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Sex differences in extracellular and intracellular calcium-mediated vascular reactivity to vasopressin in rat aorta

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Abstract

In rat thoracic aorta, contractile responses to arginine vasopressin are two-fold higher in females than in males. To determine the roles of extracellular and intracellular Ca^{2+} in this sexual dimorphism in vascular function, vascular reactivity and Ca^{2+} channel function were examined in thoracic aortae of male and female rats. In the presence of diltiazem (10 μM), maximal contraction to vasopressin was reduced to a greater extent in male (65 ± 2%) than in female aortae (38 ± 1%). Maximal contractile responses to KCl and Bay K 8644 were similar in male and female aortae. Sensitivity to KCl was slightly but significantly higher in male than in female aorta; in contrast, sensitivity to Bay K 8644 was nearly three-fold higher in males than in females. Removal of the endothelium enhanced sensitivity to KCl similarly in male and female aortae. In the presence of simvastatin (60 μM; an inhibitor of intracellular Ca^{2+} release), reactivity to vasopressin was reduced substantially in female (42 ± 1%) but unaltered in male aortae. Removal of the endothelium enhanced the inhibitory effect of simvastatin in both female (73 ± 2%) and male aortae (41 ± 2%). These findings demonstrate that male aortae depend more upon extracellular Ca^{2+} influx, whereas female aortae depend more upon intracellular Ca^{2+} release for vasopressin-induced contraction.

Keywords: Calcium; Sexual dimorphism; Vasopressin; Simvastatin; Diltiazem; (Rat)

1. Introduction

Arginine vasopressin is a potent vasoconstrictor and there is abundant evidence that endogenous vasopressin plays a role in the regulation of cardiovascular function and arterial blood pressure, particularly in hypovolemic states (Cowley, 1982; Share, 1988). Recent studies have established that significant sexual dimorphism exists in the vascular reactivity to vasopressin in the rat, both in the thoracic aorta and mesenteric vasculature (Stallone et al., 1991; Stallone, 1995). Maximal contractile response and sensitivity of the female aorta to vasopressin are more than twice those of the male aorta. Similarly, maximal vasoconstrictor response of the isolated, perfused mesenteric vasculature is substantially higher in females than in males. This sexual dimorphism in vascular function is not a uniform phenomenon, but rather is vasoconstrictor-specific, since maximal contractile response of the female aorta to the α_1-adrenoceptor agonist phenylephrine is half that of the male aorta (Stallone et al., 1991). More recent studies have established that male–female differences in nitric oxide (NO) release are the primary mechanism underlying these sex differences in vascular reactivity to vasopressin and phenylephrine in the rat aorta, and that both estrogen and testosterone are important regulators of this endothelial mechanism (Stallone, 1993, 1994).

Although previous studies have established that the gonadal steroid hormones may regulate vascular function through the modulation of NO and endothelial function, little is known about the effects of these hormones on vascular smooth muscle Ca^{2+} regulation. Since cytosolic free Ca^{2+} plays a central role in mediating vascular smooth muscle contraction, the possible roles of intracellular and extracellular Ca^{2+} in sex differences in vascular function are of great interest. A variety of studies have demonstrated that the relative contributions of these two sources of Ca^{2+} to vascular smooth muscle contraction differ with the species and vasoconstrictor agonists studied. For example, in both the rabbit and rat, α_1-adrenoceptor mediated

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vascular contraction depends upon both the rapid release of intracellular Ca^{2+} from the sarcoplasmic reticulum and the slower entry of extracellular Ca^{2+} via vascular smooth muscle membrane Ca^{2+} channels; however, the relative contributions of these two mechanisms differ between species. In the rabbit, larger conduit vessels such as the aorta are more dependent upon intracellular Ca^{2+} release, whereas smaller resistance vessels such as the mesenteric arterioles are more dependent upon extracellular Ca^{2+} entry (Cauvin et al., 1982a,b). In contrast, in the rat, both the aorta and mesenteric resistance vessels are more dependent upon extracellular Ca^{2+} entry than on intracellular Ca^{2+} release for α_1-adrenergic-induced vascular contraction (Cauvin and Malik, 1984).

Relatively few studies have examined the relative contributions of intracellular versus extracellular Ca^{2+} pools to vasopressin-induced contraction of vascular smooth muscle. Vasopressin-induced contraction of resistance vessels appears to depend primarily upon extracellular Ca^{2+} influx, since removal of extracellular Ca^{2+} or administration of Ca^{2+} channel antagonists (diltiazem and nimodipine) nearly abolish vasopressin-induced vasoconstriction in the isolated perfused rat kidney (Cooper and Malik, 1984). In contrast, in cultured vascular smooth muscle cells derived from larger conduit vessels (rat aorta), removal of extracellular Ca^{2+} only reduced vasopressin-induced increases in cytosolic Ca^{2+} by about half; thus, both extracellular influx and intracellular release contributed to the increases in cytosolic Ca^{2+} (Capponi et al., 1985; Wallnofer et al., 1987; Berman et al., 1994).

The gonadal steroid hormones may alter cytosolic Ca^{2+} and contraction in smooth muscle by interfering with the entry of Ca^{2+} or by modifying intracellular Ca^{2+} pools. In vascular smooth muscle, the presence of high circulating levels of female sex steroid hormones appears to enhance the responsiveness of the rat aorta to epinephrine by increasing the uptake of Ca^{2+} into intracellular stores, whereas testosterone appears to increase the contribution of extracellular Ca^{2+} to vascular smooth muscle contraction (DeFelice and Joiner, 1975). Similar effects of female sex steroids have been reported in uterine smooth muscle. Increases in both excitability and contractility of the myometrium to oxytocin, norepinephrine and other agonists occur following estrogen treatment in vivo (as reviewed by Stander and Barden, 1970; Batra, 1980, Kostrzewska et al., 1993).

