Sexual dimorphism in prostanoid-potentiated vascular contraction: roles of endothelium and ovarian steroids

CLIFFORD T. FULTON1 AND JOHN N. STALLONE2

Department of Physiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272-0095; and Michael E. DeBakey Institute for Comparative Cardiovascular Science and Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4466

Revised 25 February 2002; accepted in final form 3 July 2002

Fulton, Clifford T., and John N. Stallone. Sexual dimorphism in prostanoid-potentiated vascular contraction: roles of endothelium and ovarian steroids. Am J Physiol Heart Circ Physiol 283: H2062–H2073, 2002.—The effects of constrictor prostanoid (CP) pathway inhibitors on vascular reactivity to vasopressin (VP) and phenylephrine (PE) were examined in thoracic aortas of male, female, and ovariectomized (OVX) female Sprague-Dawley rats. Maximal contractile response of control (Cont) aortas to VP was markedly higher in females (3,885 ± 332 mg/mg ring wt) than in males (810 ± 148 mg). Indomethacin (Indo; 10 μM) attenuated maximal response to VP in females (3,043 ± 277 mg) but not in males. SQ-29,548 (SQ; 1 μM) attenuated maximal response to VP in females (3,042 ± 290 mg) to a similar extent as Indo. Dazoxiben (Daz; 10 μM) alone had no effect, but Daz + SQ attenuated maximal contractile response to VP to a similar extent as SQ alone. Removal of the endothelium in female aortas attenuated contractile responses to VP in Cont aortas. OVX attenuated maximal contractile response to VP in Cont aortas (2,093 ± 329 mg) and abolished the attenuating effects of Indo. Indo, SQ, and Daz exerted identical effects on contractile responses of male, female, and OVX female aortas to PE. These findings establish the following in the rat aorta: 1) CP, probably thromboxane and/or endoperoxide, is responsible for ~25–30% of contractile responses of females, but not males, to VP and PE; 2) CP production by the female aorta is primarily endothelial in origin; and 3) ovarian steroids modulate production and/or actions of CP in female aortas.

Constrictor prostanoids; thromboxane; vasoconstriction; vascular reactivity; aortic rings; vasopressin; endoperoxide

Significant male-female differences exist in the responses of the vasculature to vasoactive hormones, both in vivo and in vitro. There is increasing evidence that the gonadal steroid hormones play an important role in these differences through the modulation of vascular responsiveness to vasoconstrictor as well as vasodilator substances. Endothelium-derived relaxing factor (nitric oxide (NO)) and vasodilatory prostanoids (prostacyclin) are major products of the endothelium known to be involved in the modulation of local vascular function (46, 50). The presence of gonadal steroid hormone receptors in both the endothelium (12) and vascular smooth muscle (25) of blood vessels suggests that male-female differences in vascular function may involve gonadal steroid modulation of the release and/or vascular actions of NO and prostanoids.

Recent studies (51, 52) have established that vasopressin (VP)-induced contractions of the rat aorta are three- to fourfold higher in females than in males, primarily due to the greater production of endothelium-derived NO in males than in females. Testosterone appears to play a primary role in the regulation of this endothelial mechanism because gonadectomy of male rats enhances contractile responses of the aorta to VP and eliminates the potentiating effect of NO synthase (NOS) inhibition (52), and virtually identical changes are seen in androgen receptor-deficient male rats (56). Inhibition of NOS reduces substantially but does not abolish this sex difference in vascular reactivity in the rat aorta, suggesting that another mechanism is responsible for the remaining male-female difference in reactivity to VP (51). Interestingly, inhibition of cyclooxygenase with indomethacin (Indo) attenuates contractile responses to VP in female but not in male rat aortas, suggesting that a constrictor prostanoid may be responsible for the sex difference in vascular reactivity to VP that persists in the presence of NOS inhibition (51). Similarly, Indo decreases vascular responsiveness to phenylephrine (PE) in the female rat aorta (58) and to norepinephrine in the ovariectomized (OVX) female rabbit aorta after estrogen treatment in vivo (39). These data suggest that sex differences in the vascular actions of some vasoconstrictor agonists may involve agonist-induced release of constrictor prostanoids in female but not in male vasculature.

The possibility that constrictor prostanoids may potentiate vasoconstrictor responsiveness of the systemic vasculature in females is of particular interest because it is generally accepted that constrictor prostanoids,

Address for reprint requests and other correspondence: J. N. Stallone, Dept. of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M Univ., College Station, TX 77843-4466 (E-mail: jstellone@cvm.tamu.edu).

0363-6135/02 $5.00 Copyright © 2002 the American Physiological Society http://www.ajpheart.org

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
while important in pathological states such as hypertension (40), play little or no role in the regulation of normal vascular tone (8, 41) except in the pulmonary vasculature (7, 18, 59, 61). Furthermore, epidemiological evidence that the incidences of primary vascular diseases involving excessive vasoconstriction are several fold higher in premenopausal women than in men (11, 24, 60, 63) suggests that a common mechanism of vasospasm, which may involve constrictor prostanoids, may be responsible (5, 10, 17, 64).

Therefore, in the present investigation, the role of constrictor prostanoids in the sexual dimorphism in vascular reactivity to VP was examined in the thoracic aortas of male and female rats. Because previous studies (7, 18, 35, 39, 51, 52, 57) have established the importance of both the endothelium and gonadal steroid hormones in the regulation of vascular function, the relationships between these factors and constrictor prostanoid function were also examined. To determine the specificity of sex-related differences in constrictor prostanoid potentiation of vascular reactivity, the role of this prostanoid system in responses of male and female aortas to a nonpeptide vasoconstrictor, the α1-adrenergic agonist PE, was also examined.

