Estrogen potentiates vasopressin-induced contraction of female rat aorta by enhancing cyclooxygenase-2 and thromboxane function

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Li, Min and John N. Stallone. Estrogen potentiates vasopressin-induced contraction of female rat aorta by enhancing cyclooxygenase-2 and thromboxane function. Am J Physiol Heart Circ Physiol 289: H1542–H1550, 2005. First published June 3, 2005; doi:10.1152/ajpheart.01024.2004.—To determine the roles of estrogen and constrictor prostanoids in vasopressin (VP)-induced contraction of female rat aorta, vascular reactivity to VP was determined in thoracic aortas of intact, ovariectomized, and ovariectomized + estrogen-replaced female rats in the presence of indomethacin (Indo), NS-398, SQ-29,548, or vehicle control. The effects of estrogen on vascular reactivity to the thromboxane A2 analog U-46619 were also examined. Maximal contractile response to VP in intact female rats (5,567 ± 276 mg/mg of aortic ring wt) was markedly attenuated by ovariectomy (2,485 ± 394 mg; P < 0.001) and restored by estrogen replacement with 17β-estradiol (5,059 ± 194 mg; P > 0.1). Indo and NS-398 significantly attenuated maximal responses to VP in intact female rats to a similar extent [3,176 ± 179 (P < 0.0001) and 3,258 ± 152 mg (P < 0.0001), respectively]. Ovariectomy abolished and estrogen replacement restored the inhibitory effects of Indo, NS-398, and SQ-29,548. Contractile responses of rat aorta to U-46619 were significantly greater (P < 0.0001) in females (5,040 ± 238 mg) than in males (3,679 ± 96 mg). Ovariectomy markedly attenuated (3,923 ± 84 mg; P < 0.01) and estrogen replacement restored (5,024 ± 155 mg; P > 0.1) responses to U-46619 in female aortas. These data reveal that estrogen is an important regulator of the contractile function of female rat aorta to VP, which appears to potentiate both cyclooxygenase-2 and constrictor prostanoid function in the vascular wall.

arginine vasopressin; constrictor prostanoids; indomethacin; NS-398; SQ-29,548; U-46619; vascular reactivity; vasoconstriction

Vasopressin (VP) is an extremely potent vasoconstrictor that functions in the short- and long-term control of blood pressure during hypovolemic states such as hemorrhage or dehydration (34, 47). Previous studies revealed that a marked sexual dimorphism exists in the vascular reactivity of the rat to VP. Contractile responses of female rat aorta to VP are three- to fourfold greater than those of male aorta (19, 51, 52, 54), and similar sex differences exist in the reactivity of rat mesenteric vasculature to VP (1, 53, 56). The presence of gonadal steroid hormone receptors in both endothelium (2, 13, 14) and vascular smooth muscle (VSM; Refs. 2, 25, 37, 39, 42) of the vascular wall suggests that female gonadal steroid hormones may influence VSM and/or endothelial functions and thereby mediate sex differences in vascular reactivity to VP and other vasoconstrictors. Previous studies also established that VP-stimulated release of nitric oxide (NO) is much greater in male rat aorta and is responsible for the markedly attenuated responsiveness to VP compared with females (51, 52). Interestingly, even in the presence of NO synthase (NOS) inhibition, contractile responses of rat aorta to VP are still significantly higher in females, and the cyclooxygenase (COX) inhibitor indomethacin (Indo) attenuates contractile responses to both VP (51) and the α-adrenergic agonist phenylephrine (PE; Ref. 19) in female but not male aorta. These data suggest that constrictor products of COX may contribute to the enhanced vascular reactivity of female rat aorta to both VP and PE.

In the present investigation, the effects of estrogen on the contractile responses of thoracic aorta to VP were examined in intact, ovariectomized, and ovariectomized + estrogen-replaced female Sprague-Dawley rats. The role of COX products, especially thromboxane A2 (TXA2), in modulating vascular reactivity to VP was determined by the use of COX pathway inhibitors. The role of estrogen in modulating constrictor prostanoid function was further examined by determining vascular reactivity to the TXA2 analog U-46619 in thoracic aortas of male and female rats.

MATERIALS AND METHODS

Animals

Age-matched (14–18-wk old) female and male Sprague-Dawley rats (Harlan; Houston, TX) were used in all studies. The rats were housed in vivarium facilities at the College of Veterinary Medicine (Laboratory Animal Resources and Research facility) with controlled temperature (22–24°C), relative humidity (~50%), and lighting (12:12-h light-dark cycle). The animals were segregated by sex and housed in pairs in standard plastic laboratory rat cages. Tap water and standard laboratory rat chow (Harlan; Indianapolis, IN) or an alfalfa- and soy-free diet (7% corn oil diet; Harlan TekLad) was provided ad libitum. This latter special diet is free of phytosterogens, which are often present in commonly used standard laboratory rat chow (45, 46) and have been reported to confound the effects of ovariectomy on vascular reactivity to VP (19). All experiments were reviewed and approved by the Texas A&M University Laboratory Animal Care Committee.