The purpose of the present investigation was to determine whether male–female differences exist in the relative contributions of extracellular Ca^{2+} influx and intracellular Ca^{2+} release to vasopressin-induced contraction of the rat aorta. The selective Ca^{2+} channel antagonist diltiazem and simvastatin were used to pharmacologically inhibit extracellular Ca^{2+} entry and the release of Ca^{2+} from inositol trisphosphate (IP_3)-sensitive stores in the sarcoplasmic reticulum, respectively. Additional experiments were performed with KCl and with the Ca^{2+} channel agonist Bay K 8644 to evaluate male–female differences in voltage-operated Ca^{2+} channel function in vascular contraction of the rat aorta.

2. Materials and methods

2.1. Experimental animals

Age-matched male and female Sprague–Dawley rats (12 to 16 weeks of age) were obtained from Zivic-Miller Laboratories (Zelienople, PA). The rats were housed in standard plastic laboratory rat cages and were segregated by sex at the Northeastern Ohio Universities College of Medicine Comparative Medicine vivarium facilities. 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. All experimental procedures performed in these studies were reviewed and approved by the Northeastern Ohio Universities College of Medicine Institutional Animal Care and Use Committee.

2.2. Preparation of isolated vascular tissue

The thoracic aorta was removed from rats after sacrifice by decapitation, and placed in chilled Krebs–Henseleit–bicarbonate solution (KHB, 4°C) gassed with 95% O_2/5% CO_2. Composition of the KHB solution was (in mM): 118.0 NaCl, 25.0 $NaHCO_3$, 10.0 glucose, 4.74 KCl, 2.5 $CaCl_2$, 1.18 $MgSO_4$, and 1.18 KH_2PO_4 (pH = 7.40, osmolality = 292 ± 1 mosm/kg H_2O). Fat and connective tissue were removed and the mid-thoracic region was cut into rings (3 mm long). During preparation of the rings, extreme care was taken to avoid stretching the tissue or touching the lumenal surfaces to preserve endothelial integrity, which was evaluated functionally in all experiments (as described below). Aortae intended for assessment of endothelial function were stripped of endothelium prior to mounting by gently rubbing the lumenal surface with a frayed nylon string (Stallone, 1993). After preparation, the aortic 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-tension transducer (Grass FT-03D) for the measurement of isometric tension. The transducer was connected to a polygraph (Gould 2600S) for continuous recording of aortic tension.

Immediately after mounting, the aortic rings were suspended in water-jacketed tissue baths containing 15 ml of KHB warmed (37°C) and continuously gassed with 95% O_2/5% CO_2. Passive tension was slowly adjusted (over 30 min) to 2.50 g (optimal passive tension for male and female rat aortae; Stallone et al., 1991) and the aortic rings were equilibrated for 60–90 min. During this time, the solution in the baths was replaced with freshly gassed,

warmed KHB every 20 min. After equilibration, the aortic rings were stabilized by two successive near-maximal contractions with phenylephrine (10^{-6} M). After the second phenylephrine contraction reached a stable contractile tension, the endothelium-dependent vasodilator acetylcholine (10^{-7} M) was added to the baths to assess endothelial integrity. Endothelium-intact aortae that did not respond to acetylcholine normally and endothelium-denuded aortae that responded to acetylcholine were excluded from the study. The tissue baths were then rinsed twice with KHB and the aortic rings were allowed to relax fully and re-equilibrate (30 min) prior to further experimentation. After completion of all experiments, the rings were dried (80°C) and weighed to the nearest 0.01 mg.

2.3. Effects of diltiazem on reactivity of male and female aortae to vasopressin

The effects of the Ca^{2+} channel antagonist diltiazem on vasopressin-induced contraction were evaluated in male and female rat aortae to determine the role of extracellular Ca^{2+} in this sexual dimorphism in vascular function. After the initial stabilization and re-equilibration periods, paired endothelium-intact aortic rings from male and female rats were pretreated with either diltiazem (10 μM) or its vehicle (KHB) for 30 min prior to obtaining a concentration-response to arginine vasopressin (10^{-11}–10^{-6} M). Arginine vasopressin was added to the baths in a cumulative manner to obtain a concentration-response for each ring, allowing a stable plateau tension to be attained at each concentration. The concentration of diltiazem used in these experiments approximated the reported IC_{50} for contraction of the rat aorta (Godfraind et al., 1986)

2.4. Reactivity of male and female rat aortae to KCl

To determine whether sex differences exist in voltage-operated Ca^{2+} channel (VOC) function in the rat aorta, contractile responses to KCl were assessed in male and female rat aortae. As in the previous experiments, these vessels were stabilized by two maximal contractions, but with 80 mM KCl–KHB instead of phenylephrine. After the initial stabilization and re-equilibration periods, a concentration-response to KCl (5–80 mM) was obtained from paired endothelium-intact and endothelium-deleted male and female rat aortae. KCl 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.

2.5. Reactivity of male and female aortae to Bay K 8644

To further assess male–female differences in Ca^{2+} channel function, concentration-response effects of Bay K 8644, a VOC agonist, were assessed in male and female rat aortae. As in the previous experiments, these vessels were stabilized by two maximal contractions, but with 80 mM KCl–KHB instead of phenylephrine. After the initial stabilization and re-equilibration periods, endothelium-intact male and female rat aortae were partially depolarized by the addition of KHB containing 19.5 mM KCl. After a stable contractile tension was attained, Bay K 8644 (10^{-9}–10^{-6} M) was added to the baths in a cumulative manner to obtain a concentration-response for each ring, allowing a stable plateau tension to be attained at each concentration. Contractile responses to Bay K 8644 were corrected for the minor contractile response to 19.5 mM KCl (approximately 212–360 mg/mg ring weight). This experimental protocol was performed under sodium-vapor lighting to protect the stability of Bay K 8644 during the entire course of the experiments.