MATERIALS AND METHODS

Animals

Age-matched male and female Sprague-Dawley rats (4–12 wk old), obtained from either Zivic-Miller Laboratories (Zelienople, PA) or Harlan Sprague Dawley (Indianapolis, IN), were used in the present study. The rats were housed in pairs in standard plastic laboratory rat cages and were segregated by gender at the Northeastern Ohio Universities College of Medicine Comparative Medicine Unit or the Texas A&M University Laboratory Animal Resources and Research vivarium facilities. Both temperature (21–26°C) and lighting (12:12-h light-dark cycle) were controlled. Standard laboratory rat chow (Purina; St. Louis, MO, or Harlan) and tap water were provided ad libitum. At 5 wk of age, some female rats underwent bilateral ovariectomy. The rats were anesthetized with a mixture of ketamine HCl (50 mg/kg) and chloral hydrate (150 mg/kg ip) and were ovariectomized using standard sterile surgical techniques. OVX and sham-OVX female rats were maintained for 8–11 wk until experimentation. At the time of experimentation, male, female, and OVX female rats were 13–16 wk old, and body weights averaged 512 ± 17 (n = 14), 260 ± 5.0 (n = 58), and 348 ± 8 g (n = 37), respectively (means ± SE). The female rats were studied without regard to phase of the estrous cycle because previous studies (54) established that reactivity of female aortas to VP or to the α1-adrenergic agonist PE does not vary significantly during the estrous cycle. All surgical and experimental procedures used in these studies were reviewed and approved by the Northeastern Ohio Universities College of Medicine Institutional Animal Care and Use Committee or the Texas A&M University Laboratory Animal Care Committee.

Preparation of Vascular Tissue

Rats were euthanized by rapid decapitation and the thoracic aortas were removed and placed in chilled Krebs-Henseleit bicarbonate (KHB) solution (4°C), which was continuously gassed with 95% O2-5% CO2. The KHB solution was composed of (in mM) 118.0 NaCl, 25.0 NaHCO3, 10.0 glucose, 4.74 KCl, 2.50 CaCl2, 1.18 MgSO4, and 1.18 KH2PO4 (pH 7.40, osmolality, 292 ± 1 mosmol/kg H2O). The aortas were cleaned of all adipose and connective tissue and the midthoracic region was cut into rings (3 mm long). Extreme care was taken to avoid stretching the vessels or touching the luminal surfaces to preserve the integrity of the endothelium, which was evaluated functionally in all experiments (as described below). In some experiments, the endothelium was removed before the experiments by gently passing a frayed nylon string through the aortic lumen before it was sectioned into rings (51). Successful removal of the endothelium was confirmed functionally in all experiments (see Experimental Protocols).

Two, three, or four adjacent aortic rings were prepared from each animal and were studied in paired, triplicate, or quadruplicate fashion, depending on the experiment. The rings were mounted on two 25-gauge stainless steel wires; one was attached to a stationary stainless steel rod and micrometer and the other was attached to a force displacement transducer (model FT-03D, Grass) for measurement of isometric tension. The transducers were connected to a polygraph (model 2600S; Gould) for a continuous record of contractile tension. Immediately after being mounted, the aortas were immersed in water-jacketed organ baths filled with 15.0 ml of KHB solution, maintained at 37°C, and continuously gassed with 95% O2–5% CO2. The aortic rings were gradually stretched (over a 30-min period) to an optimal passive tension of 2.50 g for male, female, and OVX female aortas (52, 54) and then equilibrated for 90 min. During the equilibration period, the KHB solution in the organ baths was replaced with fresh KHB solution every 20 min. The 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 (1 × 10−6 M, final concentration). After each contraction reached a stable plateau tension, the endothelium-dependent vasodilator ACh was added to the baths (1 × 10−7 M) to assess functional integrity of the endothelium. The baths were rinsed twice and the aortas were allowed to reequilibrate with fresh KHB solution for 30–45 min before further experimentation. Aortic rings to be used in PE experiments were stabilized by a single near-maximal contraction to PE (1 × 10−6 M) to avoid possible tachyphylaxis to PE during the subsequent cumulative concentration-response experiments.

Experimental Protocols

Effects of constrictor prostanoid pathway inhibitors. The effects of constrictor prostanoid pathway inhibitors on vascular reactivity to VP and PE were examined by obtaining cumulative concentration responses to either VP (10−11–10−6 M) or PE (10−11–10−5 M) in endothelium-intact aortas in the presence of the following: 1) the cyclooxygenase inhibitor Indomethacin (Indo) (10 μM) or vehicle (control) in paired male or female aortas; 2) Indo (10 μM), the specific endoperoxide (PGH2) + thromboxane A2 (TXA2) receptor antagonist SQ-29,548 (SQ; 1 μM), or vehicle control (0.03% EtOH) in triplicate female aortic rings; 3) the thromboxane synthase inhibitor dazoxiben (Daz; 10 μM), Daz + SQ (10 and 1 μM respectively), or vehicle control in triplicate female aortic rings. All aortas were pretreated with the inhibitors for 20 min before experimentation. After the concentration responses to VP or PE, the baths were rinsed twice with KHB and allowed to reequi-
liberate 20–30 min before the contractile responses to 80 mM KCl were obtained from the same experimental groups.

