Androgen-receptor defect abolishes sex differences in nitric oxide and reactivity to vasopressin in rat aorta

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Stallone, John N., Ronald L. Salisbury, and Clifford T. Fulton. Androgen-receptor defect abolishes sex differences in nitric oxide and reactivity to vasopressin in rat aorta. J Appl Physiol 91: 2602–2610, 2001.—Contractions of rat thoracic aorta to vasopressin (VP) are threefold higher in females (F) than in males (M), primarily because nitric oxide (NO) attenuation of contraction is greater in M. To determine the role of the androgen receptor (AR) in this mechanism, vascular reactivity to VP was examined in thoracic aorta of the testicular-feminized male (Tfm) rat, which has an X-linked, recessive defect in AR function in affected M. Maximal contraction of normal aortas to VP was fourfold higher in F (4,128 ± 291 mg/mg ring wt) than in M (971 ± 133 mg); maximal response of Tfm (3,967 ± 253 mg) was similar to that of normal F. N\(^\text{G}\)-nitro-L-arginine methyl ester increased maximal response to VP threefold in M but had no effect in F or Tfm. In contrast, maximal contraction of normal aortas to phenylephrine was 43% higher in M (4,011 ± 179 mg) than in F (2,808 ± 78 mg); maximal response of Tfm (2,716 ± 126 mg) was similar to that of normal F. N\(^\text{G}\)-nitro-L-arginine methyl ester increased maximal response to phenylephrine by >50% in F and Tfm but had no effect in M. Maximal contractile response to 80 mM KCl did not differ among M, F, or Tfm. Thus androgens and normal vascular AR function are important in the greater NO-mediated attenuation of reactivity to VP in M than in F rat aorta, which may involve specific modulation of endothelial VP signal transduction pathways and NO release by androgens. These data also establish the importance of the Tfm rat as a model to study the effects of androgens on cardiovascular function.

Keywords: aorta; arginine vasopressin; endothelium-derived relaxing factor; gonadal steroid hormones; testicular-feminized rat; vasoconstriction

ACCUMULATING epidemiological, clinical, and experimental animal data suggest that the gonadal steroid hormones play an important role in normal cardiovascular regulation and in the pathogenesis of cardiovascular disease. For example, the incidences of coronary artery disease and systemic hypertension are higher in men than in age-matched premenopausal women (26, 53), whereas diseases involving excessive vascular tone, such as Raynaud’s disease (6) and primary pulmonary hypertension (54), occur more frequently in premenopausal women than in men. Similarly, in genetic and nongenetic rat models of hypertension, males develop higher blood pressure than females, and this sexual dimorphism is reduced or eliminated by gonadectomy (4, 10, 20, 43). The presence of gonadal steroid hormone receptors in the vasculature (7, 16), in the heart (25, 51), and in regions of the medulla oblongata involved in cardiovascular regulation (17, 50) suggests that these sex differences in cardiovascular function and blood pressure could result from the integrated effects of the gonadal steroids to modulate peripheral vasoconstriction, cardiac output, and/or the central nervous system (sympathetic outflow) in sexually dimorphic ways. The posterior pituitary hormone vasopressin (VP) is an extremely potent vasoconstrictor, and there is abundant evidence that endogenous VP plays an important role in the regulation of cardiovascular function and arterial blood pressure, particularly in hypovolemic states such as dehydration and hemorrhage (28, 29, 42). More recent studies have established that substantial sexual dimorphism exists in the cardiovascular actions of VP, which may involve its effects on the heart, vasculature, and/or central nervous system (42). There is increasing evidence that the gonadal steroid hormones play a particularly important role in the acute and long-term modulation of vascular function, and the presence of gonadal steroid hormone receptors in both the endothelium (7) and vascular smooth muscle (VSM; Ref. 19) of blood vessels suggests that gonadal steroid modulation of vascular responsiveness may involve the action of these hormones on one or both of these tissues. A variety of earlier studies demonstrated that significant male-female differences exist in vascular reactivity (9, 21, 31, 47, 57). More recent studies have clearly established that estrogen exerts effects on both endothelial nitric oxide (NO) function (12, 16, 37, 45) and VSM contractile function (32, 49,
that contribute to sex differences in vascular tone (for review, see Ref. 11). In contrast, far less is known about the effects of testosterone on the regulation of endothelial and VSM mechanisms. Whereas several studies have demonstrated that testosterone increases the density of VSM α1-adrenergic (32) and thromboxane (18, 40) receptors, very little is known about the effects of the androgens on endothelial or VSM function.