2.6. Effects of simvastatin on reactivity of male and female aortae to vasopressin

The effects of simvastatin on vasopressin-induced contraction were evaluated in male and female rat aortae to determine the role of intracellular Ca^{2+} in this sexual dimorphism in vascular function. Simvastatin, an inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, also inhibits IP_3-sensitive Ca^{2+} release from the sarcoplasmic reticulum in rat aortic vascular smooth muscle (Escobales et al., 1996; Ng et al., 1994). After the initial stabilization and re-equilibration periods, which were performed in KHB containing 1 mM Ca^{2+}, separate groups of endothelium-intact or endothelium-deleted aortic rings from male and female rats were pretreated with either simvastatin (60 μM) or its vehicle-control (0.5% dimethyl sulfoxide (DMSO)) for 40 min. Arginine vasopressin was then 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.

2.7. Chemical reagents and drugs

The following drugs were used in the study: arginine vasopressin (Bachem, Torrance, CA), acetylcholine chloride, diltiazem hydrochloride and phenylephrine hydrochloride (Sigma, St. Louis, MO), Bay K 8644 (Research Biochemicals International, Natick, MA), and simvastatin (generously provided as a gift by Merck Research Laboratories, Rahway, NJ). All drug solutions were prepared fresh daily (except for arginine vasopressin, which was diluted daily from aliquots of 10^{-3} M stock solution stored at −70°C); these working solutions were kept on ice during the experiments. Stock solutions of the drugs were prepared in KHB (acetylcholine and arginine vasopressin), KHB with 100 μM ascorbic acid (phenylephrine), 25% methanol (Bay K 8644), or DMSO (simvastatin). Diltiazem was added to KHB to produce a final concentration of 10^{-5} M. The final concentration of

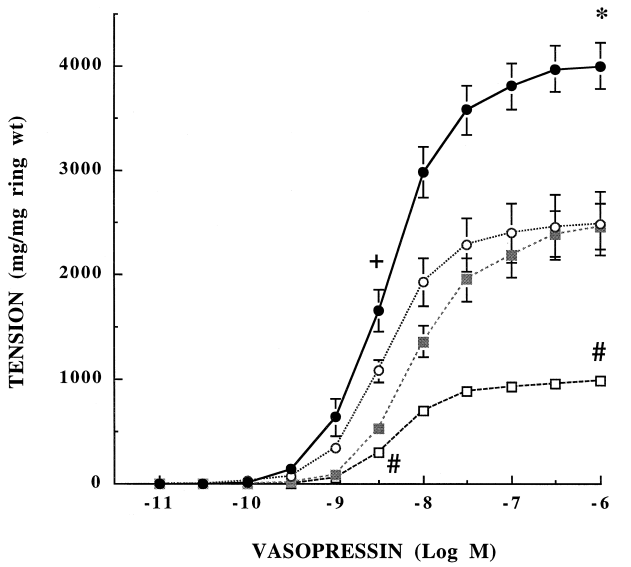

Fig. 1. Concentration-response curves for arginine vasopressin in endothelium-intact thoracic aortae of male and female rats in the presence of either diltiazem (10 μM) or its vehicle-control (KHB). (■) Male-control, $n=9$; (□) male-diltiazem, $n=9$; (●) female-control, $n=10$; (○) female-diltiazem, $n=10$. Contractile tension has been normalized by dry weight of aortic rings. Each point represents the mean ± S.E. (n = number of animals). Statistically significant differences exist in female-control vs. female-diltiazem, male-control, and male-diltiazem at the maximal concentration (10^{-6} M) of arginine vasopressin ($^{*}P \leq 0.006$); in female-control vs. male-control and male-diltiazem at the middle concentration (3×10^{-9} M) of arginine vasopressin ($^{+}P \leq 0.0001$); and in male-diltiazem vs. male-control and female-diltiazem at middle and maximal concentrations of arginine vasopressin ($^{\#}P \leq 0.002$).

the carriers did not exceed 0.5% (DMSO) or 0.33% (methanol). The concentrations of all chemicals and drugs are expressed as the final concentrations in the tissue baths. All other chemical compounds were obtained from Sigma or Fisher Scientific (Fair Lawn, NJ) and were of reagent grade quality.

2.8. Data analysis

All data are expressed as means ± S.E.; n denotes the number of animals studied. Contractile responses to arginine vasopressin, Bay K 8644, and KCl have been normalized by dry weight of the aortic rings and are expressed as milligrams tension per milligram ring weight. The concentrations of arginine vasopressin, Bay K 8644 or KCl producing 50% of the maximal response (EC_{50}) were calculated individually from the concentration-response curve of each aortic ring and reported as the geometric mean ± S.E. for the particular experimental group. Male and female data groups were analyzed by sex (male vs. female) and experimental treatment (vehicle-control vs. drug treatment or endothelium-intact vs. endothelium-deleted) using a two-way analysis of variance (ANOVA) to detect significant differences, followed by unpaired t-tests to distinguish significant differences among the means of male and female data groups. To correct for the increase in Type I error associated with multiple comparisons, a modified Bonferroni test was employed (Keppel, 1982).

