Estrogen Replacement Suppresses a Prostaglandin H Synthase–Dependent Vasoconstrictor in Rat Mesenteric Arteries

Sandra T. Davidge, Yunlong Zhang

Abstract—There is evidence that estrogen can upregulate nitric oxide (NO) synthase expression. There is also evidence that NO increases the activity of prostaglandin H synthase (PGHS). Our initial hypothesis was that removal of ovarian steroids would decrease endothelium/NO-dependent relaxation responses but that estrogen replacement would increase NO and PGHS activity, leading to increased vasodilation. Resistance-sized (<250 μm) mesenteric arteries from ovariectomized Sprague-Dawley rats without and with 17β-estradiol replacement (0.15 or 0.5 mg/pellet, 60-day release) for 4 weeks were studied in a myograph system. The vasodilator response to methacholine, an endothelium-dependent muscarinic agonist, was reduced in the arteries of the ovariectomized rats compared with estradiol-replaced rats. In the presence of PGHS inhibitors (meclofenamate, valeryl salicylate, and NS-398) or a thromboxane A2 (TxA2)/prostaglandin H2 (PGH2) receptor blocker (SQ-29548), there was no longer a significant difference among the groups. Contrary to our initial hypothesis, inhibition of the PGHS pathway significantly enhanced the relaxation response in the arteries from the ovariectomized rats, which was similar to the response in the arteries from estradiol-replaced rats, indicating that a PGHS-dependent vasoconstrictor had modified the response to methacholine. Confirming these data, in response to exogenous arachidonic acid, arteries from ovariectomized rats exhibited constriction, whereas the arteries from the estradiol-replaced rats exhibited vasodilation. In the ovariectomized rats, pretreatment with inhibitors of the PGHS pathway reversed the vasoconstriction to a vasodilation. In addition, the vasoconstrictor response to the thromboxane mimetic U-46619 as well as PGH2 was enhanced in endothelium-denuded arteries from the ovariectomized rats compared with the estradiol-replaced rats. These data demonstrate that removal of ovarian steroids increased endothelium-mediated PGHS-dependent vasoconstriction that was associated with augmented sensitivity of the TxA2/PGH2 receptor. Chronic estrogen replacement in the ovariectomized rat suppressed this PGHS-dependent vasoconstrictor response. (Circ Res. 1998;83:388-395.)

Key Words: estrogen, vascular ■ endothelium ■ nitric oxide ■ prostaglandin

There is an inherent vascular protection in women that is affected by changes in their hormonal milieu. The incidence of cardiovascular disease is markedly lower in cycling, premenopausal women and estrogen-replaced postmenopausal women than in men or untreated postmenopausal women.1 Although it is clear that estrogen replacement has a protective effect on vascular function, the biological basis for this is unclear.

A major regulation of vascular tone is through release of endothelium-derived vasoactive agents, such as nitric oxide (NO) and prostaglandin H synthase (PGHS)-dependent products.2 NO is a potent vasorelaxant that also inhibits platelet aggregation,3,4 whereas PGHS-dependent products act as relaxing and contracting factors as well as regulators of platelet aggregation.5,6

NO is synthesized by oxidation of a guanidino nitrogen of l-arginine in a reaction catalyzed by the enzyme NO synthase.3,4 Reports of elevated protein levels of endothelial NO synthase as well as increased NO production in response to 17β-estradiol have led to the speculation that the beneficial effects of estrogen are probably due to NO.7,8 However, the effect of estrogen to be cardioprotective is likely more complex and involves numerous regulatory systems.