Animal Preparations

Animals were randomly separated into four experimental groups: males, intact females (Int-F), ovariectomized females (Ovx-F), and ovariectomized + estrogen-replaced females (Ovx+ER-F). Because previous studies established that contractile responses of female rat aorta to VP or PE do not differ with phase of the estrous cycle (54), female rats were studied without regard to estrous cycle status. For each experimental group, sample sizes of 5–9 rats were used to account for known levels of statistical variability in vascular experiments in vitro. Bilateral ovariectomy of female rats was performed at 4–5 wk of age using standard methods. Half of the Ovx-F rats received estrogen replacement therapy (ERT) beginning at 8–9 wk of age.
age for 21–28 days using 17β-estradiol (two 0.05 mg, 60-day release pellets). Previous studies have shown that this dose produces physiological plasma levels of 17β-estradiol in Ovx-F rats (61).

**Preparation of Isolated Thoracic Aortas**

Animals were killed by rapid decapitation in accordance with American Veterinary Medical Association guidelines (3). Trunk blood was collected at the time of decapitation for radioimmunoassay (RIA) of plasma 17β-estradiol (see RIA of plasma estradiol). The thoracic aorta was rapidly but gently removed to avoid stretching or damaging the endothelium and was placed in chilled (4°C) Krebs-Henseleit bicarbonate solution (KHB). The KHB was composed of (in mM): 118.0 NaCl, 25.0 NaHCO3, 10.0 glucose, 4.74 KCl, 2.5 CaCl2, 1.18 MgSO4, and 1.18 KH2PO4 (pH 7.40; osmolality, 292 mosmol/kgH2O). The aorta was cleaned of all adipose and connective tissue, and the mid-thoracic region was cut into rings (3 mm long). Aortic rings were studied either with the endothelium intact (Endo+) or denuded (Endo−), which was accomplished by gently passing a frayed nylon string through the lumen (51). The rings were carefully mounted on two 25-gauge stainless steel wires; the upper one was attached to a force-displacement transducer (model PT-03D; Grass Instruments; Quincy, MA) for measurement of isometric tension, and the lower one was attached to a stationary stainless steel rod and micrometer that allowed for adjustment of passive tension in the aortic rings. The transducers were connected to a polygraph (model 2600S; Gould; Cleveland, OH) to provide a continuous record of contractile tension.

**Isometric Tension Studies**

Immediately after being mounted, aortic rings were immersed in water-jacketed organ baths filled with 15.0 ml of warmed (37°C) KHB and continuously gassed with 95% O2-5% CO2. Passive tension was then gradually (over a 30-min period) increased to 2.50 g, which is the optimal tension for male aortas and both intact and ovariectomized female aortas as established in previous studies (51, 52, 54). The rings were allowed to equilibrate for 90 min, and the KHB was replaced with fresh gassed and warmed KHB every 20 min. Passive tension was adjusted to maintain 2.50 g throughout the equilibration and experimental periods. After the equilibration period, the aortic rings were stabilized by two successive near-maximal contractions with PE (10−6 M final concentration). After each contraction reached a stable plateau tension, the endothelium-dependent vasodilator acetylcholine (ACh) was added to the baths (10−5 M), which is the optimal concentration of male rats was similar to that of Ovx-F rats (61).

**Experimental Protocols**

**Effects of prostanoid pathway inhibitors.** The effects of constrictor prostanoid pathway inhibitors on vascular reactivity to VP were examined by obtaining cumulative concentration responses to VP (10−11 to 10−6 M) in Endo+ aortic rings from Int-F rats in the presence of the following: 1) the nonselective COX inhibitor Indomethacin (Indo) (10 μM); 2) the COX-2-selective inhibitor NS-398 (NS; 10 μM); 3) the PGH2/TxA2 receptor antagonist SQ-29,548 (SQ; 1 μM); or 4) vehicle control (0.1% DMSO). Duplicate or triplicate rings prepared from each aorta were pretreated with inhibitors or vehicle 20 min before the concentration responses to VP were obtained. To examine whether the effects of the COX-2-selective inhibitor were agent specific, cumulative concentration responses to VP were also obtained in the presence of a second, chemically dissimilar COX-2-selective inhibitor, niflumic acid (NA; 10 μM).

**Effects of estrogen on prostanoid pathway function.** To determine the effects of estrogen on vascular reactivity and constrictor prostanoid function in female rat aortas, cumulative concentration responses to VP (10−11 to 10−6 M) were obtained in Endo+ aortic rings prepared from Ovx-F and Ovx+ER-F rats. Duplicate or triplicate rings from each aorta were pretreated with Indo (10 μM), NS (10 μM), or vehicle control.