3. Results

3.1. Effects of diltiazem on reactivity of male and female aortae to vasopressin

Contractile responses to arginine vasopressin were markedly higher in female than in male rat aortae throughout the range of concentrations studied (Fig. 1). At the maximal concentration of arginine vasopressin (10^{-6} M), maximal contractile tension of females averaged 4011 ± 225 mg/mg ring weight, whereas that of males only averaged 2469 ± 226 mg/mg ring weight ($P \leq 0.0001$). Inhibition of Ca^{2+} channel function with diltiazem (10 μM) attenuated contractile responses to arginine vasopressin in both female and male rat aortae; however, contractile responses were reduced to a greater extent in male than in female aortae (Fig. 1). Thus, pretreatment

Table 1
EC_{50} and corresponding half-maximal contractile responses to arginine vasopressin in endothelium-intact and -denuded thoracic aortae of male and female rats, pretreated with either diltiazem, simvastatin or their vehicle-controls

Treatment	EC_{50} (nM)	Half-maximal tension (mg/mg ring wt.)	n
Diltiazem: endothelium-intact			
Male			
Control	9.70 ± 1.29^{a}	1234 ± 113^{a}	9
Diltiazem	5.53 ± 0.49^{b}	496 ± 37^{b}	9
Female			
Control	$4.59 \pm 0.77^{b,c}$	2006 ± 113^{c}	10
Diltiazem	3.67 ± 0.31^{c}	$1249 \pm 156^{a,d}$	10
Simvastatin: Endothelium-intact			
Male			
Vehicle	5.49 ± 0.33^{d}	612 ± 51^{e}	8
Simvastatin	7.78 ± 0.90^{d}	668 ± 46^{e}	8
Female			
Vehicle	4.98 ± 0.79^{d}	1451 ± 130^{f}	8
Simvastatin	5.71 ± 0.23^{d}	852 ± 51^{g}	8
Simvastatin: Endothelium-denuded			
Male			
Vehicle	3.94 ± 0.49^{e}	1121 ± 112^{h}	3
Simvastatin	6.14 ± 0.44^{f}	666 ± 9^{i}	3
Female			
Vehicle	1.92 ± 0.34^{g}	1980 ± 14^{j}	3
Simvastatin	4.01 ± 0.28^{e}	$545 \pm 80^{i,k}$	3

Values are means ± S.E.; n = number of animals. EC_{50}, concentration of agonist producing 50% of the maximal contractile response. $^{a-k}$Within each column (EC_{50} or half-maximal tension) and within each treatment (Diltiazem or Simvastatin), mean values without common superscript are significantly different (Diltiazem, $0.0036 \geq P \geq 0.0001$; Simvastatin: endothelium-intact, $0.0089 \geq P \geq 0.0001$; Simvastatin: endothelium-denuded, $0.0146 \geq P \geq 0.0001$).

with diltiazem reduced maximal contractile tension of male aortae by 65 ± 2% (to 992 ± 74 mg/mg ring weight). In contrast, diltiazem reduced maximal contractile tension of female aortae by only 38 ± 1% (to 2497 ± 312 mg/mg ring weight). The differences in contractile tension in control vs. diltiazem-treated male and female aortae are highly significant at both the middle (3×10^{-9} M; $P \leq 0.02$) and maximal (10^{-6} M; $P \leq 0.0006$) concentrations of arginine vasopressin (Fig. 1).

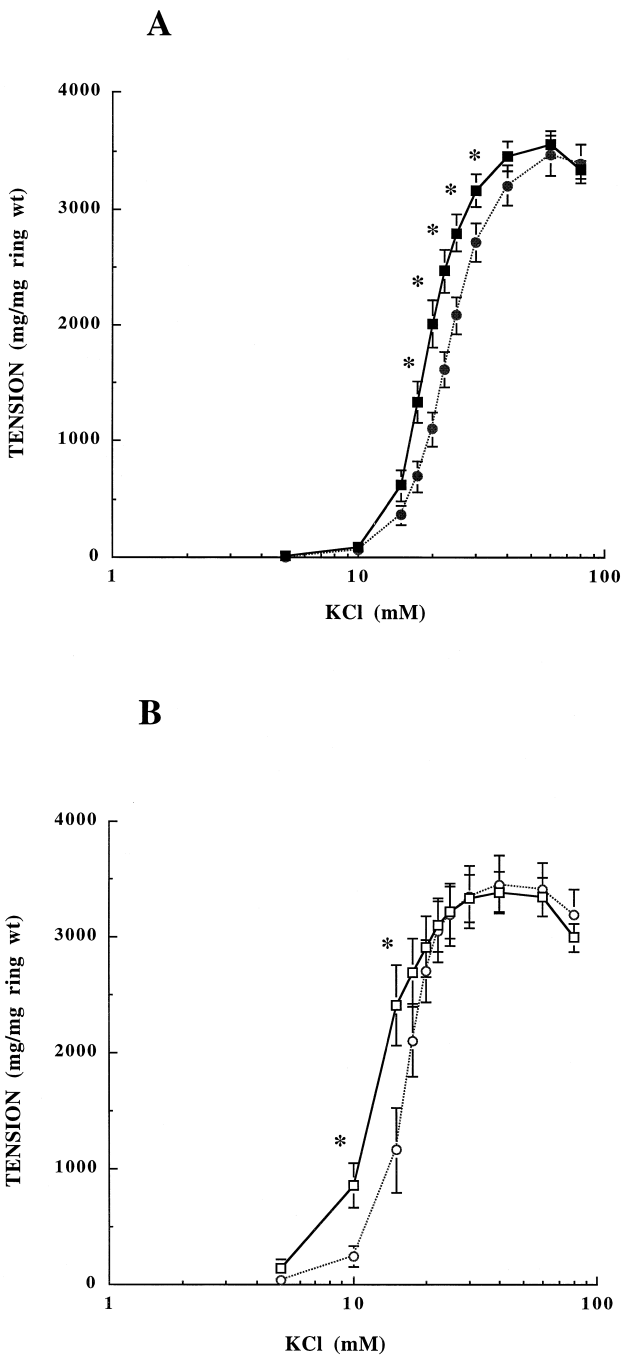

Fig. 2. Concentration-response curves for KCl in endothelium-intact (A) and endothelium-denuded (B) thoracic aortae of male and female rats. In panel A, (■) male, $n = 9$ and (●) female, $n = 10$. In panel B, (□) male, $n = 9$ and (○) female, $n = 10$. Contractile tension has been normalized by dry weight of aortic rings. Each point represents the mean ± S.E. (n = number of animals). In endothelium-intact aortae (panel A) and in endothelium-denuded aortae (panel B), contractile responses to KCl were higher in male than in female rat aortae at the lower concentrations (* $P \leq 0.0065$) and contractile sensitivity to KCl was higher in males than in females ($P \leq 0.001$).