Substantial male-female differences exist in the vascular reactivity to VP in the rat thoracic aorta (44, 47). The maximal contractile response of female aortas to VP is threefold greater than that of male aortas; in contrast, maximal response of female aortas to the α1-adrenergic agonist phenylephrine (PE) is one-half that of male aortas. These findings established that the sexual dimorphism in vascular reactivity of the rat aorta is not a uniform phenomenon but, rather, is quite vasoconstrictor specific, and that the greater release of endothelial NO in male than in female aortas is the primary mechanism underlying the sex differences in vascular responsiveness to VP (44). Furthermore, the sexual differences in modulation of VP- and PE-induced contraction of the rat aorta by NO are not uniform in nature, strongly suggesting that substantial male-female differences exist in basal and/or agonist-stimulated NO release by the aorta (44). Subsequent vascular reactivity studies on gonadectomized male and female rats revealed that both estrogen and testosterone appear to be important regulators of this endothelial mechanism and are responsible, at least in part, for the vasoconstrictor-specific sexual dimorphism in NO release and vascular reactivity to VP and PE in the rat aorta (45). These findings strongly suggested that estrogen-enhanced release of NO was the primary mechanism underlying the attenuation of PE-induced contraction in the female aorta, whereas testosterone-enhanced release of NO was responsible for the attenuation of VP-induced contraction of the male aorta, although gonadal steroid replacement experiments were not performed in this study.

The testicular-feminized male (Tfm) rat exhibits an X-linked, recessive defect in androgen-receptor (AR) function in affected males (41, 48, 56), similar to the human testicular feminization syndrome. Although genotypically male (XY), these rats develop phenotypically as female and thus serve as a natural “knockout” model to study AR-mediated effects on vascular function. Therefore, in the present investigation, the Tfm rat was employed to further examine the role of the androgens in the sexual dimorphism in vascular reactivity to VP in the rat thoracic aorta. Contractile responses to VP and NO function were examined in the Tfm rat and in normal male and female siblings. To determine the specificity of AR-mediated differences in vascular function, reactivity and NO function of Tfm, male, and female aortas to a nonpeptide vasoconstrictor, the α1-adrenergic agonist PE, were also examined.

MATERIALS AND METHODS

Experimental animals. Age-matched Tfm rats and their normal male and female siblings (24–26 wk old), bred by Dr. Ronald L. Salisbury of the Department of Biology, University of Akron, were used in the present study. The Tfm rat, derived from the King-Holtzmann strain, exhibits an X-linked, recessive defect in AR function in affected males (41, 48). More recent studies have identified the molecular defect in the Tfm rat as a single transition mutation within the steroid-binding domain of the AR, thus preventing normal binding of androgens to the AR (56). Females carrying the mutant gene responsible for this defect are unaffected by it (because they also carry a normal copy of the gene), whereas one-half of their male offspring receive the defective gene. Although genotypically male (XY), these rats develop phenotypically as female and thus serve as a natural knockout model to study AR-mediated effects on vascular function.

The rats were housed in pairs in standard plastic laboratory rat cages and were segregated by sex (male, female, or Tfm) at the Northeastern Ohio Universities College of Medicine Comparative Medicine Unit vivarium facilities. Both temperature (21–26°C) and lighting (12:12-h light-dark cycle) were controlled. Purina laboratory chow (Purina Mills, St. Louis, MO) and tap water were provided ad libitum. At the time of experimentation, Tfm, male, and female rats were 24–26 wk old, and body weight averaged 356 ± 13.0 (SE) g (n = 21 rats), 458 ± 26.0 g (n = 20 rats), and 284 ± 16.3 g (n = 20 rats), respectively. Normal female rats were studied without regard to phase of the estrous cycle, because previous studies established that reactivity of female aortas to VP or to the α1-adrenergic agonist PE does not vary significantly during the estrus cycle (47). 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.

Preparation of vascular tissue. Tfm, male, and female rats were killed by decapitation, and the thoracic aortas were removed and placed in chilled Krebs-Henseleit-bicarbonate (KHB) solution (4°C) and gassed with 95% O2-5% CO2. The aortas were cleaned of all adipose and connective tissue, and the midthoracic region was cut into rings to avoid stretching the tissue or touching the luminal surfaces to preserve the endothelium, which was evaluated functionally in all experiments (as described below). The rings were cleaned of all adipose and connective tissue, and the midthoracic region was cut into rings (3 mm long). Extreme care was exercised during preparation of the rings to avoid stretching the tissue or touching the luminal surfaces to preserve the endothelium, which was evaluated functionally in all experiments (as described below). Two adjacent aortic rings were studied from each animal in paired fashion. The rings were mounted on two 25-gauge stainless steel wires: the lower one was attached to a stationary stainless steel rod, and the upper one was attached to a force-displacement transducer (Grass FT-03D) for the measurement of isometric tension. The transducer was connected to a polygraph (Gould 2600S) for a continuous record of blood vessel tension.