**Effects of endothelium in female aorta.** To determine the role of the endothelium in the contractile responses of the female aorta to VP and PE, cumulative concentration responses to either VP (10^{-11}–10^{-6} M) or PE (10^{-11}–10^{-7} M) were obtained from endothelium-intact [Endo(+)] and endothelium-denuded [Endo(–)] aortas. Two pairs of rings were prepared from each female aorta, and one ring of each pair was denuded of its endothelium before being mounted. One pair of aortic rings was pretreated with Indo (10 μM), whereas the remaining pair served as vehicle controls. This allowed comparison of the effects of cyclooxygenase inhibition in both Endo(+) and Endo(–) rings prepared from each female aorta.

**Effects of ovariectomy on constrictor prostanoid function in female aorta.** To determine the effects of ovarian steroid hormones on constrictor prostanoid function in the female rat aorta, cumulative concentration responses to either VP or PE were obtained in aortic rings prepared from O VX female rats. Triplicate rings from each aorta were pretreated with Indo (10 μM), SQ (1 μM), or vehicle (control).

**Chemical reagents and drugs.** The following drugs were used in the study: arginine VP (Bachem; Torrance, CA); PE HCl, ACH HCl, and Indo (all from Sigma; St. Louis, MO); SQ (Cayman; Ann Arbor, MI); and Daz (generously provided by Pfizer Pharmaceuticals; Kent, UK). All drug solutions were prepared fresh daily (except for VP, which was diluted daily from aliquots of 1 × 10^{-3} M stock solution stored at −70°C), and SQ, which was diluted daily from a 2.58 × 10^{-3} M stock solution that was prepared weekly and stored at 4°C. ACH, VP, and PE solutions were kept on ice during the experiments. Stock solutions of the drugs were prepared in KHB solution (VP and ACH), KHB with 100 μM ascorbic acid (PE), 100% EtOH (SQ), double-distilled water (Daz) or 0.05 M Na₂CO₃ (Indo). Indo, SQ, and Daz were added to the baths to produce final concentrations of (in μM) 10 Indo, 1 SQ, and 10 Daz. The final vehicle concentrations were 0.038% EtOH (SQ) and 0.36 mM Na₂CO₃ (Indo). Either VP or PE was added to the organ baths in volumes of 100–200 μl to produce the desired concentrations (expressed as final molar concentrations in the bath solutions). All other chemical compounds were obtained from Sigma or Fisher Scientific (Fair Lawn, NJ) and were of the highest reagent grade quality.

**Data Analysis**

All data are expressed as means ± SE; n indicates the number of animals studied. Contractile responses to VP and PE were normalized by dry weight of the aortic rings and expressed as milligrams contractile force per milligram ring weight. The concentration of VP or PE producing 50% of the maximal response (EC₅₀) was calculated individually from the log concentration-response curve of each aortic ring and reported as the mean ± SE for the particular experimental group. Male and female data groups were analyzed by gender (male vs. female) and by experimental treatment (e.g., control vs. Indo) using two-way analysis of variance (ANOVA) to detect significant differences among the treatment groups, followed by Dunnett’s modification of the t-test to distinguish significant differences between any two means of the data groups. Differences between means were accepted as significant if P ≤ 0.05.

**RESULTS**

**Effects of Constrictor Prostanoid Pathway Inhibitors**

A comparison of the contractile responses of male and female aortas revealed a marked sexual dimorphism in vascular reactivity. Differences in contractile responses between male and female aortic rings were highly significant (0.0001 ≤ P ≤ 0.0055) throughout the concentration response to VP (Fig. 1, Table 1). The maximal contractile response to VP was nearly fivefold higher (P = 0.0001) in female (3,885 ± 332 mg/mg ring wt) than male aortas (810 ± 148 mg), and sensitivity (EC₅₀) was also significantly (P = 0.033) greater in female (5.44 ± 0.80 nM) than in male (9.72 ± 2.51 nM) aortas. Inhibition of cyclooxygenase with Indo attenuated the contractile responses of female but not male aortas at the middle and higher concentrations of VP (Fig. 1). The maximal contractile response of female aortas was reduced ~26% (2,864 ± 236 mg, P = 0.013), whereas the maximal contractile response of male aortas was unchanged by Indo pretreatment (886 ± 163 mg, P = 0.699). Sensitivity to VP (EC₅₀) was unchanged in male (7.65 ± 1.06 nM, P = 0.550) or female (5.78 ± 0.52 nM, P = 0.388) aortas after pretreatment with Indo. The TxA₂/PGH₂ receptor antagonist SQ attenuated VP-induced contractions of female aortas at middle and higher concentrations to a similar extent as Indo (Fig. 2, Table 2). SQ reduced the maximal contractile response ~26% (3,042 ± 290 mg, P = 0.013), but had no effect on sensitivity (EC₅₀) to VP (4.20 ± 0.36 nM, P = 0.388). Contractile responses to 80 mM KCl were quite similar among control, Indo, and SQ groups (Table 2) and did not differ significantly (P >
Inhibition of thromboxane synthase with Daz had no significant effect on the contractile responses of female aortas; however, the combination of Daz + SQ reduced the contractile responses to VP (Fig. 3, Table 2). Maximal contractile response was attenuated ~30% after Daz + SQ pretreatment (Daz control, 3,614 ± 236 mg vs. Daz + SQ, 2,536 ± 161 mg, P = 0.001), whereas sensitivity (EC50) to VP was unchanged (Daz control, 8.36 ± 1.12 nM vs. Daz + SQ, 7.45 ± 1.42 nM, P = 0.579). Contractile responses to 80 mM KCl varied little among Daz control, Daz, and Daz + SQ groups (Table 2) and did not differ significantly (P > 0.05).