Table 2
EC_{50} and corresponding half-maximal contractile responses to KCl in endothelium-intact and -denuded thoracic aortae of male and female rats

Experimental group	EC_{50} (nM)	Half-maximal tension (mg/mg ring wt.)	n
KCl			
Male-intact	19.1 ± 0.7^{a}	1756 ± 60^{a}	9
Male-denuded	13.2 ± 1.0^{b}	1681 ± 91^{a}	9
Female-intact	23.7 ± 0.9^{c}	1750 ± 86^{a}	10
Female-denuded	$16.9 \pm 1.1^{a,d}$	1756 ± 113^{a}	10

Values are means ± S.E.; n = number of animals. EC_{50}, concentration of agonist producing 50% of the maximal contractile response. $^{a-d}$Within each column (EC_{50} or half-maximal tension), mean values without common superscript are significantly different ($0.013 \geq P \geq 0.0001$).

The EC_{50} and corresponding half-maximal tensions of control and diltiazem-treated male and female aortae are presented in Table 1. The EC_{50} of control female and male aortae averaged 4.6 ± 0.8 and 9.7 ± 1.3 nM, respectively. Pretreatment of aortae with diltiazem did not significantly alter the sensitivity of female aortae to arginine vasopressin (3.7 ± 0.3 nM; $P > 0.05$); however, the sensitivity of male aortae to arginine vasopressin was increased significantly (5.5 ± 0.4 nM; $P \leq 0.004$).

3.2. Reactivity of male and female rat aortae to KCl

Unlike the differences observed between male and female rat aortae in response to arginine vasopressin, maximal contractile responses of endothelium-intact aortae to KCl did not differ significantly between males (3511 ± 121 mg/mg ring weight) and females (3499 ± 163; $P > 0.05$; Fig. 2A). Removal of the endothelium did not alter maximal responses of male (3362 ± 182 mg/mg ring weight) or female aortae (3511 ± 226; $P > 0.05$; Fig. 2B).

The EC_{50} and corresponding half-maximal tensions for endothelium-intact and -denuded male and female aortae are presented in Table 2. The sensitivity of endothelium-intact aortae to KCl was slightly but significantly higher in males (19.1 ± 0.7 mM) than in females (23.7 ± 0.9 mM; $P \leq 0.001$). Removal of the endothelium enhanced the sensitivity to KCl to a similar extent in females (16.9 ± 1.1 mM) and males (13.2 ± 1.0 mM; $P \leq 0.0126$); thus, sensitivity of endothelium-deleted aortae did not differ between males and females.

3.3. Reactivity of male and female aortae to Bay K 8644

Maximal contractile responses to the VOC agonist, Bay K 8644 were similar to those observed with KCl. At the

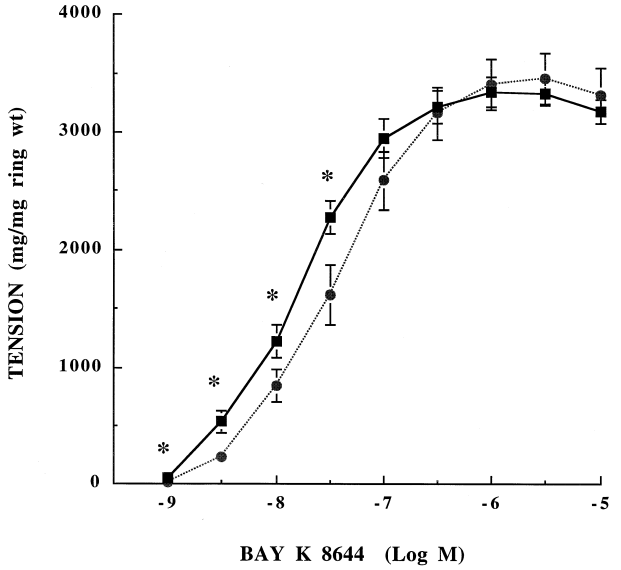

Fig. 3. Concentration-response curves for Bay K 8644 in endothelium-intact thoracic aortae of male and female rats. (■) Male, $n = 10$; (●) female, $n = 11$. Contractile tension has been normalized by dry weight of aortic rings. Each point represents the mean ± S.E. (n = number of animals). Contractile responses to Bay K 8644 were higher in male than in female rat aortae at the lower concentrations ($* P \leq 0.037$).

maximal concentration of Bay K 8644 (10^{-6} M), contractile responses of female aortae (3502 ± 219 mg/mg ring weight) did not differ significantly ($P > 0.05$) from those of male aortae (3381 ± 121). However, contractile responses of male aortae were significantly ($P \leq 0.03$) greater than those of female aortae at the lower concentrations of Bay K 8644 (10^{-9}–3×10^{-8} M; Fig. 3).

The EC_{50} and corresponding half-maximal tensions for male and female aortae are presented in Table 3. The sensitivity of male aortae to Bay K 8644 (18.4 ± 2.9 nM) was nearly three-fold higher than that of female aortae (45.0 ± 11.0 nM; $P \leq 0.02$).