**Male-female differences in vascular reactivity to U-46619 and effects of estrogen.** To determine male-female differences in vascular reactivity to TxA2 and the effects of estrogen on vascular reactivity to TxA2 in female rat aorta, cumulative concentration responses to the TxA2 analog U-46619 (1 × 10−11 M to 3 × 10−6 M) were obtained in paired Endo+ and Endo− aortic rings obtained from male, Int-F, Ovx-F, and Ovx+ER-F rats.

**RIA of plasma estradiol.** Trunk blood samples (~5–6 ml) were collected from rats at the time of decapitation into 13×100-mm glass culture tubes coated with 150 U of sodium heparin. Blood samples were centrifuged (10,000 g for 5 min), and the resultant plasma was separated and stored at −70°C until RIA for plasma 17β-estradiol levels was performed. Estradiol concentrations were measured in unextracted plasma in duplicate using a double-antibody RIA (Diagnostic Products; Los Angeles, CA) validated for measurements of rat plasma (61). The limit of detection of this assay is ~2.0 pg/ml in this laboratory.

**Chemical Reagents and Drugs**

The following reagents and drugs were used: 17β-estradiol (Innovative Research of America; Sarasota, FL), U-46619 (9,11-dideoxy-9x,11α-methanoepoxy prostaglandin F2α), NS, and NA (Cayman Chemical; Ann Arbor, Michigan), arginine VP (Bachem; Torrance, CA), PE hydrochloride, ACh chloride, and Indo (Sigma Chemical; St. Louis, MO). Stock solutions of all drugs were prepared fresh daily except for VP (which was diluted daily from aliquots of 1 × 10−8 M stock solution stored at −70°C) and NS (which was diluted daily from 1 mg/ml stock solution stored at −20°C).

**Data Analysis**

All data are expressed as means ± SE; n indicates the number of animals studied. Contractile responses to VP and U-46619 were normalized by dry weight of the aortic rings and are expressed as milligrams of contractile force per milligram of ring weight. The concentration of VP or U-46619 that produced 50% of the maximal response (EC50) was calculated individually from the log concentration-response curve of each aortic ring, and these values are reported as the mean ± SE for the particular experimental group. U-46619 data groups were analyzed by sex (male vs. female) and by experimental treatment (e.g., Endo+ vs. Endo−) using two-way ANOVA to detect significant differences among the treatment groups and subsequently by pair-wise Student’s t-tests to distinguish significant differences between any two means of the data groups. VP data groups were analyzed by sex (Int-F vs. Ovx-F vs. Ovx+ER-F) and by experimental treatment (control vs. Indo− vs. NS) using two-way ANOVA and Student’s t-tests as described. U-46619 and VP data groups involving the effects of ovariectomy were analyzed by sex alone (Int-F vs. Ovx-F vs. Ovx+ER-F) using one-way ANOVA and Student’s t-tests as described. Differences between any two means were accepted as significant if P < 0.05.

**RESULTS**

**Effects of Ovariectomy and Estrogen Replacement on Vascular Reactivity to VP**

Plasma 17β-estradiol concentrations are summarized in Table 1. Compared with Int-F rats, ovariectomy reduced plasma estradiol concentration by 97% (P < 0.05), whereas 17β-estradiol replacement restored the plasma concentration to levels similar to those of Int-F rats (P > 0.05). Plasma estradiol concentration of male rats was similar to that of Ovx-F rats (P > 0.05).

In rats fed the phytoestrogen-free diet, ovariectomy attenuated contractile responses to VP throughout the concentration-response curve; in contrast, ERT restored the contractile responses to VP (Fig. 1). Maximal contractile responses to VP
were reduced 55% by ovariectomy (P < 0.001) and were fully restored by ERT compared with Int-F rats (P > 0.1; Table 2). Sensitivity to VP (EC50) was not altered by ovariectomy (P > 0.1), although ERT produced a slight but significant increase in sensitivity to VP compared with ovarioectomized rats (P < 0.01; Table 2). In preliminary experiments, reactivity to VP in aortas obtained from Ovx-F rats fed the standard (phytoestrogen-containing) laboratory rat chow did not differ from that of Int-F rats (P > 0.05; data not shown).