Immediately after mounting, the aortic rings were suspended 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. Before the start of the experiments, the blood vessels were stretched gradually (over a 30-min period) to an optimal passive tension of 2.50 g and equilibrated for a period of 60–90 min. During the equilibration period, the bathing solution in the organ baths was replaced with freshly gassed, warmed KHB solution every 20 min. The passive tension was adjusted (when necessary) to maintain 2.50 g throughout the equilibration and experimental periods. This optimal passive tension for Tfm, male, and female aortic rings was determined in preliminary experiments and was

J Appl Physiol • VOL 91 • DECEMBER 2001 • www.jap.org
identical to that for male and female aortas as determined in previous studies (44, 45, 47).

**Experimental protocol.** After the equilibration period, the aortic rings were stabilized by two successive near-maximal contractions with PE (1 × 10⁻⁶ M; see Refs. 5, 15, 47). 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 then changed twice, and the tissues were allowed to reequilibrate for 30–45 min. After the second reequilibration period, arginine VP was added to the baths in a cumulative manner to obtain a concentration-response curve for each ring, allowing a stable plateau tension to be attained at each concentration. The effects of NO synthase (NOS) inhibition on vascular reactivity to VP were assessed by the addition of N⁶-nitro-L-arginine methyl ester (L-NAME; 250 μM) to the tissue bath KHB of one aortic ring of each pair during the second reequilibration period and during subsequent contractions with PE and VP. The other ring of each pair served as the control with the addition of L-arginine (2.5 mM; the precursor for NO synthesis; Ref. 36) to the tissue bath KHB. Both L-NAME and N⁶-monomethyl-L-arginine competitively inhibit endothelial NOS (eNOS) to a similar extent, and both compounds exert qualitatively similar enantiomerically specific effects on vascular tissues both in vitro and in vivo, which are at least partially reversible in the presence of excess L-Arg (33, 34, 38).

To determine the specificity of any AR-mediated effects on vascular reactivity and NO function, the contractile responses of the thoracic aorta to a nonpeptide vasconstrictor, the α₁-adrenergic agonist PE, were examined in a separate group of Tfm, male, and female rats. Concentration-response curves for paired Tfm, male, and female aortas were obtained in the presence and absence of eNOS inhibition after a protocol identical to that for VP, except that, after the 60- to 90-min equilibration period, aortic rings were stabilized by only one near-maximal contraction with PE (1 × 10⁻⁶ M) to avoid possible tachyphylaxis to PE during the subsequent cumulative concentration-response curve.

**Chemical reagents and drugs.** The following drugs were used in the study: arginine VP (Bachem, Torrance, CA), L-Arg, L-NAME, and PE hydrochloride (all from Sigma Chemical, St. Louis, MO). All drug solutions were prepared fresh daily (except for VP, which was diluted daily from a 1 × 10⁻³ M stock solution stored at −70°C); VP and PE solutions were kept on ice during the experiments. Stock solutions of the drugs were prepared in KHB solution (VP, L-Arg, and L-NAME) or KHB with 0.1 mM ascorbic acid (PE). L-NAME was added to KHB to produce a final concentration of 250 μM; VP or PE were 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 Chemical or Fisher Scientific (Fair Lawn, NJ) and were of 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 have been normalized by dry weight of the aortic rings and are expressed as milligrams of tension per milligram ring weight. The concentration of VP or PE producing EC₅₀ was calculated individually from the concentration-response curve of each aortic ring and reported as the mean ± SE for the particular experimental group. Data groups were analyzed by sex (Tfm vs. male vs. female) and experimental treatment (L-NAME vs. L-Arg) using two-way analysis of variance to detect significant differences, followed by paired or unpaired t-tests to distinguish significant differences among the means of Tfm, male, and female data groups. To correct for the increase in type I error associated with multiple comparisons, a Bonferroni modification of the t-test was employed (24).