3.4. Effects of simvastatin on reactivity of male and female aortae to vasopressin

Rat aortae were pretreated with simvastatin to determine the relative importance of IP_3-mediated intracellular Ca^{2+} release to arginine vasopressin-induced contraction of male and female aortae. Pretreatment of endothelium-intact aortae with simvastatin substantially reduced the contractile responses to arginine vasopressin in female but not in male aortae (Fig. 4). Thus, simvastatin attenuated the maximal contractile tension of female aortae by $42 \pm 1\%$ (1704 ± 102 mg/mg ring weight) but had no significant effect on male aortae (Fig. 4). The concentration-response curves for control and simvastatin-treated male aortae were virtually superimposable and maximal contractile tensions did not differ (1224 ± 102 vs. 1336 ± 91 mg/mg ring weight, respectively; $P > 0.05$). The differences in maximal contractile tension in control female vs. simvastatin-treated female aortae are highly significant at both the middle (3×10^{-9} M; $P \leq 0.0004$) and maximal (10^{-6} M; $P \leq 0.0008$) concentrations of arginine vasopressin. Removal of the endothelium enhanced the inhibitory effect of simvastatin on arginine vasopressin-induced contraction of female aortae and unmasked an inhibitory effect of simvastatin on male aortae. Thus, pretreatment of endothelium-denuded aortae with simvastatin reduced the maximal contractile response of female aortae by $73 \pm 2\%$ and that of male aortae by $41 \pm 2\%$.

The EC_{50} and corresponding half-maximal tensions of simvastatin-treated male and female aortae are presented in

Table 3
EC_{50} and corresponding half-maximal contractile responses to Bay K 8644 in endothelium-intact thoracic aortae of male and female rats

Experimental group	EC_{50} (nM)	Half-maximal tension (mg/mg ring wt.)	n
Bay K 8644			
Male	18.4 ± 2.9^{a}	1691 ± 60^{a}	10
Female	45.0 ± 11.0^{b}	1751 ± 110^{a}	11

Values are means ± S.E.; n = number of animals. EC_{50}, concentration of agonist producing 50% of the maximal contractile response. $^{a-b}$Within each column (EC_{50} or half-maximal tension), mean values without common superscript are significantly different ($P \leq 0.019$).

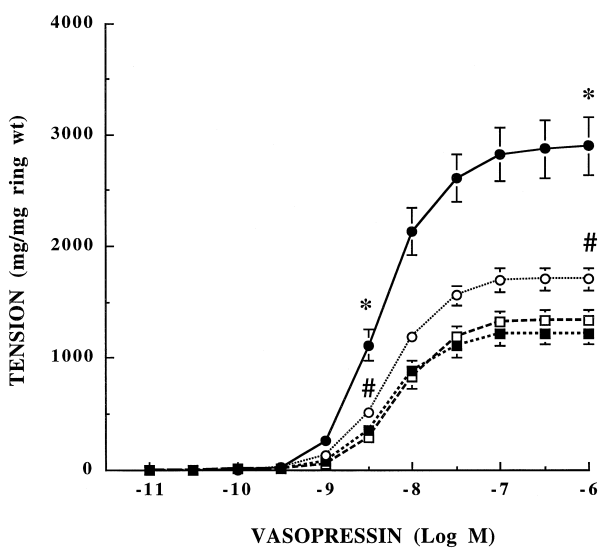

Fig. 4. Concentration response curves for arginine vasopressin in endothelium-intact thoracic aortae of male and female rats in the presence of either Simvastatin (60 μM) or its vehicle-control (0.5% DMSO). (■) male vehicle-control, (0.5% DMSO, $n = 8$); (□) male-Simvastatin, $n = 8$. (●) female vehicle-control, (0.5% DMSO, $n = 8$); (○) female-Simvastatin, $n = 8$. Contractile tension has been normalized by dry weight of aortic rings. Each point represents the mean ± S.E. (n = number of animals). Statistically significant differences exist in female-control vs. female-Simvastatin, male-control and male-Simvastatin at middle concentration (3×10^{-9} M) and maximal concentration (10^{-6}) of arginine vasopressin ($* P \leq 0.001$); and in female-Simvastatin vs. male-control and male-Simvastatin at middle and maximal concentrations of arginine vasopressin ($^{\#}P \leq 0.0100$).

Table 1. The sensitivity of endothelium-intact control male and female aortae to arginine vasopressin averaged 5.49 ± 0.33 and 4.98 ± 0.79 nM, respectively. Pretreatment with simvastatin did not alter the sensitivity of male or female aortae to arginine vasopressin (7.78 ± 0.90 and 5.71 ± 0.23 nM, respectively; $P > 0.05$). The sensitivity of endothelium-denuded control male and female aortae to arginine vasopressin averaged 3.94 ± 0.49 and 1.92 ± 0.34 nM, respectively. Pretreatment with simvastatin significantly reduced the sensitivity of endothelium-denuded male (6.14 ± 0.44 nM) and female aortae (4.01 ± 0.28 nM) to arginine vasopressin ($P \leq 0.015$; Table 1).

4. Discussion

In the present investigation, vascular reactivity and Ca^{2+} channel function were examined in the thoracic aortae of male and female rats to determine the roles of extracellular and intracellular Ca^{2+} in the sexual dimorphism in vascular responsiveness to vasopressin in the rat. The findings reveal that substantial male–female differences exist in the relative contributions of extracellular Ca^{2+} influx and intracellular Ca^{2+} release to vasopressin-induced contraction of the rat aorta. Thus, the male aorta appears to depend more upon extracellular Ca^{2+} influx, whereas the female aorta appears to depend more upon intracellular Ca^{2+} release for vasopressin-induced contraction.

4.1. Effects of diltiazem on reactivity of male and female aortae to vasopressin

Inhibition of Ca^{2+} channel function attenuated vasopressin-induced contraction of both male and female rat aortae in the present study; however, the inhibitory effects of diltiazem were substantially greater in male than in female aortae. These findings suggest that vasopressin-induced contraction exhibits a relatively greater dependence upon extracellular Ca^{2+} influx in male than in female rat aortae.