PGHS-dependent products are known to have important physiological and pathophysiological roles in vascular function. PGHS is the enzyme that converts arachidonic acid to prostaglandin endoperoxide H2 (PGH2). There are 2 known PGHS isoforms, PGHS-1 and PGHS-2, with both being reported in endothelial cells.9 Cell-specific isomerization or reduction of PGH2 then occurs, yielding the major biological eicosanoids, such as prostacyclin and thromboxane (Tx).5,6

The functional effect of estrogen regarding the PGHS-dependent pathway is not clear, with reports of no effect10–12 or of a vasorelaxer component13 that modulates adrenergic vasoconstriction. In addition, the influence of NO on the PGHS pathway is still emerging. In cultured endothelial cells,
we have reported that NO activates PGHS.14 Another study has reported an in vivo regulation of PGHS-2 by NO.15 Consequently, the effect of estrogen on the NO pathway may have a role in increasing PGHS-dependent relaxation, thereby conferring a protective action on the vascular system.

Our hypothesis for the present study was that removal of ovarian steroids would decrease endothelium/NO-dependent relaxation responses but that estrogen replacement would increase NO and PGHS activity, leading to increased vasodilation.

Materials and Methods

Animal Model

Female Sprague-Dawley rats were obtained from Harlan, Indianapolis, Ind, at 10 weeks of age. One week later, ovarioectomy was performed. At the time of ovarioectomy, 2 groups of rats received a 17β-estradiol pellet (0.15 or 0.5 mg, 60-day release, Innovative Research of America) subcutaneously. Another group received a placebo (Innovative Research of America). After 4 weeks, the rats were killed under light anesthesia with methohexital sodium (50 mg/kg body wt), and blood was collected by heart puncture for measurement of estradiol levels.17

An experimental design was performed. At the time of ovariectomy, 2 groups of rats received a 17β-estradiol pellet (0.15 or 0.5 mg, 60-day release, Innovative Research of America) subcutaneously. Another group received a placebo (Innovative Research of America). After 4 weeks, the rats were killed under light anesthesia with methohexital sodium (50 mg/kg body wt), and blood was collected by heart puncture for measurement of estradiol levels. 17β-Estradiol was measured by use of a radioimmunoassay kit (Diagnostic Products Corp). 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

A section of the mesentery 5 to 10 cm distal to the pylorus was rapidly removed and placed in ice-cold HEPES-buffered PSS (HEPES-PSS). Mesenteric arteries averaging <250 μm in diameter were dissected free from surrounding adipose tissue, cut into 1.8-mm lengths, and threaded onto 20-μm wires. These wires were attached to 2 polyacrylamide blocks in an isotropic myograph system (Kent Scientific Corp). The blocks rested in 5-mL glass-jacketed organ baths with HEPES-PSS solution kept at 37°C. Four separate baths were used to study arterial segments simultaneously. Force production was recorded on a data acquisition system (Workbench, Strawberry Tree Inc).

Resting Length-Tension Curve

After mounting, the arteries were stretched to ~0.2 mN per millimeter vessel length (1 mN=102 mg) and allowed to equilibrate for 1 hour in HEPES-PSS buffer. The arteries were then given a conditioning stretch of ~0.6 mN. A resting length tension curve was generated for each vessel. The arterial circumference that was used to perform the dose-response curves was obtained by using Laplace’s law. With this equation, Lmax is calculated from the exponential curve fit of tension versus circumference. Lmax is defined as the circumference the vessel would have at a transmural pressure of 100 mm Hg.

We have found from our previous studies that the point on the dose-response curve obtained at 0.8 Lmax provides maximum active force generation with minimum passive tension.

Solutions and Drugs

The HEPES-PSS solution used in these experiments contained (mmol/L) sodium chloride 142, potassium chloride 4.7, magnesium sulfate 1.17, calcium chloride 1.56, potassium phosphate 1.18, HEPES 10, and glucose 5.5. The HEPES-PSS solution was maintained at a pH of 7.4. Stock solutions of phenylephrine (1-phenylephrine hydrochloride), methacholine (acetyl-β-methylcholine chloride) (both from Sigma Chemical Co), and meclofenamate (Warner Lambert, Ann Arbor) were prepared in water at a concentration of 10 mmol/L for each experiment. Stock solutions of 10 mmol/L SQ-29548, valeryl salicylate, NS-398, U-51605, arachidonic acid, U-46619, prostaglandin E2 (PGE2), and PGH2 (all from Cayman Chemical Co) were prepared in ethanol. Appropriate dilutions of all stocks were obtained using HEPES-PSS.