**RESULTS**

**Effects of NOS inhibition on concentration response of Tfm, male, and female rat aortas to VP.** There was a marked sexual dimorphism in the contractile response of the endothelium-intact rat aorta to VP (Fig. 1). Contractile tension produced by male aortas was one-fourth of that produced by female aortas through the entire range of VP concentrations studied (10⁻¹¹-10⁻⁶ M). In
contrast, contractile responses of Tfm aortas were remarkably similar to those of female aortas (Fig. 1). At the maximal concentration of VP (1 × 10⁻⁶ M), male aortas averaged 971 ± 133 mg/mg ring wt, whereas female and Tfm aortas averaged 4,128 ± 291 and 3,967 ± 253 mg/mg ring wt, respectively (Table 1). Inhibition of NOS with l-NAME markedly potentiated contractile responses in male aortas through the entire range of VP concentrations, increasing maximal contractile tension by nearly threefold (2,578 ± 48 mg/mg ring wt; Fig. 1, Table 1). In contrast, inhibition of NOS had much less effect in female and Tfm aortas; although contractile tension increased significantly in both groups at lower concentrations of VP, maximal tension was unaffected in either female (4,205 ± 303 mg/mg ring wt) or Tfm (4,023 ± 285 mg/mg ring wt) aortas (P > 0.05; Fig. 1, Table 1). The differences in contractile responses in control (l-Arg-treated) male vs. female and Tfm aortas and l-NAME-treated male vs. female and Tfm aortas are highly significant (P ≤ 0.0002) at both the middle (1 × 10⁻⁸ M) and maximal (1 × 10⁻⁶ M) concentrations of VP. The differences in contractile tension in l-NAME-treated male vs. l-NAME-treated female aortas are also highly significant (P ≤ 0.007) at both the middle and maximal concentrations of VP, whereas the differences in l-NAME-treated male vs. l-NAME-treated Tfm aortas are significant (P ≤ 0.0085) only at the maximal concentration of VP (Fig. 1, Table 1).

The EC₅₀ (sensitivity) of control (l-Arg-treated) male and female aortas was nearly identical, whereas the sensitivity of Tfm aortas was slightly but significantly (P ≤ 0.016) lower (Table 1). Pretreatment with l-NAME increased the sensitivity of female and Tfm aortas to VP slightly but significantly (P < 0.012) but had no effect on male aortas (P > 0.05; Table 1).

Relaxation responses to ACh were used to assess the functional integrity of the endothelium in Tfm, male, and female aortas. A single concentration of ACh (1 × 10⁻⁷ M) in aortas precontracted with a near-maximal concentration of PE (1 × 10⁻⁶ M) produced similar (P > 0.05) relaxations in Tfm (47 ± 3.9% of PE contraction), male (44 ± 4.9%), and female (48 ± 5.1%) aortas; pretreatment of paired Tfm, male, and female aortas with l-NAME abolished relaxation responses to ACh.

Contractile responses of Tfm, male, and female aortas to 80 mM KCl were obtained after the concentration-response experiments with VP to determine the specificity of sex- and AR-mediated differences in aortic reactivity. Maximal contractions to 80 mM KCl were similar in Tfm, male, and female aortas (P > 0.05); pretreatment of paired aortas with l-NAME enhanced contractile response of male aortas by >20% (P < 0.01) but did not alter responses of female or Tfm aortas (Table 1).

Effect of NOS inhibition on concentration response of Tfm male and female rat aortas to PE. The contractile responses of the endothelium-intact rat aorta to PE differ substantially among Tfm, male, and female aortas (Fig. 2); however, the sexual dimorphism is opposite to that observed in response to VP. Thus contractile tension produced by male aortas was 40% higher than that produced by female or Tfm aortas through the entire range of PE concentrations studied (10⁻¹⁰ to 10⁻⁵ M). At the maximal concentration of PE (1 × 10⁻⁵ M), male aortas averaged 4,011 ± 179 mg/mg ring wt, whereas female and Tfm aortas averaged 2,809 ± 78 and 2,716 ± 126 mg/mg ring wt, respectively. Inhibition of NOS in Tfm, male, and female aortas with l-NAME also resulted in substantial sex- and AR-mediated differences in the potentiation of contractile responses to PE, which were opposite to those observed in response to VP. Thus pretreatment of endothelium-intact aortas with l-NAME enhanced contractile responses of female and Tfm aortas through the entire concentration response range of PE and increased maximal tension to 4,417 ± 157 and 4,258 ± 125 mg/mg ring wt, respectively (Fig. 2, Table 2). In contrast, l-NAME had much less effect on male aortas and did not alter maximal contractile tension. The differences in contractile tension in control (l-Arg-treated) male vs. female and Tfm aortas are highly significant (P < 0.0001) at both the middle (1 × 10⁻⁷ M) and maximal (1 × 10⁻⁵ M) concentrations of PE. The differences in contractile tension in l-Arg-treated female and Tfm aortas vs. l-NAME-treated female and Tfm aortas are also highly significant (P < 0.0001) at both the middle and maximal concentrations of PE, whereas the difference between l-Arg-treated male vs. l-NAME-treated male aortas is only significant (P < 0.0001) at the middle concentration of PE (Fig. 2, Table 2).