Previous studies have demonstrated that vasopressin can induce contraction of vascular smooth muscle by increasing cytosolic intracellular Ca^{2+} concentration through several possible mechanisms. First, vasopressin can stimulate extracellular Ca^{2+} entry via ROC and VOC in vascular smooth muscle (Capponi et al., 1985; Wallnofer et al., 1987; Berman et al., 1994; Dumont and Lamontagne, 1995). Second, vasopressin can also open non-selective cation channels in vascular smooth muscle, as reported for norepinephrine (Van Breemen et al., 1987). Third, vasopressin can also cause rapid increases in cytosolic intracellular Ca^{2+} concentration through stimulation of phosphoinositide hydrolysis and the resultant IP_3-mediated release of Ca^{2+} from the sarcoplasmic reticulum (Capponi et al., 1985; Escobales et al., 1996). Stimulation of extracellular Ca^{2+} entry has been identified as the primary mechanism of vasopressin-induced vasoconstriction in several vascular preparations, including the isolated, perfused rat kidney (Cooper and Malik, 1984) and isolated rat aorta (Dumont and Lamontagne, 1995), although both of these studies utilized vascular tissues obtained from male rats.

Notably, the reduced effects of diltiazem observed in the intact female aorta in the present study completely corroborate the earlier findings of Capponi et al. (1985) that aortic vascular smooth muscle cells obtained from female rats still exhibited a rise in cytosolic free Ca^{2+} concentration in response to vasopressin following removal of extracellular Ca^{2+} or pretreatment with the VOC antagonist nifedipine. Together, these findings suggest that in the absence of extracellular Ca^{2+} entry, vasopressin-induced increases in cytosolic free Ca^{2+} and vascular smooth muscle contraction involve IP_3-mediated release of intracellular Ca^{2+} from the sarcoplasmic reticulum, and that there is a greater dependence upon this mechanism in female than in male rat aortae. Indeed, although Ca^{2+} channel blockade reduced vasopressin-induced increases in both contractile tension in the female aorta in the present study and cytosolic Ca^{2+} concentration in cultured female aortic vascular smooth muscle cells in the previous study, 50–70% of the maximal responses to vasopressin still remained in both preparations. Male–female differences in the effect of the Ca^{2+} channel antagonist verapamil, similar to those observed in the present study, have also been reported for U46619-induced contraction of rat pulmonary artery (Cunard et al., 1986).

The relatively greater dependence upon extracellular Ca^{2+} influx for contraction of the male aorta in the present study may involve both ROC and VOC, since findings from several studies revealed that diltiazem can inhibit ROC as well as VOC function in arterial vascular smooth muscle (Van Breemen et al., 1981; Cauvin et al., 1984), and the concentration of diltiazem used in the present study (10 μM) inhibits both KCl- and norepinephrine-induced contractions (Cauvin et al., 1984). Furthermore, vasopressin was reported to increase Ca^{2+} entry through dihydropyridine-sensitive (VOC) Ca^{2+} channels in both rat aorta (Dumont and Lamontagne, 1995) and rat mesenteric artery (Noguera et al., 1997). These findings, together with the sex difference in the effect of diltiazem observed in the present study, strongly suggest that greater numbers of VOC and/or ROC exist in male than in female rat aortae.

4.2. Reactivity of male and female aortae to KCl and Bay K 8644

To determine the role of VOC in the prominent sexual dimorphism in the contribution of extracellular Ca^{2+} to vasopressin-induced contraction of the rat aorta, contractile responses to KCl were examined in male and female aortae. Although maximal responses did not differ between

the sexes, sensitivity to KCl was 24% greater in male than in female aortae. Removal of the endothelium slightly but significantly potentiated contractile responses to KCl and increased sensitivity of both male and female aortae to a similar extent (30%); thus, the greater sensitivity of the male aorta to KCl-induced contraction persisted in the absence of the endothelium. The effect of the endothelium on KCl contractions is consistent with the abundant evidence that this tissue modulates vascular reactivity to vasoconstrictor agonists in vitro (Carrier and White, 1985; Godfraind et al., 1985; Gruetter et al., 1988; Miller and Vanhoutte, 1990; Stallone, 1993). The results of the KCl experiments are in perfect agreement with those of the diltiazem experiments, which demonstrated a substantially greater inhibitory effect of this Ca^{2+} channel blocker in male than in female aortae.

Although the results of the KCl experiments suggest that a small but significant male–female difference exists in VOC-mediated Ca^{2+} influx, these findings may be complicated by the effects of KCl-induced depolarization on other vascular smooth muscle cell membrane ion channels, such as the delayed-rectifier and Ca^{2+}-dependent K^+ channels. Activation of these K^+ channels by KCl-induced depolarization would be expected to attenuate VOC-mediated contractile responses to extracellular KCl, particularly at lower concentrations of extracellular K^+, which may interfere with the accurate assessment of male–female differences in VOC function.

To assess these sex differences in VOC function more directly, contractile responses to VOC agonist Bay K 8644 (Hwang and van Breeman, 1985) were evaluated in male and female aortae. Sex differences in the reactivity of the endothelium-intact aorta to Bay K 8644 were similar to those of KCl but were more substantial. Thus, maximal contractile responses to Bay K 8644 did not differ, but sensitivity to the VOC agonist was nearly threefold higher in male than in female aortae. These data provide more conclusive evidence that greater numbers of VOC exist in male than in female rat aortae and are in uniform agreement with the results of the KCl and diltiazem experiments. Together, these data provide convincing evidence that the relatively greater dependence upon extracellular Ca^{2+} influx for vasopressin-induced contraction of the male rat aorta involves greater numbers of both VOC and ROC.

4.3. Effects of simvastatin on reactivity of male and female aortae to vasopressin

The cholesterol synthesis inhibitor simvastatin is also reported to inhibit IP_3-dependent Ca^{2+} mobilization in rat aortic vascular smooth muscle cells by depletion of IP_3-sensitive Ca^{2+} stores in the sarcoplasmic reticulum (Ishikawa et al., 1995; Escobales et al., 1996). Increases in cytosolic Ca^{2+} concentration in response to the vasoconstrictor agonists angiotensin II and vasopressin are nearly abolished in the presence of acute micromolar concentrations of simvastatin, which release Ca^{2+} from a thapsigargin-sensitive intracellular compartment (Escobales et al., 1996).