Experimental Design

Mesenteric arteries were studied from the 3 groups of rats (n=5 or 6 rats per group) for each experimental protocol. Cumulative doses of phenylephrine (0.3 to 10 μmol/L) were administered. The data from the dose-response curves were fitted to the Hill equation, from which a straight line was generated by linear least squares regression analysis. The mean effective concentration that produced a 50% constriction (EC50) was determined from this line for each individual artery. The EC50 of phenylephrine was used to constrict arteries in order to achieve a baseline from which subsequent relaxation responses were measured. After completion of each dose-response curve, a 30-minute recovery period was allowed, during which the baths were changed every 10 minutes with fresh HEPES-PSS. Cumulative doses of the muscarinic agonist, methacholine (0.01 to 1 μmol/L) were administered to assess endothelium-dependent relaxations. To determine whether the responses to methacholine were dependent on the endothelium, one segment of a divided artery was denuded of its endothelium before the methacholine dose-response curve. Endothelium removal was performed mechanically using a human hair threaded through the lumen of the artery and rubbed back and forth.16

To determine the role for endogenous vascular prostaglandins in modulating the relaxation responses, methacholine dose-response curves were repeated in the absence or presence of the specific blockers in the PGHS cascade. The studies performed involving the absence and presence of inhibitors were conducted sequentially on the same artery. The reproducibility of repeating curves for these experiments was determined in a preliminary set of experiments designed to test for tachyphaxis. Meclofenamate (1 μmol/L) was administered to block cyclooxygenase conversion of arachidonic acid. Valeryl salicylate (3 mmol/L) and NS-398 (10 μmol/L) were administered to preferentially inhibit PGHS-1 and PGHS-2, respectively. U-51605 (10 μmol/L) was used to inhibit TXA2 synthase; SQ-29548 (1 μmol/L) was used to block the TXA2/PGH2 receptor. All inhibitors were preincubated with the arteries 15 minutes before the beginning of a dose-response curve.

Another series of experiments was conducted to determine the response of mesenteric arteries to an exogenous source of arachidonic acid. The response to arachidonic acid (0.01 to 5 μmol/L) was obtained after preconstricting the arteries with phenylephrine to 50% of the maximum response. After a 30-minute recovery period, one segment of the divided artery from each rat had the arachidonic acid doses repeated in the presence of specific PGHS inhibitors, valeryl salicylate, and NS-398. The arachidonic response was repeated in the presence or absence of endothelium.

Contractions to increasing concentrations of PGE2, U-46619 (TXA2 mimetic), or PGH2 were obtained in arteries absent of endothelium to determine sensitivity of the response of arteries from ovariectomized compared with estradiol-replaced rats.

Data Analysis

ANOVA was used to determine the statistical difference of the parameters between the control and estradiol-replaced rats shown in the Table. A 2-way ANOVA with repeated measures was used to compare the response to a drug before and after specific inhibitors. Pairwise comparison was then conducted using the Student-Newman-Keuls test. Differences among means were considered significant at P<0.05.

Results

Animal Model

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

**Vascular Responses**

Before relaxation-response curves were generated, arteries were preconstricted by their EC$_{50}$ of phenylephrine. There was no significant difference in the EC$_{50}$ of phenylephrine among the experimental groups. There was a decreased response to methacholine in the ovariectomized rats compared with the estradiol-replaced rats (Figure 1A). Chronic estrogen replacement in the rat dose-dependently improved the relaxation response, as evidenced by a significant decrease in the EC$_{50}$ to methacholine (Figure 1A, insert). For all groups of rats, relaxation response to methacholine was abolished by endothelium removal. Time-control data indicated that dose-response curves to methacholine could be repeated at least 3 times with a variance of $<4\%$.