PE-induced contractions of male and female aortas also exhibited a marked sexual dimorphism similar to that in response to VP. The maximal contractile response to PE was nearly 50% higher in female (3,785 ± 369 mg) than in male (2,611 ± 69 mg) aortas (P = 0.026; Fig. 4, Table 1), whereas sensitivity (EC50) to PE was nearly identical (P = 0.779) in male (0.197 ± 0.043 μM) and female (0.172 ± 0.056 μM) aortas. Indo significantly attenuated the contractile responses of female but not male aortas at middle and higher concentrations of PE (Fig. 4). The maximal contractile response of female aortas was reduced ~30% (2,660 ± 160 mg, P = 0.007), whereas the maximal contractile response of male aortas was unchanged by Indo (2,388 ± 97 mg, P = 0.091). Sensitivity to PE did not differ significantly in male (0.179 ± 0.028 μM, P = 0.556) or female (0.203 ± 0.057 μM, P = 0.205) aortas in the presence of Indo. The effects of Indo were mimicked by SQ, which attenuated the maximal contractile response of female aortas to PE by 33% (2,426 ± 161 mg, P = 0.007), but had no significant effect on sensitivity to PE (0.105 ± 0.011 μM, P = 0.205; Fig. 5, Table 3). Contractile responses to 80 mM KCl were quite similar among control, Indo, and SQ groups (Table 3) and did not differ significantly (P > 0.05). Again, Daz

Table 1. EC50 and corresponding maximal contractile responses to arginine vasopressin and phenylephrine in thoracic aortas of male and female rats pretreated with indomethacin or its vehicle control

<table>
<thead>
<tr>
<th>Group</th>
<th>EC50, nM</th>
<th>Contractile force, mg/mg ring wt</th>
<th>n</th>
<th>EC50, nM</th>
<th>Contractile force, mg/mg ring wt</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vasopressin</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>Phenylephrine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>9.72 ± 2.51a</td>
<td>810 ± 148c</td>
<td>8</td>
<td>5.44 ± 0.80b</td>
<td>3,855 ± 332d</td>
<td>7</td>
</tr>
<tr>
<td>Indo</td>
<td>7.65 ± 1.06b,c</td>
<td>886 ± 163c</td>
<td>8</td>
<td>5.78 ± 0.52b</td>
<td>2,864 ± 236c</td>
<td>7</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>0.197 ± 0.042f</td>
<td>2,611 ± 69c</td>
<td>6</td>
<td>0.172 ± 0.056f</td>
<td>3,785 ± 368b</td>
<td>6</td>
</tr>
<tr>
<td>Indo</td>
<td>0.179 ± 0.025f</td>
<td>2,388 ± 97e</td>
<td>6</td>
<td>0.203 ± 0.057f</td>
<td>2,860 ± 160e</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. EC50, concentration of agonist producing 50% of the maximal contractile response; Cont, vehicle control; Indo, indomethacin (10 μM). *Within columns and rows for each agonist and experimental treatment (control versus Indo), mean values for EC50 or maximal contractile force without common superscript are significantly different (control versus Indo and male versus female, 0.033 ≥ P ≥ 0.0001). Data are derived from Figs. 1 and 4. EC50 values for phenylephrine are in μM.

Table 2. EC50 and corresponding maximal contractile responses to arginine vasopressin and 80 mM KCl in thoracic aortas of female rats pretreated with dazoxiben, indomethacin, SQ-29,548, or vehicle control

<table>
<thead>
<tr>
<th>Group</th>
<th>EC50, nM</th>
<th>Contractile Force to AVP, mg/mg ring wt</th>
<th>Contractile Force to 80 mM KCl, mg/mg ring wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont</td>
<td>4.86 ± 0.72a</td>
<td>4,126 ± 308b</td>
<td>2,275 ± 165b</td>
</tr>
<tr>
<td>Indo</td>
<td>5.19 ± 0.57a</td>
<td>3,043 ± 277b</td>
<td>2,160 ± 178b</td>
</tr>
<tr>
<td>SQ</td>
<td>4.20 ± 0.36b</td>
<td>3,042 ± 290b</td>
<td>2,457 ± 165b</td>
</tr>
<tr>
<td>Daz Cont</td>
<td>8.36 ± 1.12b</td>
<td>3,614 ± 236b</td>
<td>2,581 ± 170b</td>
</tr>
<tr>
<td>Daz</td>
<td>7.67 ± 0.91b</td>
<td>3,779 ± 244b</td>
<td>2,849 ± 116b</td>
</tr>
<tr>
<td>Daz + SQ</td>
<td>7.45 ± 1.42b</td>
<td>2,536 ± 161b</td>
<td>2,644 ± 144b</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. AVP, arginine vasopressin; Daz, dazoxiben (10 μM); Daz Cont, vehicle control for Daz; SQ, SQ-29,548; Daz + SQ, dazoxiben (10 μM) + SQ-29,548 (1 μM). *Within columns, for each experimental treatment (control versus Indo versus SQ-29,548 and (Daz control versus Daz versus Daz + SQ), mean values for EC50, contracture to AVP, or contraction to KCl without common superscripts are significantly different (0.013 ≥ P ≥ 0.001). Data are derived from Figs. 2 and 3.
alone had no significant effect on the contractile responses of female aortas to PE; however, the combination of Daz and SQ significantly reduced the contractile responses to PE (Fig. 6, Table 3). Maximal contractile response to PE was attenuated >40% in the presence of Daz + SQ (Daz control, 3,983 ± 366 mg vs. Daz + SQ, 2,244 ± 358 mg, P = 0.02), whereas sensitivity (EC50) was unchanged (Daz control, 0.292 ± 0.067 μM vs. Daz + SQ, 0.185 ± 0.050 μM; P = 0.225). Contractile responses to 80 mM KCl varied little among Daz control, Daz, and Daz + SQ groups (Table 3) and did not differ significantly (P > 0.05).