Table 1. Maximal contractile tension and EC₅₀ values of testicular-feminized male, normal male, and female rat thoracic aortas in response to arginine vasopressin and 80 mM KCl

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>Maximal Tension to AVP, mg/mg ring wt</th>
<th>AVP EC₅₀, nM</th>
<th>Maximal Tension to 80 mM KCl, mg/mg ring wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tfm + l-Arg</td>
<td>10</td>
<td>3,967 ± 253a</td>
<td>9.47 ± 1.08b</td>
<td>2,862 ± 147b,a</td>
</tr>
<tr>
<td>Tfm + l-NAME</td>
<td>10</td>
<td>4,023 ± 285c</td>
<td>5.02 ± 0.88b</td>
<td>3,202 ± 199b,a</td>
</tr>
<tr>
<td>NM + l-Arg</td>
<td>9</td>
<td>971 ± 133a</td>
<td>5.86 ± 1.12b</td>
<td>2,799 ± 110b,a</td>
</tr>
<tr>
<td>NM + l-NAME</td>
<td>9</td>
<td>2,578 ± 48b</td>
<td>5.96 ± 1.61b</td>
<td>3,386 ± 183b,a</td>
</tr>
<tr>
<td>NF + l-Arg</td>
<td>9</td>
<td>4,128 ± 291a</td>
<td>5.86 ± 0.94b</td>
<td>2,809 ± 116b,a</td>
</tr>
<tr>
<td>NF + l-NAME</td>
<td>9</td>
<td>4,205 ± 303a</td>
<td>2.98 ± 0.42b</td>
<td>3,177 ± 124b,a</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. AVP, arginine vasopressin; NF, normal female; NM, normal male; Tfm, testicular feminized male; l-NAME, N⁰-nitro-l-arginine methyl ester (250 μM); l-Arg, l-arginine (2.5 mM, control for l-NAME). a,b,Within each data column, mean values without common superscript are significantly different (P ≤ 0.010).
The sensitivity (EC$_{50}$) of control (L-Arg-treated) Tfm, male, and female aortas was similar and did not differ significantly ($P > 0.05$; Table 2). Pretreatment with L-NAME dramatically increased the sensitivity of female and Tfm aortas to PE by eightfold ($P \leq 0.0001$), whereas the sensitivity of male aortas increased by only fourfold ($P \leq 0.0028$; Table 2).

Relaxation responses to ACh were used to assess the functional integrity of the endothelium in Tfm, male, and female aortas. A single concentration of ACh (1 $\times$ 10$^{-7}$ M) in aortas precontracted with a near-maximal concentration of PE (1 $\times$ 10$^{-6}$ M) produced similar ($P > 0.05$) relaxations in Tfm (31 $\pm$ 5.0% of PE contraction), male (33 $\pm$ 3.2%), and female (37 $\pm$ 3.2%) aortas; pretreatment of paired Tfm, male, and female aortas with L-NAME abolished relaxation responses to ACh.

Contractile responses of Tfm, male, and female aortas to 80 mM KCl were obtained after the concentration-response experiments with PE to determine the specificity of sex- and AR-mediated differences in aortic reactivity. Maximal contractions to 80 mM KCl were similar in Tfm, male, and female aortas ($P > 0.05$); pretreatment of paired aortas with L-NAME enhanced contractile response of female and Tfm aortas by $\sim$25% ($P \leq 0.0117$) but did not alter responses of male aortas (Table 2). Mean responses to 80 mM KCl for a given experimental group (e.g., normal males) differed slightly between PE and VP study groups; however, none of the differences in means between PE and VP studies were statistically significant ($P > 0.05$), with the exception of the L-Arg-treated Tfm aortas. Mean values for this experimental group differed slightly but significantly ($P < 0.05$) between PE (2,439 $\pm$ 178 mg; Table 2) and VP (2,862 $\pm$ 147 mg; Table 1) studies. The apparently lower response of the PE group to 80 mM KCl was mainly the result of 3 of 11 rats that exhibited unusually low responses to 80 mM KCl, despite normal responses to PE; thus it seems highly unlikely that their responses to KCl adversely altered the validity of the observed differences in the concentration responses of Tfm aortas to VP vs. PE.