In the presence of meclofenamate, an inhibitor of PGHS activity, there was no longer a significant difference among the groups (Figure 1B). The ovariectomized rats and the 0.15 mg/pellet estradiol–replaced rats shifted the curves to be similar to the 0.5 mg/pellet estradiol–replaced rats, suggesting that a PGHS-dependent vasoconstrictor modified the relaxation responses to methacholine.

Figure 2 depicts the EC$_{50}$ values for the relaxation response to methacholine alone and methacholine in the presence of specific inhibitors to the PGHS pathway. In the ovariectomized rats (Figure 2A), valeryl salicylate (PGHS-1 inhibitor), NS-398 (PGHS-2 inhibitor), or SQ-29548 (TxA$_2$ synthase inhibitor) significantly reduced the EC$_{50}$ to methacholine (enhanced the relaxation response), whereas U-51605 (TxA$_2$ synthase inhibitor) had no effect. Similar results were obtained for the 0.15 mg/pellet estradiol–replaced rats (Figure 2B). In the 0.5 mg/pellet estradiol–replaced rats (Figure 2C), the PGHS pathway inhibitors did not have a significant effect on the relaxation response to methacholine. The PGHS pathway inhibitors normalized the EC$_{50}$ of the ovariectomized rats and the 0.15 mg/pellet estradiol–replaced rats to be similar to the EC$_{50}$ of the 0.5 mg/pellet estradiol–replaced rats.

**Arachidonic Acid Response**

Arteries from ovariectomized rats resulted in vasoconstriction in response to exogenous arachidonic acid, whereas the arteries from the estradiol-replaced rats exhibited vasodilation (Figure 3). There was no vascular response to arachidonic acid in the absence of endothelium from any of the groups of rats (data not shown). Pretreatment with valeryl salicylate or NS-398 (inhibitors of PGHS-1 and PGHS-2, respectively) reversed the vasoconstriction in the ovariectomized rats to a vasodilation (Figure 4A) but caused a further relaxation in the 0.15 mg/pellet estradiol–replaced rats (Figure 4B). The PGHS inhibitors had no effect on the arachidonic acid.

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**Table:**

<table>
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<th>Group</th>
<th>n</th>
<th>Sera Estradiol pg/mL</th>
<th>Uterine Weight g</th>
</tr>
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<tr>
<td>Ovariectomized</td>
<td>10</td>
<td>5.83±0.35</td>
<td>0.11±0.004</td>
</tr>
<tr>
<td>Estradiol-replaced (0.15 mg/pellet)</td>
<td>12</td>
<td>12.2±0.51*</td>
<td>0.52±0.05*</td>
</tr>
<tr>
<td>Estradiol-replaced (0.5 mg/pellet)</td>
<td>12</td>
<td>66.5±5.69*†</td>
<td>0.61±0.04*</td>
</tr>
</tbody>
</table>

Values are mean±SE.

*P<0.05 vs ovariectomized rats; †P<0.05 vs 0.15 mg/pellet estradiol–replaced rats.

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**Figure 1.** Concentration-response curves to methacholine in the absence (A) or presence (B) of meclofenamate for mesenteric arteries from ovariectomized (OVX, n=5, ○), 0.15 mg/pellet estradiol–replaced (0.15 mg/p, n=5, □), and 0.50 mg/pellet estradiol–replaced (0.5 mg/p, n=6, ▼) rats. Responses are expressed as a percentage of relaxation from phenylephrine-preconstricted levels. Insert, Bar graphs depicting EC$_{50}$ values for methacholine among the groups of rats. Data represent mean±SEM. *P<0.05 vs OVX; †P<0.05 vs 0.15 mg/p.
responses in the arteries of the 0.5 mg/pellet estradiol-replaced rats (Figure 4C).