**Effects of Ovariectomy and Estrogen Replacement on Prostanoid Pathway Inhibitors**

In Int-F rat aortas, VP produced concentration-dependent contractions with a maximal response of 5,567 ± 276 mg/mg of ring wt and an EC50 of 8.0 ± 1.2 nM (Fig. 2A; Table 2). Both the nonselective COX inhibitor Indo and the COX-2-selective inhibitor NS significantly attenuated contractile responses to VP at middle and higher concentrations (Fig. 2A; Table 2). The maximal contractile responses were reduced by 43 (P < 0.001) and 41% (P < 0.001) by Indo and NS, respectively, compared with the control group. Sensitivity (EC50) of the female aortas to VP was unchanged after pretreatment with either Indo (P = 0.165) or NS (P = 0.180; Table 2) compared with the control group. The chemically dissimilar COX-2-selective inhibitor NA produced attenuating effects on the contractile responses to VP that were qualitatively and quantitatively similar to those of NS (P > 0.05), thereby reducing maximal response to VP by 47% (P < 0.001; data not shown).

In rats fed the phytoestrogen-free diet, ovariectomy attenuated contractile responses to VP throughout the concentration-response curve and abolished the attenuating effects of Indo and NS (Fig. 2B; Table 2). In contrast, ERT fully restored the contractile responses to VP and the attenuating effects of Indo and NS (Fig. 2C; Table 2). Maximal contractile responses to VP were reduced 55% by ovariectomy (P < 0.001) and restored by ERT compared with Int-F rats (P > 0.1; Table 2). In Int-F rats, the differences in maximal contractile responses among control, Indo–, and NS-treated aortas were highly significant (P < 0.001; Table 2); in contrast, in Ovx-F rats, maximal contractile responses of control, Indo–, and NS-treated aortas did not differ significantly (P > 0.05; Table 2). With ERT, significant differences in maximal contractile responses among control, Indo–, and NS-treated aortas were fully restored (P < 0.001; Table 2). Sensitivity to VP (EC50) in Int-F, Ovx-F, and Ovx+ER-F rats did not differ significantly among control, Indo–, or NS-treated aortas (P > 0.1; Table 2) with the exception of control aortas from Ovx+ER-F rats, which exhibited a slight but significant increase in sensitivity to VP (P < 0.031; Table 2). In preliminary experiments, reactivity to VP in aortas obtained from Ovx-F rats fed the standard (phytoestrogen-containing) laboratory rat chow did not differ from that of Int-F rats (P > 0.05; data not shown), and the inhibitory effects of Indo and NS were not abolished by ovariectomy.

In rats fed the phytoestrogen-free diet, ovariectomy attenuated contractile responses to VP throughout the concentration-response curve and abolished the attenuating effects of SQ (Fig. 3). In contrast, in Ovx+ER-F rats, ERT fully restored the contractile responses to VP and the effect of SQ to attenuate contractile responses to VP (Fig. 3). Maximal contractile responses to VP were reduced 51% by ovariectomy (2,485 ± 394 mg; P < 0.001) compared with ERT in Ovx+ER-F rats (5,058 ± 193 mg). In Ovx-F rats, maximal contractile responses of control (2,485 ± 394 mg) and SQ-treated (1,721 ± 172 mg) aortas did not differ (P > 0.05); in contrast, in Ovx+ER-F rats, maximal contractile responses of control (5,058 ± 193 mg) aortas were reduced by 31% in SQ-treated aortas (3,471 ± 206 mg; P < 0.001). Sensitivity to VP (EC50) in Ovx-F rats did not differ significantly between control (9.19 ± 1.51 nM) and SQ-treated (7.26 ± 0.80 nM) aortas (P > 0.1). In Ovx+ER-F rats, ERT increased sensitivity to VP slightly in control aortas (4.90 ± 0.40 nM), and this was unchanged in SQ-treated aortas (4.77 ± 0.53 nM; P > 0.10).

**Male-Female Differences in Vascular Reactivity to U-46619 and Effects of Ovariectomy and Estrogen**

Contractile responses of Endo+ aortic rings to U-46619 were significantly higher in females than in males in the upper half of the concentration-response curve (Fig. 4). The maximal contractile response of Endo+ aortic rings was significantly higher in female than in male rat aortas (P < 0.0001; Fig. 4; Table 3). Deletion of the endothelium potentiated the contractile responses of both female and male rat aortas to a similar extent; thus contractile responses of Endo+ females were significantly lower than those of Endo- females.
ably attenuated contractile responses of female Endo

Sensitivity of both female and male aortas to a similar vasculature (53, 56). Previous studies also demonstrated that anesthetized rats (1) and in isolated perfused rat mesenteric arterioles of males (51, 54). Similar sex differences in the vascular reactivity to VP have been observed in mesenteric arterioles of males (51, 54).

In the present investigation, the roles of estrogen and constrictor prostanoids in the contractile responses of rat aorta to VP were determined in female Sprague-Dawley rats. The results reveal that endogenous estrogen is an important regulator of the contractile responses of female rat aorta to VP, which appears to potentiate both COX-2 and constrictor prostanoid function in the vascular wall.