Effects of Endothelium

Removal of the endothelium from female aortas attenuated VP-induced contractions and abolished the effects of Indo compared with Endo (+) rings from the same aortas (Fig. 7, Table 4). The differences in maximal contractile response between Endo (+) (4,505 ± 209 mg) and Endo (−) (3,469 ± 318 mg; P = 0.033) control aortas and between Endo (+) control (4,505 ± 209 mg) and Endo (+) Indo-treated (2,905 ± 334 mg; P = 0.002) aortas were highly significant. However, there were no significant differences between the maximal contractile responses of Endo (−) control (3,469 ± 318 mg) and Endo (−) Indo-treated aortas (3,582 ± 198 mg, P = 0.950). Sensitivity (EC50) to VP did not differ significantly between Endo (+) control (6.75 ± 0.67 nM) and Endo (−) control (3.63 ± 0.83 nM; P = 0.307) or

Table 3. EC50 and corresponding maximal contractile responses to phenylephrine pretreated with Daz, indomethacin, SQ-29548, or vehicle control in thoracic aortas of female rats

<table>
<thead>
<tr>
<th>Group</th>
<th>EC50, μM</th>
<th>Contractile Force to PE, mg/mg ring wt</th>
<th>Contractile Force to 80 mM KCl, mg/mg ring wt</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont</td>
<td>0.187 ± 0.031</td>
<td>3,638 ± 257</td>
<td>2,827 ± 139</td>
<td>9</td>
</tr>
<tr>
<td>Indo</td>
<td>0.168 ± 0.021</td>
<td>2,735 ± 177</td>
<td>2,653 ± 131</td>
<td>9</td>
</tr>
<tr>
<td>SQ</td>
<td>0.105 ± 0.011</td>
<td>2,426 ± 161</td>
<td>2,554 ± 157</td>
<td>9</td>
</tr>
<tr>
<td>Daz-Cont</td>
<td>0.292 ± 0.067</td>
<td>3,983 ± 366</td>
<td>2,937 ± 129</td>
<td>6</td>
</tr>
<tr>
<td>Daz</td>
<td>0.269 ± 0.079</td>
<td>3,291 ± 430</td>
<td>2,616 ± 145</td>
<td>6</td>
</tr>
<tr>
<td>Daz + SQ</td>
<td>0.185 ± 0.050</td>
<td>2,244 ± 358</td>
<td>2,664 ± 182</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. PE, phenylephrine.

Within columns, for each experimental treatment (control versus Indo versus SQ-29,548) and (Daz control versus Daz versus Daz + SQ), mean values for EC50, contraction to PE, or contraction to 80 mM KCl without common superscript are significantly different (0.007 ≤ P ≤ 0.028). Data are derived from Figs. 5 and 6.
between Endo(+)-treated (4.05 ± 1.01) and Endo(-)-treated aortas (3.07 ± 0.53; P = 0.650).

Removal of the endothelium from female aortas did not alter maximal contractile responses to PE (P = 0.205); however, sensitivity (EC50) to PE was increased threefold in control aortas (Endo(+)-control, 0.177 ± 0.015 μM vs. Endo(-)-control, 0.053 ± 0.010 μM; P = 0.023; Table 4). As with VP, removal of the endothelium completely eliminated the attenuating effect of Indo on responsiveness to PE compared with Endo(+)-rings from the same aortas (Fig. 8, Table 4). The difference in maximal contractile response between Endo(+)-control (3.059 ± 156 mg) and Endo(+)-Indo-treated aortas (2.150 ± 187 mg) was highly significant (P = 0.008), whereas maximal responses of Endo(-)-control (3.357 ± 149 mg) and Endo(-)-Indo-treated aortas (3.135 ± 300 mg) did not differ significantly (P = 0.527).

**Effects of Ovariectomy**

Ovariectomy attenuated the contractile responses to PE and abolished the attenuating effects of both Indo and SQ compared with intact female aortas (Fig. 9, Table 5). In intact female aortas, the differences in maximal contractile response among control (3.638 ± 257 mg), Indo- (2.375 ± 77 mg), and SQ-treated aortas (2.426 ± 161 mg) were highly significant (P = 0.007); in contrast, in OVX females, the maximal contractile responses of control (2.995 ± 137 mg), Indo- (2.800 ± 133 mg), and SQ-treated aortas (2.893 ± 112 mg) did not differ significantly (P = 0.488). Sensitivity (EC50) to PE was not altered by OVX and did not differ significantly among control, Indo-, or SQ-treated OVX aortas (P = 0.170).

