appears to mediate the regulation of arterial wall thickness and distensibility.

**Western immunoblot.** There was no significant difference in mesenteric eNOS protein expression among the nonpregnant, placebo-treated pregnant, and tamoxifen-treated pregnant groups (arbitrary densitometry values were 2.72 ± 0.17, 2.48 ± 0.24, and 2.21 ± 0.29, respectively).

**DISCUSSION**

Previous studies have suggested that estrogen increases NO-dependent relaxation during pregnancy (25) and that the estrogen receptor antagonist tamoxifen prevents a pregnancy-associated increase in NOS activity in a variety of tissues (38, 39). However, to the best of our knowledge, this is the first study to demonstrate the efficacy of endogenous estrogen on vascular function in a model of pregnancy. Estrogen, a hormone of pregnancy, has been shown to upregulate eNOS expression (19) and increase NO-mediated vasodilation (32). Accordingly, an increased level of NO-mediated vasodilation has been reported in a number of in vivo and in vitro studies during pregnancy (reviewed in Ref. 34). We therefore hypothesized that during pregnancy endogenous estrogen increases eNOS expression and NO-mediated vascular relaxation as well as stimulates vascular remodeling to increase arterial distensibility. In agreement with our hypothesis, estrogen inhibition during pregnancy precluded vascular remodeling, reduced the NO-mediated component of ACh-induced vasorelaxation, and increased the pressor response to PE. Thus endogenous estrogen is a critical factor in mediating pregnancy-induced changes to both active and passive properties of resistance arteries.

The importance of physiological vascular adaptations during pregnancy, including a generalized state of vasodilation and a depressed response to vasoconstrictors, becomes evident in conditions such as preeclampsia, a condition where the vascular response to pregnancy is impaired (10). However, the mechanisms for the hemodynamic changes in pregnancy remain relatively unknown. The elucidation of such processes could greatly enhance the treatment of vascular complications in pregnancy as well as further our understanding of the vascular system in general.

The observation that estrogen receptor antagonism did not alter eNOS expression yet suppressed NO-mediated relaxation suggests that estrogen may have been affecting the rate at which NO is scavenged rather than synthesized. Superoxide anions can scavenge NO and form the cytotoxic substance peroxynitrite (4). It has previously been demonstrated in endo-
thelial cell cultures and intact aortas that estrogen does not enhance eNOS activity but rather increases the release of bioactive NO through the suppression of superoxide anions (1, 3). Indeed, estrogen is capable of significantly reducing superoxide anion production (29) as well as upregulating superoxide dismutase (16), an enzyme that competes with NO for superoxide anions (22). Such effects are in accordance with the present findings of an NO-mediated increase in ACh-induced relaxation and a decrease in PE-induced vasoconstriction in the placebo-treated pregnant but not tamoxifen-treated pregnant group. Alternatively, estrogen may increase eNOS activity and consequently elevate NO production. Finally, it is important to note that the increased role of NO in regulating vessel function during pregnancy cannot be attributed to increased smooth muscle sensitivity because all three groups were equally responsive to the exogenous NO donor SNP.

In addition to the active regulation of vessel function, the above hypothesized mechanisms of estrogen increasing NO availability could also contribute to the vascular remodeling observed in pregnancy. Models of NOS inhibition have demonstrated that arteries become thicker and less distensible when NO levels are reduced (2, 18, 35). Thus increased NO availability may be one of the mechanisms through which estrogen reduces arterial wall thickness and increases vessel distensibility. Such effects further augment the cardiovascular adaptations that accompany pregnancy. A variety of other mechanisms may also be responsible for the estrogen-mediated effects of pregnancy on arterial wall structure. Estrogen has been found to decrease vascular smooth muscle cell proliferation in a variety of models (31, 36). The attenuation of gene expression of proteins that modulate cell metabolism is one mechanism whereby estrogen could attenuate vascular cell proliferation (14, 20). Reduced expression of adhesion molecules is another mechanism whereby estrogen may suppress the atherosclerotic processes of the endothelium and vascular smooth muscle (31, 36). The attenuation of gene expression of proteins that modulate cell metabolism is one mechanism whereby estrogen could attenuate vascular cell proliferation (14, 20).

In summary, our data demonstrate the importance of both endogenous estrogen and NO in the vascular adaptations that occur during rat pregnancy. NO was found to be a dominant factor determining the decreased pressor responses to PE and the increased sensitivity to endothelium-dependent relaxation in pregnancy. These NO-mediated effects were estrogen dependent, as the pregnancy-associated changes in vessel responsiveness were eliminated by the estrogen receptor antagonist tamoxifen. Estrogen was also responsible for the vascular remodeling associated with pregnancy because arteries from pregnant animals were less thick and more distensible than arteries from either the nonpregnant or tamoxifen-treated pregnant groups. Thus we conclude that endogenous estrogen mediates a variety of cardiovascular adaptations during pregnancy.

The authors acknowledge Cecile Phan for work with Western immunoblots. This study was supported by the Medical Research Council of Canada. S. T. Davidge is a Scholar of the Heart and Stroke Foundation of Canada and Alberta Heritage Foundation for Medical Research.

REFERENCES


4. Beckman JS, Beckman TW, Chen J, Marshall PA, and Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 87: 1620–1624, 1990.