Effects of Prostanoid Pathway Inhibitors

To determine the role of constrictor prostanoid function in VP-induced contraction of female rat aorta, the effects of COX inhibitors were examined. The nonselective COX inhibitor Indo significantly attenuated the contractile responses of female rat aorta to VP by 10.220.33.5 on July 5, 2017 http://ajpheart.physiology.org/ Downloaded from

Table 2. EC50 values and corresponding maximal contractile responses to arginine VP in thoracic aortas of female Sprague-Dawley rats pretreated with indomethacin, NS-398, or vehicle control

<table>
<thead>
<tr>
<th>Experimental Treatment</th>
<th>Int-F (5)</th>
<th>Ovx-F (6)</th>
<th>Ovx-ER-F (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50, nM</td>
<td>Maximal contractile force, mg/mg of ring wt</td>
<td>EC50, nM</td>
<td>Maximal contractile force, mg/mg of ring wt</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>8.0±1.2d,e</td>
<td>5,567±276a</td>
<td>9.2±1.5d,e</td>
</tr>
<tr>
<td>Indomethacin (10 μM)</td>
<td>6.1±0.4d,f</td>
<td>3,176±179a,b,c</td>
<td>7.5±0.7d,e</td>
</tr>
<tr>
<td>NS-398 (10 μM)</td>
<td>10.9±1.6d,e</td>
<td>3,258±152b</td>
<td>9.0±1.0d,e</td>
</tr>
</tbody>
</table>

Values are means±SE; n, no. of animals in parentheses. EC50, concentration of vasopressin (VP) producing 50% of maximal contractile response. Data are derived from Figs. 1 and 2. *Within columns and rows for each group (Int vs. Ovx vs. Ovx+ER) or experimental treatment (control vs. indomethacin vs. NS-398), mean values with different superscripts are significantly different (0.0001 ≤ P ≤ 0.031).

Discussed

In the present investigation, the roles of estrogen and constrictor prostanoids in the contractile responses of rat aorta to VP were determined in female Sprague-Dawley rats. The results reveal that endogenous estrogen is an important regulator of the contractile responses of female rat aorta to VP, which appears to potentiate both COX-2 and constrictor prostanoid function in the vascular wall.

Effects of Prostanoid Pathway Inhibitors

To determine the role of constrictor prostanoid function in VP-induced contraction of female rat aorta, the effects of COX inhibitors were examined. The nonselective COX inhibitor Indo significantly attenuated the contractile responses of female rat aorta to VP by 43%. Interestingly, the chemically dissimilar COX-2-selective inhibitors NS and NA both attenuated contractile responses to VP to a similar extent as Indo (41 and 47%, respectively), which strongly suggests that COX-2 but not COX-1 contributes to the constrictor prostanoid-mediated potentiation of contractile responses of female rat aorta to VP.

Previous studies established that the contractile responses of rat aorta to VP are three- to fourfold higher in females than in males (51, 54). Similar sex differences in the vascular reactivity to VP have been observed in mesenteric arterioles of anesthetized rats (1) and in isolated perfused rat mesenteric vasculature (53, 56). Previous studies also demonstrated that VP-stimulated release of NO is much greater in male rat aorta and is responsible for the markedly attenuated responses to VP compared with female aorta (51, 52). Interestingly, even in the presence of NOS inhibition, contractile responses to VP are still higher in females, and several studies have revealed that this remaining sex difference in reactivity to VP involves enhanced constrictor prostanoid function in the female vascular wall (19, 51). The nonselective COX inhibitor Indo and the PGH2/TxA2 receptor antagonist SQ both attenuated contractile responses of female but not male rat aorta to VP and the α-adrenergic agonist PE to the same extent (19), which demonstrated that constrictor prostanoids play a significant role in potentiating the contractile responses of the female but not the male systemic vasculature to VP and PE. However, these studies did not examine the underlying mechanism in detail. Thus the present study is the first to establish the involvement of a constitutive form of COX-2 in the constrictor prostanoid-mediated potentiation of the contractile responses of the systemic vasculature in female rat aorta to VP.

COX-1 and -2 are enzyme isoforms that are encoded by different genes (30, 31). COX-1 is the constitutive isoform present in many cells and is a housekeeping enzyme (50, 65). Conversely, COX-2 is an inducible isoform activated by inflammatory agents (28, 40); however, COX-2 is also constitutively expressed in some tissues such as kidney and aorta (38, 40, 64) and may play a role in regulating normal renal function and blood flow. The present study provides clear functional evidence that a constitutive form of COX-2 is, in fact, involved in the regulation of systemic vascular function in female rats.