**PGE**$_2$, **U-46619**, and **PGH**$_2$

**Vasoconstrictor Responses**

PGE$_2$ induced similar vasoconstriction in endothelium-denuded arteries from ovariectomized and estradiol-replaced rats (Figure 5A). U-46619 and PGH$_2$ evoked greater vasoconstrictor responses in the arteries from ovariectomized compared with estradiol-replaced rats (Figure 5, panels B and 5C, respectively). U-46619, but not PGH$_2$, also induced a significantly greater vasoconstriction from arteries of the 0.15 mg/pellet estradiol-replaced rats compared with the 0.5 mg/pellet estradiol-replaced rats.

**Discussion**

Experiments were designed to test the hypothesis that removal of ovarian steroids would decrease endothelial/NO-dependent relaxation responses but that estrogen replacement would increase NO and PGHS activity, leading to increased vasodilation. Contrary to our hypothesis, the principal effect of estrogen replacement was to suppress PGHS-dependent vasoconstriction. The present experiments demonstrate that ovariectomy in the rat altered endothelium-dependent function as a result of an increased PGHS-dependent vasoconstriction that was associated with an augmented sensitivity of the PGH$_2$/TxA$_2$ receptor. Estrogen replacement prevented this increased vasoconstrictor response.

Many studies, including our own, indicate that the beneficial effect of estrogen is attributed, in part, to a direct effect on endothelium function. There is a number of reports suggesting that NO production and thus vasodilation is increased due to estrogen. In our study design, methacholine (an acetylcholine analogue) was used as an endothelium/NO-dependent relaxing agent to evaluate vascular function. We observed an increased relaxation response to methacholine in the mesenteric arteries of the estrogen-replaced rats compared with the ovariectomized rats in the absence of PGHS inhibition. These data could have been interpreted to reflect an increased endothelium/NO-dependent relaxation response. However, when PGHS activity was inhibited, the relax-
In the present study, pretreatment with valeryl salicylate or NS-398, to inhibit PGHS-1 and PGHS-2, respectively, increased the relaxation response in the ovariectomized rats, indicating that both PGHS-1 and PGHS-2 are important contributors to the PGHS-dependent vasoconstriction. Little is known regarding the regulation of activity or expression PGHS-1 and PGHS-2 by estrogen. In agreement with our vascular function data, it has been described that ovariectomy enhances but that estrogen replacement inhibits PGHS-2 expression and activity through bone marrow factors (primarily interleukin-1α). In the endometrial tissue of ewes treated with sex steroids, estrogen lowered PGHS mRNA but may have also increased the stability of the enzyme. In contrast, however, Wu et al recently reported that estradiol increases PGHS-2 mRNA and protein in the myometrium of nonpregnant sheep, suggesting a complexity of estrogen regulation depending on the tissue bed, animal model, and experimental design of the study.

In the endothelium, there is limited and contradictory data regarding estrogen and the PGHS pathway. In cell culture, estradiol has been shown to either have no effect or increase prostacyclin production in cultured human vascular endothelial cells. In vivo treatment of female rabbits with the synthetic estrogen, ethinyl estradiol, results in diminished in vivo vascular production of prostacyclin. These data could reflect a decrease in PGHS activity due to estrogen, which is similar to our findings. In our study, it was not possible to measure eicosanoid production released into the organ baths from the mesenteric arteries because of their size (<250 μm in diameter). We did, however, administer exogenous arachidonic acid to substantiate that the arteries from the ovariectomized rats had an increased capacity for a PGHS-dependent vasoconstriction. Furthermore, the addition of exogenous arachidonic acid should also indicate whether vascular differences in the groups of rats is due to differences in phospholipase activity that regulates the availability of arachidonic acid or to differences in cyclooxygenase activity. Previous reports on the vascular effects of exogenous arachidonic acid have been in conflict, depending on species, tissue, or the experimental model of the disease studied. Specific to estrogen replacement, arachidonic acid has been shown to augment endothelium-dependent contractions in rabbit aorta. In contrast, our data demonstrated a dose-dependent vasoconstriction only in arteries from ovariectomized rats and a vasorelaxation in the mesenteric arteries of estradiol-treated rats. The presence of a PGHS inhibitor eliminated the difference among the groups. These data further indicate that the increased vasoconstrictor response was not due to alterations of arachidonic acid availability but rather an effect at the level of PGHS-dependent products.