In initial experiments with VP, ovariectomy failed to consistently alter contractile responses to this agonist or the attenuating effects of either Indo or SQ. Subsequent ovariectomy experiments using rats obtained from several suppliers (Zivic-Miller and Harlan) also yielded variable results with no significant effect on reactivity to VP [P > 0.500; 3.826 ± 129 mg OVX female (n = 17) vs. 4.126 ± 308 mg intact female (n = 9), maximal response]. These variable findings subsequently led to consideration of the possible effects of dietary phytoestrogens, which are often contained in the alfalfa and soy components commonly used as sources of protein in many types of standard laboratory rat chow (41, 42). These substances can interact with the estrogen receptor (31, 42) and thus may have masked the effects of ovariectomy on vascular reactivity to VP. Indeed, when a subsequent group of female rats was maintained on an alfalfa- and soy-free diet (replaced with casein as the major source of protein) (7% corn oil diet, Harlan Tek-Lad), ovariectomy produced a dramatic attenuation of the contractile responses to VP and abolished the attenuating effects of both Indo and SQ (Fig. 10, Table 5), similar to the effects observed for PE. Thus, in intact female aortas, the differences in maximal contractile response to VP among control (4.126 ± 308 mg), Indo- (3.043 ± 277 mg), and SQ-treated aortas (3.042 ± 290 mg) were highly significant (P = 0.013). In contrast, in OVX females, maximal contractile responses of control (2.093 ± 329 mg), Indo- (1.511 ± 152 mg), and SQ-treated aortas (1.721 ± 72 mg) did not differ significantly (P = 0.109). Sensitivity (EC50) to VP was not altered by OVX and did not differ significantly among control, Indo-, or SQ-treated OVX aortas (P = 0.150).

**DISCUSSION**

The results of the present study demonstrate that dramatic male-female differences exist in vascular responsiveness of the rat aorta to both VP and PE, which
are dependent on both the endothelium and the ovarian steroid hormones. The greater responsiveness of the female aorta is due to the release of constrictor prostanoids, which appear to be primarily regulated by the effects of the ovarian steroid hormones on the endothelium. In contrast, the lesser responsiveness of the male aorta to VP is mainly due to the release of NO, as established in previous studies (51, 52).

Effects of Constrictor Prostanoid Pathway Inhibitors

In the present study, Indo attenuated maximal contractile responses to both VP and PE by ~26% and ~30%, respectively, in the female aorta, but had no significant effect on the male aorta. The TxA2/PGH2 receptor antagonist SQ attenuated contractile responses of the female aorta to both VP and PE to the same extent as Indo (26% and 33% of the maximum, respectively). This identical pattern of attenuation reveals that constrictor prostanoids (TxA2 and/or PGH2) mediate approximately one-fourth to one-third of the contractile effects of VP and PE in the female rat aorta, but play no role in the male rat aorta. Inhibition of thromboxane synthase with Daz alone had no significant effect on the contractile responses to VP or PE in the female aorta; however, Daz + SQ attenuated the maximal contractile responses to VP (~30%) and to PE (~40%). These data do not eliminate TxA2 as the constrictor prostanoid released by the female aorta, because it is conceivable that PGH2, which can bind to the TxA2 receptor on vascular smooth muscle, may have accumulated in the presence of thromboxane synthase inhibition (14, 62). Therefore, these data include PGH2 rather than exclude TxA2 as the constrictor prostanoids likely to potentiate the contractile responses of the female rat aorta.

Maximal contractile responses to VP were approximately fivefold higher in female than in male aortas in
the present study. This gender difference in vascular reactivity to VP is consistent with previous studies of the rat aorta (51, 52, 54) and the rat mesenteric vasculature studied both in situ (1) and in vitro (53, 57). Several previous studies suggested that sex differences in responsiveness of the rat aorta to both VP and PE might involve constrictor prostanoids; however, these studies did not examine this mechanism in any detail. Thus inhibition of cyclooxygenase attenuated contractile responses to VP in female but not male aortas (51). Several previous studies suggested that sex differences in responsiveness of the female but not male systemic vasculature to VP and PE.

Effects of Endothelium in Female Aorta

Although the results of past and present studies reveal that constrictor prostanoids contribute significantly to the contractile responses of the female aorta, the site of their synthesis has been unknown. Therefore, the relative contributions of the endothelium and vascular smooth muscle to the constrictor prostanoid system in the female aorta were examined. 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. In the absence of the endothelium, pretreatment with Indo did not alter contractile responses of vascular smooth muscle alone. Removal of the endothelium had similar effects on the vascular responses to PE and Indo, but also significantly increased the sensitivity of the female aorta to PE. Thus PE exerts a similar effect as VP to stimulate constrictor prostanoid release from the endothelium. The increased sensitivity to PE in the absence of the endothelium can most likely be explained by a concomitant loss of endothelium-derived NO release by the female aorta, as demonstrated in previous studies (51, 52). These findings clearly establish an endothelial origin for the agonist-stimulated release of the constrictor prostanoids that potentiate contractile responses of the female rat aorta to VP and PE.