### DISCUSSION

In the present investigation, vascular reactivity and NO function were examined in the thoracic aorta of the Tfm rat. The observed differences in the concentration responses of Tfm aortas to VP vs. PE.

### Table 2. Maximal contractile tension and EC$_{50}$ values of testicular-feminized male, normal male, and female rat thoracic aortas in response to phenylephrine and 80 mM KCl

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>n</th>
<th>Maximal Tension to PE, mg/mg ring wt</th>
<th>PE EC$_{50}$, $\mu$M</th>
<th>Maximal Tension to 80 mM KCl, mg/mg ring wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tfm + L-Arg</td>
<td>11</td>
<td>2,716 $\pm$ 126$^a$</td>
<td>0.204 $\pm$ 0.047$^a$</td>
<td>2,439 $\pm$ 178$^b$</td>
</tr>
<tr>
<td>Tfm + L-NAME</td>
<td>11</td>
<td>4,258 $\pm$ 125$^a$</td>
<td>0.024 $\pm$ 0.003$^b$</td>
<td>3,078 $\pm$ 92$^a$</td>
</tr>
<tr>
<td>NM + L-Arg</td>
<td>11</td>
<td>4,011 $\pm$ 179$^a$</td>
<td>0.193 $\pm$ 0.031$^a$</td>
<td>3,011 $\pm$ 150$^{a,c}$</td>
</tr>
<tr>
<td>NM + L-NAME</td>
<td>11</td>
<td>4,347 $\pm$ 140$^a$</td>
<td>0.051 $\pm$ 0.016$^b$</td>
<td>3,061 $\pm$ 137$^a$</td>
</tr>
<tr>
<td>NF + L-Arg</td>
<td>11</td>
<td>2,809 $\pm$ 78$^a$</td>
<td>0.258 $\pm$ 0.058$^a$</td>
<td>2,537 $\pm$ 172$^{a,b,c}$</td>
</tr>
<tr>
<td>NF + L-NAME</td>
<td>11</td>
<td>4,417 $\pm$ 157$^a$</td>
<td>0.094 $\pm$ 0.007$^a$</td>
<td>3,142 $\pm$ 139$^a$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n$ = no. of animals. PE, phenylephrine. $^a$–$^c$Within each data column, mean values without common superscript are significantly different ($P \leq 0.012$).

J Appl Physiol • VOL 91 • DECEMBER 2001 • www.jap.org
Tfm rat, a breed with a sex-linked, recessive defect in AR function in affected males, to determine the role of the AR in the sexually dimorphic vascular responsiveness to VP in the rat aorta. The results clearly establish the importance of androgens and normal AR function in the striking male-female differences that exist in endothelial NO-mediated attenuation of vascular reactivity to VP in the rat aorta and that the effects of androgens on the endothelium are qualitatively and quantitatively vasoconstrictor specific in nature.

**Effects of NOS inhibition on concentration response of Tfm, male, and female rat aortas to VP.** Contractile responses of Tfm aortas to VP and the effects of NOS inhibition were remarkably different from those of normal male and were nearly identical to those of normal female aortas. In both past and present studies, inhibition of NOS in aortas of normal male rats resulted in a marked potentiation of the contractile responses to VP. In contrast, inhibition of NOS in aortas of female rats resulted in little or no change in the contractile responses to VP (44). In previous studies, gonadectomy of normal male rats markedly potentiated contractile responses to VP in endothelium-intact aortas and virtually eliminated the NO-mediated attenuation of VP-induced contractions prominent in the aortas of intact male rats, whereas gonadectomy of female rats did not appreciably alter the minor effects of NO in female aortas (45). Interestingly, in the present study, the contractile responses to VP in the aortas obtained from Tfm rats, which are unable to respond to androgens, are virtually identical to those of gonadectomized male rats in the previous study (45). Taken together, these findings clearly establish the importance of androgens and normal AR function in the regulation of endothelial NO function and vascular reactivity to VP in the male rat aorta.