Comparable to the ovariectomized rat, an increase of PGHS-dependent vasoconstriction has been observed in animal models of hypertension, diabetes, and oxidative stress. Although some reports have suggested that a deficiency of NO contributes to the pathogenesis of hypertension, there are data indicating that the release of NO is normal and that a concomitant release of a PGHS-dependent endothelium-derived contracting factor accounts for the impaired relaxation. Relevant to our study, in female com-
pared with male spontaneously hypertensive rats, there is both an increased NO-dependent response as well as decreased PGHS-dependent vasoconstriction. It is possible that estrogen has a beneficial role to slow the progression of hypertension through suppression of PGHS-dependent vasoconstriction.

In the spontaneously hypertensive rat model, it has been suggested that the endothelium-derived PGHS-dependent vasoconstrictor is likely to be PGH₂.33,35 Our data for the ovariectomized rats agree with the finding of a concomitant release of a PGHS-dependent endothelium-derived contractile factor. The presence of a TxA₂/PGH₂ receptor antagonist resulting in a response similar to PGHS inhibition indicated that the PGHS-dependent vasoconstrictor was eliciting a response through this receptor. Since TxA₂ and PGH₂ bind to the same receptor, the PGHS-dependent vasoconstrictor could be either TxA₂ or PGH₂. Inhibition of TxA₂ synthase with U-51605 did not alter the relaxation response to methacholine in the ovariectomized rats, suggesting that TxA₂ may not be responsible for the PGHS-dependent vasoconstriction. However, inhibition of a specific synthase may shunt the pathway to other eicosanoids produced through isomerization or reduction of PGH₂.

Along with the observed increase of PGHS-dependent vasoconstriction in the ovariectomized rats, there appeared to be enhanced sensitivity of the PGH₂/TxA₂ receptor. The vascular constriction response to both PGH₂ and the TxA₂ mimetic U-46619 was enhanced, whereas there was no difference in the vascular response to PGE₂ in the ovariectomized rats compared with the estradiol-replaced rats. The

Figure 4. Bar graphs showing contractile responses to arachidonic acid (1 μmol/L) alone (open bar) and arachidonic acid in the presence of valeryl salicylate (PGHS-1 inhibitor) or NS-398 (PGHS-2 inhibitor) (hatched bars) in mesenteric arteries from ovariectomized (n=5) (A), 0.15 mg/pellet estradiol-replaced (n=6) (B), and 0.50 mg/pellet estradiol-replaced (n=6) (C) rats. Bars represent mean±SEM. *P<0.05 vs arachidonic acid alone.
increased sensitivity to PGH₂ in the ovariectomized rats is similar to the PGH₂ hypersensitivity observed in the aortas of spontaneously hypertensive rats. Furthermore, a recent study reported a decrease in contractility of guinea pig coronary arteries to the TxA₂ mimetic U-46619 in estrogen-replaced guinea pigs; however, unlike our data, their data indicated that NO mediated the reduced contractility. In our study, using endothelium-denuded mesenteric arteries from Sprague-Dawley rats, both PGH₂ and U-46619 induced a vasoconstriction that was greater in ovariectomized rats compared with estradiol-replaced rats. These data suggest that estrogen has the potential to suppress a PGHS-dependent vasoconstrictor response via the PGH₂/TxA₂ receptor.

In summary, estrogen-replacement in an ovariectomized rat suppressed PGHS-dependent vasoconstriction. The present results provide a novel observation that may explain, in part, the vascular physiology in postmenopausal women. Furthermore, these data provide another potential mechanism for the protective effect of estrogen that should be further studied for development of alternative therapeutic approaches for cardiovascular disease in men and women.

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References


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