In the present study, acute inhibition of intracellular Ca^{2+} mobilization with simvastatin attenuated contractile responses to vasopressin substantially in female but not in male endothelium-intact rat aorta. These findings suggest that Ca^{2+} release from IP_3-sensitive intracellular stores contribute more to vasopressin-induced contraction in female than in male aortic vascular smooth muscle. The lack of effect of simvastatin in male aortae is consistent with the finding that extracellular Ca^{2+} influx contributes more to vasopressin-induced contraction in male than in female aortae. Removal of the endothelium in the male aorta unmasked an attenuating effect of simvastatin on the contractile responses to vasopressin that was absent from the endothelium-intact male aorta; however, the attenuating effects of simvastatin in endothelium-denuded preparations were still substantially greater in female than in male aortae, further supporting the idea that Ca^{2+} release from IP_3-sensitive intracellular stores contribute more to vasopressin-induced contraction in female than in male aortic vascular smooth muscle.

The mechanism underlying this sexual dimorphism in the modulatory effect of the endothelium on the vascular action of simvastatin is uncertain. Previous studies have established that vasopressin-induced contraction of the endothelium-intact rat aorta is greater in females than males, primarily due to greater agonist-stimulated release of NO by the male aorta (Stallone, 1993, 1994). Weisbrod et al. (1997) recently reported that NO enhances Ca^{2+} uptake by the sarcoplasmic reticulum; thus, the modulatory effect of the endothelium on simvastatin action may reflect the effect of NO to oppose the Ca^{2+}-depleting effect of simvastatin on IP_3-sensitive intracellular stores. The greater effect of the endothelium to modulate the effects of simvastatin in male than in female aortae in the present study is entirely consistent with the markedly greater vasopressin-stimulated release of NO by the male aorta reported previously (Stallone, 1993, 1994).

4.4. Gonadal steroid regulation of vascular calcium

The pronounced male–female differences in the contributions of extracellular and intracellular Ca^{2+} to vasopressin-induced contraction of aortic vascular smooth muscle observed in the present study could result, at least in part, from the effects of the gonadal steroid hormones on vascular smooth muscle. The presence of gonadal steroid hormone receptors in the vasculature (Horwitz and Horwitz, 1982; Tamaya et al., 1993) provides suggestive evidence that these hormones may influence vascular smooth muscle function and there is functional evidence that the gonadal steroids may influence Ca^{2+} regulation in various types of smooth muscle. In a study involving male,

female, and lactating female rats, female rat aortic strips contracted equally to both KCl and epinephrine, whereas male aortic strips contracted more to KCl than to epinephrine (DeFelice and Joiner, 1975). The greater contractile response of female aortic strips to epinephrine in this study was enhanced further in lactating females previously exposed to higher circulating levels of female sex steroids. These findings suggest that endogenous female sex steroids enhance binding or sequestration of intracellular Ca^{2+}, which increases tissue response to catecholamines (Cauvin et al., 1982a,b, 1984; Cauvin and Malik, 1984), whereas androgens may enhance extracellular Ca^{2+} entry and contractile responses to KCl. Similar effects of female sex steroids have been reported in uterine smooth muscle; increases in both excitability and contractility of myometrium to oxytocin, norepinephrine, and other agonists occur following estrogen treatment in vivo (as reviewed by Stander and Barden, 1970, Batra, 1980 and Kostrzewska et al., 1993). These findings are entirely consistent with those of the present study, which demonstrate that vasopressin-induced contraction of rat aortic vascular smooth muscle is more dependent upon extracellular Ca^{2+} entry in males but more dependent upon intracellular Ca^{2+} release in females. Male–female differences in the time-course of the contractile responses to vasopressin observed in past (Stallone et al., 1991) and the present studies provide further support for this idea. Thus, vasopressin-induced increases in contractile tension in male aortae, which appear to rely primarily upon extracellular Ca^{2+} entry, develop much more slowly than the contractile responses of female aortae, which appear to depend more upon the rapid mobilization of intracellular Ca^{2+}. Consistent with these observations, pre-treatment of female aortae with simvastatin, an inhibitor of IP_3-mediated intracellular Ca^{2+} release, slowed the contractile responses to vasopressin to a time-course similar to that of male aortae.

In conclusion, the results of the present study demonstrate that the relative contributions of extracellular and intracellular Ca^{2+} to vasopressin-induced contraction differ markedly in male and female rat aortae. Thus, contraction of the male aorta appears to depend more upon the influx of extracellular Ca^{2+} via VOC and ROC, whereas the release of intracellular Ca^{2+} from IP_3-sensitive stores appears to be the primary source of Ca^{2+} for contraction of the female aorta. The substantially greater inhibitory effect of the Ca^{2+} channel inhibitor diltiazem in the male aorta and of the intracellular Ca^{2+} inhibitor simvastatin in the female aorta provide strong support for this idea. Species and regional vascular differences in the relative contributions of extracellular and intracellular Ca^{2+} to alpha adrenergic-mediated vascular contraction (Cauvin et al., 1982a,b; Cauvin and Malik, 1984) provide further support for the concept that the sources of cytosolic Ca^{2+} for vascular smooth muscle contraction could vary between sexes. Previous studies on uterine and vascular smooth muscle suggest that the gonadal steroid hormones, particularly estrogen, may influence vascular smooth muscle contractility through modulation of the sarcoplasmic reticulum and other organelles involved in the regulation of intracellular Ca^{2+}. Further studies to examine the role of the gonadal steroid hormones in the modulation of extracellular and intracellular Ca^{2+} pathways are currently underway.

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