The strikingly different patterns of attenuation of the contractile responses to VP by endothelial NO observed in Tfm, male, and female rat aortas in the present and previous (44) studies suggest that greater amounts of NO are released by normal male than by normal female aortas. In the normal male aorta, basal and agonist-induced NO release attenuated the contractile effects of VP throughout the concentration-response curve and reduced the maximal response to VP substantially. In contrast, in the normal female aorta, the effects of basal NO (but little or no agonist-induced NO) are most apparent at lower concentrations of VP, thereby reducing the sensitivity but not the maximal response to VP. The loss of VP-induced NO release in the Tfm rat aorta, which occurs in the absence of normal AR function, results in a concentration response similar to that of normal female or gonadectomized male rat aortas (45). The possibility that VP-stimulated NO release occurs in the male rat aorta is supported by pharmacological evidence from Yamada et al. (55), who reported that the V2-selective VP receptor agonist desmopressin induced NO-dependent relaxation of the rat aorta and from earlier studies by Katusic et al. (22), which established that VP causes endothelium-dependent relaxation of the canine basilar artery, which was later shown to involve the release of NO (8). Alternatively, it is possible that the apparent differences in NO between male and female aortas in the present study result from sex differences in the responsiveness of VSM to NO; however, studies by Kauser and Rubanyi (23) demonstrated that relaxation responses to the NO donor sodium nitroprusside do not differ between male and female rat aortas.

In the absence of NO synthesis, maximal contractile responses remained significantly higher in normal female and Tfm aortas than in the normal male aorta; thus other endothelial and/or VSM mechanisms also may be involved in the greater responsiveness of the female aorta to VP. Recent studies by Fulton and Stallone (13) demonstrated that endothelial PG endoperoxide (PGH2) and/or thromboxane A2 are responsible for nearly one-third of the contractile response to VP in the female aorta and that this mechanism is dramatically upregulated by androgenic ovarian steroid hormones (probably estrogen; Ref. 13). Thus the substantial sex differences in contractile responses of the rat aorta to VP appear to result from both the testosterone-dependent attenuating effect of NO release in the male aorta and the estrogen-dependent potentiating effect of constrictor prostanoid release in the female aorta; however, other as of yet unidentified endothelial mechanisms may also be involved.

**Effects of NOS inhibition on concentration response of Tfm, male, and female rat aortas to PE.** Similar to VP, there is a marked sexual dimorphism in the vascular responsiveness to the α1-adrenergic agonist PE and in the attenuation of PE-induced contraction by NO (Fig. 2); however, the dimorphism is opposite to that observed with VP. These findings indicate that sexually dimorphic vasoconstriction of the rat aorta is a vasoconstrictor-specific phenomenon and that sexual differences in the modulation of vasoconstrictor action by NO are not uniform in nature, strongly suggesting that substantial male-female differences exist in basal and/or agonist-stimulated NO release in the rat aorta, in agreement with earlier studies (44, 45). Interestingly, the contractile responses to PE in Tfm aortas, in the absence of normal AR function, were extraordinarily different from those of normal male and were nearly identical to those of normal female aortas. These data suggest that testosterone enhances vascular reactivity to PE primarily by reducing the release of endothelial NO in response to PE; in contrast, ovarian steroids (probably estrogen) appear to reduce aortic reactivity to PE primarily by enhancing the release of NO (45), an effect that has been confirmed in other studies (16, 37). Thus sex differences in the release of endothelial NO are the primary mechanism underlying the sexual dimorphism in vascular reactivity to PE, as well as VP, in the rat aorta.

**Gonadal steroid regulation of eNOS in male and female aortas.** The results of the present study clearly establish that androgens exert important regulatory effects on endothelial NO release in the male rat aorta, which are vasoconstrictor specific and appear to in-
volve basal and/or agonist-stimulated release of NO. This possibility is supported by the finding of the present study that the marked VP-induced release of NO seen in the normal male aorta is abolished in the absence of normal AR function in the Tfm aorta and, furthermore, by the finding that NO-mediated attenuation of the contractile responses to PE in normal male and female aortas exhibits a fundamentally different pattern than that observed with VP. The lack of significant differences in the maximal contractile responses of Tfm, male, and female aortas to KCl in the present study (Tables 1 and 2) further supports the idea that the gonadal steroids exert agonist-specific effects on the endothelium to influence NO release, rather than general stimulatory or inhibitory effects on endothelial or VSM function of the rat aorta. In most experimental groups (normal male, normal female, or Tfm) in both the VP and PE studies, L-NAME appeared to exert a slight potentiating effect on the contractile response to 80 mM KCl (∼200–400 mg); however, these differences between L-NAME and L-Arg groups were statistically significant in only some of the groups. Similar findings were reported in two previous studies (44, 45), which appear to result from the effect of L-NAME to inhibit basal release of NO, which has a slight and variable effect on maximal contractile responses to 80 mM KCl. Overall, the responses to 80 mM KCl indicate that there is little or no male-female difference in the contractile responses of the VSM, which is shown by the lack of significant differences among L-NAME-treated normal female, normal male, and Tfm aortas, both in arginine VP and PE studies. Relaxation responses to ACh were used in the present study to assess endothelial integrity of the aortas. Although only a single concentration of ACh was tested, the lack of differences in the relaxation responses of Tfm, male, and female aortas further supports the concept that the gonadal steroids exert agonist-specific effects on endothelial NO release.