Because both the endothelium and NOS function were intact in the present studies, it is possible that constrictor prostanoid release could have been influenced by the simultaneous release of basal and agonist-induced NO release by the female aorta, as demonstrated in previous studies (51, 52). These findings clearly establish an endothelial origin for the agonist-stimulated release of the constrictor prostanoids that potentiate contractile responses of the female rat aorta to VP and PE.
intact blood vessels. In contrast, other studies (2, 13) have demonstrated that NO promotes the release of prostacyclin and/or TxA2 either in cultured endothelial cells or in intact arterioles. Most of the evidence, although controversial, suggests that the modulatory action of NO mainly involves the prostacyclin but not the TxA2 pathway. However, previous studies (51, 52, 56) of contractile function in the rat aorta, using methods identical to those in the present study, have clearly established that dramatic differences exist in the release of NO by the female aorta in response to VP versus PE. Despite the substantially greater release of NO by the female aorta in response to PE than to VP in these previous studies, constrictor prostanoid release potentiated contractile responses to both VP and PE to a nearly identical extent (26 vs. 30%) in the present study. Together, these past and present data clearly argue against any significant influence of NO on the constrictor prostanoid pathway in the present study.

**Effects of Ovariectomy on Prostanoid Pathway Function in Female Aorta**

To determine the role of ovarian steroids in the regulation of prostanoid pathway function in the female aorta, the effects of ovariectomy on reactivity to VP and PE and prostanoid function were examined. Ovariectomy dramatically attenuated the contractile responses to VP and PE (by 18–49% at maximum) and abolished the effects of both Indo and SQ. These findings reveal that agonist-induced release of constrictor prostanoids in response to VP and PE is strongly dependent on ovarian steroid hormones (probably estrogen). The results of the ovariectomy experiments further suggest that the vasopressinergic and α-adrenergic signal transduction pathways in the endothelium and/or vascular smooth muscle, although qualitatively similar in nature, exhibit differential sensitivities to the modulatory effects of the ovarian steroids (probably estrogen) on agonist-induced release of constrictor prostanoids. This conclusion is based on the probable effects of soy-derived dietary phytoestrogens (48, 49), which appeared to negate the effects of ovariectomy on vascular reactivity to VP but not PE when the rats were fed a standard soy-based laboratory rat chow but not when fed an equivalent soy-free diet. Because the phytoestrogens are known to bind to and activate the estrogen receptor, it seems likely that in the absence of ovarian steroids, the dietary phytoestrogens were responsible for the continued release of constrictor prostanoids in response to VP in the OVX female rat aorta. Indeed, earlier evidence suggests that phytoestrogens are capable of modulating prostanoid biosynthesis by interaction with cyclooxygenase (15). In contrast, PE-induced release of constrictor prostanoids in the OVX female rat aorta could not be maintained by the phytoestrogens, suggesting that the vasopressinergic signal transduction pathway is more sensitive to the modulatory effects of the ovarian steroids (estrogen). This unanticipated and interesting differential effect of estrogen on vasoconstrictor function has not been previously reported and is a potentially very important finding that bears further study.

The greater attenuation of contractile responses to VP observed with ovariectomy (~40%), compared with the effects of Indo or SQ in intact female aortas (~26%), suggests that the ovarian steroids may exert multiple effects on the contractile responses of the female aorta. Because estrogen treatment increased vascular smooth muscle binding site density and reactivity to VP in rat mesenteric arteries in a previous study (57), it is possible that ovariectomy resulted in the concomitant loss of both constrictor prostanoids from the endothelium and VP binding sites from the vascular smooth muscle in the present study. These simultaneous effects on the endothelium and vascular smooth muscle could account for the larger attenuation of contractile responses to VP in the OVX female aorta than those observed with Indo or SQ alone in the intact female aorta.

Similar effects of estrogen to modulate contractile responses of the female aorta to adrenergic agonists and arachidonic acid have been reported in previous studies. Treatment of OVX female rabbits and rats with estrogen enhanced contractile responses of the rabbit aorta to arachidonic acid andnorepinephrine (39) and of the rat aorta to PE (43). Although both studies reported that these contractile responses were attenuated by pretreatment with Indo, suggesting that constrictor prostanoids were involved, neither study identified the actual sources and/or types of prostanoids involved.

The findings of the present study are consistent with epidemiological data that the incidences of primary vascular diseases involving excessive vasoconstriction are higher in premenopausal women than in men, and may be associated with elevated vascular production and/or sensitivity to TxA2. Primary pulmonary hypertension, Raynaud’s disease, acrocyanosis, livedo reticularis, and some forms of migraine headache affect women at rates as much as fourfold higher than men (11, 24, 60, 63). Clinically, an association has been reported between Raynaud’s disease and pulmonary hypertension, migraine headache, and variant angina, which suggests that a common mechanism of vasospasm may be responsible (60). Vascular TxA2 may be the common mechanism of vasospasm, because excessive production of this prostanoid has been implicated in the pathogenesis of several primary vascular diseases in women (5, 10, 17, 19, 64) and in several animal models of pulmonary hypertension (21, 44). Furthermore, the thromboxane synthase inhibitors CGS-13,080 and Daz have been used successfully in the treatment of primary pulmonary hypertension and Raynaud’s disease, respectively (6, 45), and have been used to prevent the early stages of experimentally induced pulmonary hypertension in animals (65).