Effects of Estrogen on Constrictor Prostanoid Pathway Function in Female Aorta

To determine the role of estrogen in the regulation of the constrictor prostanoid pathway in female rat aorta, the effects of ovariectomy and ERT on vascular reactivity to VP and prostanoid function were determined. Plasma 17β-estradiol levels of Int-F and Ovx-F rats measured in the present study are consistent with previous findings (49, 61). Plasma concentrations of 17β-estradiol were attenuated drastically in Ovx-F rats and did not differ from those of male rats. ERT restored plasma estradiol levels to concentrations that did not differ from those of Int-F rats.

Ovariectomy markedly attenuated contractile responses to VP in Endo+ female rat aorta and abolished the inhibitory effects of the COX inhibitors Indo and NS as well as the PGH2/TXA2 receptor antagonist SQ. In contrast, long-term (3–4 wk) ERT in Ovx-F rats with 17β-estradiol completely

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restored contractile responses to VP and the inhibitory effects of Indo, NS, and SQ. These results reveal that constrictor prostanoid-mediated potentiation of the contractile responses of female rat aorta is strongly dependent upon the action of estradiol on the vascular wall. Estrogen receptors have been identified in both endothelium (2, 13, 14) and VSM (2, 37, 39); thus the potentiating effect of estrogen on the contractile responses to VP in female aorta may involve actions on endothelium and/or VSM.

In the present study, pharmacological blockade of the prostanoid pathway at the levels of COX and the PGH2/TxA2 receptor strongly suggests that the potentiating effect of estrogen involves upregulation of COX-2 and the subsequent constrictor prostanoid pathway, which thereby regulates both the release of and vascular reactivity to constrictor prostanoids in the vascular wall. Numerous studies support the idea that estrogen enhances vascular prostanoid production. For example, pretreatment of isolated, blood-perfused lungs of juvenile female sheep with estradiol enhances the production of prostaglandins (57). Similarly, in isolated rat lung, COX activity varies during the course of the estrous cycle and peaks during the estrogen surge at proestrus (4). Furthermore, estrogen treatment enhances TxA2 production in rat endothelial cells (66) and PGI2 production in both rat VSM cells (11) and ovine fetal pulmonary artery endothelial cells (48). The results of the present study are consistent with these previous findings and further reveal that estrogen potentiates the contractile responses to VP in female rat aorta by enhancing the activity of COX-2. This effect of estrogen also appears to involve the subsequent enhancement of the constrictor prostanoid pathway as suggested by the effects of ovariectomy and ERT on constrictor prostanoid function in past (19) and present studies. Indeed, preliminary studies reveal that VP-stimulated release of TxA2 by rat aorta is dramatically reduced by ovariectomy but is restored by ERT (33). In a previous study, removal of the endothelium attenuated the contractile responses to VP to a similar extent as either Indo or SQ and abolished the attenuating effects of Indo and SQ, which suggests the involvement of endothelium-derived constrictor prostanoids (19). Additional studies are necessary to elucidate the mechanism underlying estrogen-mediated increases in VP-stimulated constrictor prostanoid release and the origin of their production in female rat aorta.

The present study also demonstrates that estrogen not only enhances the activity of COX-2 but also potentiates reactivity of

Fig. 2. Concentration-response curves for arginine VP in endothelium-intact aortic rings prepared in triplicate from Sprague-Dawley Int-F (A), Ovx-F (B), or Ovx+ER-F (C) rats in the presence of indomethacin (Indo, 10 μM), NS-398 (NS, 10 μM), or Veh. Statistically significant differences exist in A in Veh vs. Indo (0.0001 ≤*P ≤ 0.048) and Veh vs. NS (0.0001 ≤*P ≤ 0.009) at both maximal (1 × 10−6 M) and middle (1 × 10−8 M) concentrations of VP and in C in Veh vs. Indo (0.0002 ≤*P ≤ 0.0003) and Veh vs. NS (*P < 0.0001) at both maximal and middle concentrations of VP. No statistically significant differences exist in B in Veh vs. Indo (P ≥ 0.088), in Veh vs. NS (P ≥ 0.160), or in Indo vs. NS (P ≥ 0.521) at both maximal and middle concentrations of VP.