The presence of both α-adrenergic (3) and vasoressinalgeric (22, 55) receptors in the endothelium suggests that agonist- and sex-specific release of NO results from gonadal steroid modulation of these endothelial receptors, which mediate activation of eNOS and the release of NO. Indeed, endothelial and VSM receptors for estrogen have been identified in several species (7, 16, 30, 52), and estrogen-mediated increases in eNOS activity and NO release through the endothelial receptors have been reported (16). In contrast, much less is known concerning the effects of androgens on endothelial or VSM function. Receptors for both androgens and estrogens have been identified in aortic VSM of rabbit and rat (30, 52). Whereas several studies have demonstrated that testosterone increases the density of VSM α-adrenergic (32) and thromboxane (18, 40) receptors in the rat and/or guinea pig, very little is known about the effects of the androgens on endothelial function. Taken together, the results of past (44, 45) and present studies, which employed the AR-defective Tfm rat, are the first to clearly establish the importance of androgens and normal AR function in the regulation of endothelial NO function and vascular reactivity to VP and PE in the male rat aorta. These findings strongly suggest that agonist- and sex-specific release of NO results from modulation of endothelial α-adrenergic and vasoressinalgeric receptor expression by the androgens and possibly estrogens. Alternatively, it is possible that the androgens (and estrogens) might influence other aspects of the endothelial VP receptor signal transduction pathway (e.g., G protein expression, intracellular calcium, or kinases) or might modulate the activity of eNOS. Interestingly, circulating plasma concentrations of both testosterone and estradiol are elevated in the Tfm rat (1, 35, 39) because of the lack of normal AR-mediated negative feedback at the pituitary and/or hypothalamus and the resultant increase in testosterone secretion and peripheral aromatization to estrogen. The higher plasma testosterone level is inconsequential in the Tfm rat because of the defect in AR function; however, the elevated plasma estradiol level could play a role in the regulation of vascular function in this animal, because estrogen receptors have been identified in the aorta of normal male rabbits (52), as well as rats (30), and estrogen reduces aortic reactivity to PE, primarily by enhancing the release of NO (16, 37, 45). Thus the vascular responses of the Tfm aorta to VP and PE observed in the present study, which are remarkably similar to those of the normal female aorta, may result from the increased effects of estradiol, as well as the absence of normal effects of testosterone.

Although the rat aorta is a large conduit vessel not involved in the regulation of peripheral resistance, it is well established that the functional properties of this blood vessel are more similar to peripheral resistance vessels than are those of other large vessel models (e.g., rabbit aorta; Ref. 15); 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 have also been observed in peripheral microvascular preparations, such as the rat mesenteric vasculature (2, 46, 49) and tail artery (27).

Overall vascular tone depends on the relative balance between vasoconstrictor and vasodilator pathways in VSM and/or endothelium. The male-female differences in endothelial and VSM reactivity to vasoconstrictor agonists, such as VP and PE, demonstrated in past and present studies establish the importance of the gonadal steroids as modulators of endothelial and/or VSM function in the rat and other species and that the effects of androgens and estrogens on the endothelium are not a uniform phenomenon but rather are qualitatively and quantitatively vasoconstrictor specific in nature. Thus both androgens and estrogens may act on the endothelium and/or VSM to either attenuate or potentiate vascular tone, which reveals that the effects of the gonadal steroids on vascular tone are much more complex in nature than the widely...
accepted dogma that estrogens are beneficial, whereas androgens are deleterious to vascular function.

In conclusion, the results of the present study demonstrate that male-female differences in the release of endothelial NO are the primary mechanism underlying the prominent sexual dimorphism in vascular reactivity to VP and PE in the rat aorta. More importantly, these studies are the first to clearly establish the importance of androgens and normal vascular AR function in the regulation of endothelial NO function in the male rat aorta, which are vasoconstrictor specific, and appear to involve basal and/or agonist-stimulated release of NO. Finally, these findings strongly suggest that agonist- and sex-specific release of NO results from male-female differences in the expression of endothelial α-adrenergic and vasopressinergic receptors, which appear to be regulated by the effects of androgens and estrogens, resulting in modulation of the release of NO.

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