The higher incidences of primary vascular diseases in premenopausal women suggest that estrogen and/or other ovarian steroids may be responsible for the elevated vascular TxA2 production associated with primary pulmonary hypertension, Raynaud’s disease, and
other vascular diseases involving excessive vascular tone. Indeed, oral contraceptive use in young women increases the risk of pulmonary hypertension (31, 38), myocardial infarction (36), hypertension (34), and other vascular diseases (28), and postmenopausal women undergoing estrogen replacement therapy have elevated urinary levels of both TxA2 and prostacyclin (20). Furthermore, arterial fractional turnover and plasma levels of arachidonate are higher in women than in men, and contraceptive steroids increase the turnover of arachidonate in the vascular wall (23).

Data from a variety of animal studies provide consistent support for the idea that estrogen enhances vascular TxA2 production. Thus, tissue arachidonate levels are higher in female than male rabbits (16), activity of cyclooxygenase in the rat lung varies during the estrous cycle and peaks with the estrogen surge during proestrus (3), and estrogen increases cyclooxygenase-1 gene expression in ovine fetal pulmonary artery endothelium (29). Similarly, in response to exogenous arachidonic acid, isolated female hamster lungs produce fivefold more TxB2 than 6-keto-PGF1α (61). Furthermore, estrogen treatment, both in vivo and in vitro, enhances the production of prostacyclin in the rat aorta (30) and TxA2 in the ovine pulmonary artery (59), and in cultured bovine (27), porcine (47), and rat endothelial (66) and vascular smooth muscle cells (9).

The present study clearly demonstrates that constrictor prostanoids play a greater role in vascular function in female than in male vasculature and that ovarian steroid hormones, probably estrogen, modulate their release and/or actions. However, the mechanism underlying ovarian steroid hormone upregulation of constrictor prostanoid function in the female rat aorta is unknown. It is likely that PGH2 and/or TxA2 production is greater in female than in male aortas; indeed, preliminary measurements suggest that agonist-induced release of TxA2 (as measured by radioimmunoassay of TxB2) is twofold higher in female than in male rats, and both constrictor prostanoid-potentiated contractile responses to VP and TxB2 release are upregulated in parallel by estrogen (32, 55). Vascular smooth muscle sensitivity to these constrictor prostanoids may also be enhanced in female vessels. Isolated perfused lungs from female rats exhibit a greater pressor response to the TxA2 mimetic U-46619 than those from males (18). Furthermore, acute perfusion of the pulmonary vasculature with either 17β-estradiol (10 nM) or diethylstilbestrol (10 nM) sharply potentiates the pressor responses of both male and female rat lungs to U-46619, whereas ovariecotomy attenuates the pressor responses of the female rat lung to this constrictor prostanoid analog (18). Preliminary measurements in the rat aorta also suggest that reactivity to U-46619 is greater in females than in males, and that estrogen is responsible for this sexual dimorphism in vascular function (32).

Although the rat aorta is a large conduit vessel not involved in the regulation of peripheral resistance, and its sensitivity to vasoconstrictor and vasodilator agonists, at least in vitro, is often much lower than smaller resistance-level vessels, it is well established that the functional properties of this blood vessel are more similar to peripheral resistance vessels than those of other large vessel models (e.g., rabbit aorta; Ref. 22); thus the rat aorta serves as a relevant model for the study of gonadal steroid effects on vascular function. Furthermore, many, if not all, of the male-female differences in vascular reactivity to vasoconstrictor agonists such as VP and PE identified in the rat aorta are qualitatively similar to those observed in peripheral microvascular preparations such as the rat mesenteric vasculature (1, 53, 57) and tail artery (33). The ovarian steroid-dependent constrictor prostanoid mechanism identified in the rat aorta in the present study appears to be quite relevant to the regulation of systemic blood pressure because preliminary experiments reveal that intravenous infusion of the PGH2/TxA2 receptor antagonist SQ-29,548 into conscious rats reduces mean arterial blood pressure by 15% in females but has no effect in males (4).

In conclusion, the results of the present study demonstrate that a dramatic male-female difference exists in vascular reactivity to both VP and PE. The greater responsiveness of the female rat aorta is due, in part, to the agonist-induced release of constrictor prostanoids by the female but not male aorta. This sexual dimorphism in the vascular prostanoid system is endothelium dependent and is regulated primarily by the ovarian steroid hormones, probably estrogen. Before this study, constrictor prostanoids were not believed to play a significant role in the regulation of normal vascular tone, except in the pulmonary circulation. The findings of the present study are important because the current dogma surrounding the literature states that vascular TxA2 production is important only in pathophysiologic states such as hypertension in males, but not in normal states or in females (8, 40, 41). This study clearly establishes that constrictor prostanoids play an important role in the regulation of vascular tone in the normal systemic female vasculature. Studies on the role of constrictor prostanoids in the regulation of tone in the systemic female vasculature in pathophysiologic states such as hypertension and their regulation by the ovarian steroid hormones are currently underway in our laboratory.

We gratefully acknowledge the technical assistance of Jennifer L. McRaven, Robin D. Jacquet, and Min Li.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-47432 (to J. N. Stallone).

Preliminary reports of this investigation were presented at the Experimental Biology 1998 Meeting (San Francisco, CA) and the Experimental Biology 1999 Meeting (Washington, DC), and has been published in abstract form (FASEB J 13: A385, 1998 and FASEB J 13: A524, 1999).

Present address of C. T. Fulton: Department of Biology, Southern Indiana University, 8600 University Blvd., Evansville, IN 47712-3596.

REFERENCES


47. Seillan C, Ody C, Russo-Marie F, and Duval D. Differential effects of sex-steroids on prostaglandin secretion by male and...