Fig. 3. Concentration-response curves for arginine VP in endothelium-intact aortic rings prepared in duplicate from Ovx-F and Ovx+ER-F Sprague-Dawley rats in the presence of SQ-29,548 (SQ, 1 μM) or Veh. Statistically significant differences exist in Ovx+ER-F for SQ vs. Veh (0.0001 ≤ *P ≤ 0.001) at both maximal (1 × 10−6 M) and middle (1 × 10−8 M) concentrations of VP. No statistically significant differences exist in Ovx-F for SQ vs. Veh (P ≥ 0.100) at both maximal and middle concentrations of VP.
vascular endothelium, which may be enhanced by estrogen; or effects of the constrictor prostanoid TxA2 on the vasculature. The (16), which both reveal that estrogen potentiates the contractile with those reported for the isolated rat pulmonary vasculature.

ovariectomy abolished and ERT restored the contractile responses were significantly higher in females than in males. Furthermore, ovariectomy abolished and ERT restored the contractile responses in female Sprague-Dawley rats. Statistically significant differences exist in female Endo-(#P<0.001) vs. male Endo-(P=0.008) at middle concentrations of U-46619. No statistically significant differences exist in female Endo- vs. female Endo- (P ≥ 0.111) or in male Endo- vs. male Endo- (P ≥ 0.051) at maximal concentrations of U-46619.

female aorta to the constrictor prostanoid TxA2. Thus contractile responses of female rat aorta to the stable TxA2 analog U-46619 were significantly higher in females than in males. Furthermore, ovariectomy abolished and ERT restored the contractile responses of female aorta to U-46619. The present findings are consistent with those reported for the isolated rat pulmonary vasculature (16), which both reveal that estrogen potentiates the contractile effects of the constrictor prostanoid TxA2 on the vasculature. The underlying mechanisms may include: 1) estrogen-induced increases in the density of PGH2/TxA2 receptors in rat VSM cells; 2) U-46619-stimulated release of vasoactive substance(s) from the vascular endothelium, which may be enhanced by estrogen; or 3) estrogen modulation of downstream signal transduction mechanisms involved in TxA2-stimulated VSM constriction. A previous study of cultured rat aortic VSM cells reported that the number of TxA2 binding sites per cell is higher in females than in males (36), but reactivity of the TxA2 receptors in female aorta was not assessed. Thus it is possible that the greater contractile responses to U-46619 in females in the present study, especially in Endo- aortic rings, involve enhancement of PGH2/TxA2 receptor expression by estrogen in female aortic VSM cells. Indeed, preliminary studies suggest that estrogen upregulates expression of PGH2/TxA2 receptors in female aortic VSM cells (32). Concentration-response curves with paired Endo+ and Endo− aortic rings suggest that deletion of the endothelium increases contractile responses to U-46619 in both males and females; however, the increases were not significantly different in either group. The increase in contractile responses to U-46619 after removal of the endothelium may be due to the loss of endothelial vasodilators, especially NO; however, this mechanism does not appear to contribute to sex differences in the responses of rat aorta to U-46619. Thus sex differences in U-46619-induced release of vasoactive factors from endothelium are not likely to be involved in the observed sex differences in reactivity to U-46619. Studies with human omental arteries also showed that neither inhibition of NOS by Nω-nitro-L-arginine nor removal of the endothelium affected contractile responses to U-46619 (59). Finally, estrogen may potentiate downstream signal transduction mechanisms involved in the TxA2-induced VSM contraction; in particular, the activity of VSM phospholipase C or inositol 1,4,5-trisphosphate are likely sites of regulation by estrogen that would enhance TxA2-induced contraction.

Table 3. EC_{50} values and corresponding maximal contractile responses to thromboxane A2 analog U-46619 in paired endothelium-intact and endothelium-denuded thoracic aortic rings of Sprague-Dawley rats

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<thead>
<tr>
<th>Experimental Treatment</th>
<th>Male (9)</th>
<th>Int-F (8)</th>
<th>Ovx-F (7)</th>
<th>Ovx+ER-F (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC_{50}, nM</td>
<td>Maximal contractile force, mg/mg of ring wt</td>
<td>EC_{50}, nM</td>
<td>Maximal contractile force, mg/mg of ring wt</td>
</tr>
<tr>
<td>Endo+</td>
<td>27.3±3.2^{d}</td>
<td>3.67±0.9^{e}</td>
<td>34.7±2.2^{d}</td>
<td>5.04±0.2^{d}</td>
</tr>
<tr>
<td>Endo−</td>
<td>13.0±1.7^{d}</td>
<td>4.09±2.6^{d}</td>
<td>20.4±2.6^{d}</td>
<td>5.59±2.2^{d}</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals in parentheses. Endo+, endothelium intact; Endo−, endothelium denuded. Data are derived from Figs. 4 and 5. "Within columns and rows for each group (male vs. Int-F vs. Ovx-F vs. Ovx+ER-F) or experimental treatment (Endo+ vs. Endo−), mean values with different superscripts are significantly different (0.0001 ≤ P ≤ 0.029).
The greater contractile responses of female aorta to VP also may be due to the upregulation of VP receptors in VSM cells by estrogen. In the present study, ovariectomy reduced the maximal contractile response to VP by 55%, which was greater than the effects of Indo or NS (43% and 41%, respectively) in Int-F rat aortas; thus it is possible that ovariectomy not only decreased constrictor prostanaloid function but also reduced the expression of VP binding sites in aortic VSM cells. Estrogen significantly increases the expression of VP V1α receptor mRNA in the preoptic area of young ovariectomized rats (20). Similarly, estrogen increases VP V1 receptor expression in rabbit myometrium and rat mesenteric arteries (35, 56). In the latter study, upregulation of VP V1 receptors was correlated with an increase in the vasoconstrictor responses to VP in the isolated, perfused mesenteric vascular bed. Estradiol also increased the maximal response to VP and the density of VP binding sites in the mesenteric vasculature of male rats (56). This evidence suggests that it is possible, indeed likely, that ovariectomy not only decreased constrictor prostanaloid function but also reduced the expression of VP binding sites in female aortic VSM cells.

Although most studies reveal that long-term treatment with estrogen enhances reactivity to VP or U-46619, not all studies agree with these findings. For example, long-term estrogen replacement of ovariecctomized rats has been reported to be without effect on reactivity of resistance-sized mesenteric arteries to either VP or U-46619 (68). The reason(s) for these differences is uncertain but may involve differences in the blood vessels studied and/or the dose of ERT employed.

Although rat aorta is a large conduit vessel not involved in regulation of peripheral resistance, the ovarian steroid-dependent constrictor prostanaloid mechanism identified in rat aorta in present and past (19) studies appears to be quite relevant to the regulation of systemic arterial blood pressure. Preliminary studies reveal that intravenous infusion of the PGH2/TxA2 receptor antagonist SQ into conscious normotensive rats reduces mean arterial blood pressure (MAP) by 15% in females but has no effect in males; even more striking, infusion of this antagonist into aortic coarctation-hypertensive rats reduces MAP by 34% in females but only 15% in males (5). Taken together, these data suggest that the estrogen-dependent constrictor prostanaloid mechanism in the vascular wall may play an important role in the regulation of vascular tone in females but not in males in both normal and pathophysiological states.

The findings of the present study are consistent with the extensive epidemiological data that reveal the existence of a prominent sexual dimorphism in the incidences of primary vascular diseases that involve excessive vasoconstriction. Primary pulmonary hypertension (21, 63), migraine headache (41), and Raynaud’s disease (6, 9, 22, 62) all occur in premenopausal women at rates as much as fourfold higher than in men. Furthermore, an association has been reported between Raynaud’s disease and these other afflications, which suggests that a common mechanism of vasospasm may be involved (8a). Elevated vascular production of TxA2 may be the common mediator of vasospasm in these diseases. The production of this constrictor prostanaloid is increased in patients with Raynaud’s disease (44), migraine headache (29), and pulmonary hypertension (12). Animal studies also revealed the relationship of TxA2 and the development of these diseases (10, 17). Furthermore, the thromboxane synthase inhibitors CGS-13080 and dazoxiben have been successfully used for treatment of primary pulmonary hypertension and Raynaud’s disease (8, 43).

The higher incidences of primary vascular diseases in premenopausal women suggest that estrogen and/or other ovarian steroids may be involved in the pathogenesis of primary vascular diseases in women. Indeed, the recent Heart and Estrogen-Progestin Replacement Study (HERS) reported no overall benefit of ERT, and untoward cardiovascular events including venous thrombosis actually increased significantly in the first year of the study (23, 24, 26). Similar deleterious effects of ERT on the incidences of coronary artery disease, stroke, and venous thrombosis were also reported in the more recent Women’s Health Initiative (WHI) studies (67). Furthermore, oral contraceptive use in young women is also associated with an increased risk of thrombosis (7, 15) and acute myocardial infarction (27). The influence of estrogen on constrictor prostanaloid function, as established in the present study, may contribute to these deleterious effects of estrogen on the vasculature. Indeed, postmenopausal women undergoing ERT have elevated urinary levels of both TxA2 and prostacyclin (18, 60). Similarly, preliminary studies in rat aorta suggest that estrogen upregulates the release of TxA2, and that this effect of estrogen involves increased expression of message for COX-2, TxA2 synthase, and TxA2 VSM receptor (33, 55). These findings raise the intriguing possibility that the deleterious effects of estrogen on the vascular wall and/or thrombosis that are suggested by both the Heart and Estrogen-Progestin Replacement Study (23, 24, 26) and the more recent Women’s Health Initiative studies (67) may involve the potentiating effects of estrogen on TxA2 and the constrictor prostanaloid pathway.

In conclusion, the present study is the first to establish that estrogen potentiates the contractile responses of female rat aorta to VP, and that this effect of estrogen appears to involve the enhancement of both COX-2 and constrictor prostanaloid function in the vascular wall. The mechanisms underlying the effects of estrogen on COX-2 and the constrictor prostanaloid pathway are presently being investigated in our laboratory.

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GRANTS

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REFERENCES