Gender Differences in Vascular Smooth Muscle Reactivity to Increases in Extracellular Sodium Salt

Laura A. Barron, GaChavis M. Green, Raouf A. Khalil

Abstract—Hypertension is more common in men and postmenopausal women than in premenopausal women, and gender differences in sensitivity to high dietary Na⁺ salt have been suggested; however, the vascular mechanisms involved are unclear. We investigated whether increases in the extracellular concentration of Na⁺ ([Na⁺]e) enhance the mechanisms of vascular smooth muscle contraction and whether the vascular effects of [Na⁺]e exhibit gender differences. Isometric contraction and ⁴²Ca²⁺ influx were measured in endothelium-denuded aortic strips that were isolated from intact male, intact female, castrated male, and ovariectomized (OVX) female Sprague-Dawley rats and incubated in Krebs’ solution (2.5 mmol/L Ca²⁺) containing the addition of 1, 3, 6, 10, 20, and 30 mmol/L NaCl. Increasing [Na⁺]e for 30 minutes did not increase the resting tone or ⁴²Ca²⁺ influx in any group of rats. Phenylephrine (Phe) caused concentration-dependent increases in contraction and ⁴²Ca²⁺ influx. In vascular strips from intact males, increasing [Na⁺]e by the addition of 1 to 6 mmol/L NaCl significantly increased the magnitude of Phe contraction and ⁴²Ca²⁺ influx. Further increases in [Na⁺]e by the addition of 10, 20, and 30 mmol/L NaCl increased Phe-induced ⁴²Ca²⁺ influx but inhibited Phe contraction, possibly because of excessive increases in ionic strength. Preincubation with 2,4-dichlorobenzamil (10⁻⁵ mol/L), inhibitor of the Na⁺-Ca²⁺ exchanger, or KB-R7943 (10⁻⁵ mol/L), selective inhibitor of the reverse mode of the Na⁺-Ca²⁺ exchanger, abolished the increases in Phe contraction and ⁴²Ca²⁺ influx at increasing [Na⁺]e obtained by the addition of 1 to 6 mmol/L NaCl. Preincubation in Krebs’ solution containing control [Na⁺]e plus 1 to 6 mmol/L LiCl or N-methyl-D-glucamine did not increase Phe contraction. In intact females, the Phe contraction and Ca²⁺ influx were less than those in intact males and were not enhanced with increases in [Na⁺]e. The enhancement of Phe contraction and Ca²⁺ influx with increases in [Na⁺]e were not significantly different between castrated male rats and intact male rats but were greater in OVX female rats than intact female rats. In OVX female rats or castrated male rats treated with 17β-estradiol (but not 17α-estradiol) subcutaneous implants, no significant changes in Phe contraction or Ca²⁺ influx with increases in [Na⁺]e were observed. In OVX female or castrated male rats simultaneously treated with 17β-estradiol plus the estrogen receptor antagonist ICI 182,780, the Phe contraction and Ca²⁺ influx were enhanced with increases in [Na⁺]e. Thus, in intact male rats, small physiological increases in [Na⁺]e enhance smooth muscle contraction to Phe by a mechanism involving Ca²⁺ entry, possibly via the reverse mode of the Na⁺-Ca²⁺ exchanger. This mechanism appears to be reduced in the presence of endogenous or exogenous estrogen and thereby protects female rats against excessive increases in vascular reactivity during high dietary Na⁺ intake. (Hypertension. 2002;39[part 2]:425-432.)

Key Words: arterial pressure ■ muscle, smooth, vascular ■ calcium ■ sodium ■ gender

Hypertension is more common in men and postmenopausal women than in premenopausal women, and putative vascular protective effects of endogenous estrogen have been suggested.¹⁻⁴ Although the risk of hypertension often increases with high dietary Na⁺, particularly in salt-sensitive individuals,⁵⁻⁸ gender differences in salt sensitivity have been reported.⁹ It has been shown that among elderly normotensive subjects, postmenopausal women respond to changes in Na⁺ intake with greater increases in blood pressure than those observed in men of similar age.⁹ The potential beneficial vascular effects of estrogen replacement therapy in postmenopausal women¹⁰ have further suggested a role for estrogen in protecting against hypertension, particularly for women on a high Na⁺ diet.

Gender differences in sensitivity to high dietary Na⁺ have recently been reported in experimental animals, such as the Dahl salt-sensitive rat and the deoxycorticosterone acetate (DOCA)-salt hypertensive rat,¹¹⁻¹⁴ and have been explained by gender-specific increases in sympathetic activity and norepinephrine release, reductions in prostacyclin levels, and alterations in the vascular reactivity to vasoconstrictor agonists in males compared with females.¹²⁻¹⁷ Also, in DOCA-salt hypertension, the arterial pressure rises more rapidly and reaches a higher level in male rats than in female rats, and the
course of hypertension is exacerbated by gonadectomy in female rats, suggesting that the gender differences in salt sensitivity are possibly related to endogenous gonadal hormones.11,18

During high dietary Na+ intake, several adaptive mechanisms are activated to cope with the large Na+ overload. These mechanisms include expansion of extracellular fluid volume,19 increased cardiac output,20 and increased renal blood flow.21 These adaptive mechanisms make Na+ excretion as equivalent as possible to Na+ intake and thus maintain the body Na+ and the extracellular fluid volume within normal levels. However, some studies have shown that the body Na+ could be higher during high Na+ intake than during low Na+ intake, suggesting that increases in body Na+, albeit small and perhaps temporary, could still occur during a high Na+ diet.22 Also, although the location of the possible increases in body Na+ is not entirely clear, some of the Na+ could be in the cells,23 whereas a significant portion could be in the extracellular fluid volume.19

Although high dietary Na+ could be associated with increases in vascular reactivity to vasoconstrictor agonists16,24–26 and could lead to small increases in extracellular Na+ concentration ([Na+]e), the direct effect of physiological increases in extracellular Na+ on the mechanisms of vascular smooth muscle contraction is not clearly understood. Vascular smooth muscle contraction is triggered by increases in intracellular Ca2+ that are due to Ca2+ release from the intracellular stores and Ca2+ entry from the extracellular space.27–29 Also, in activated smooth muscle, the balance between intracellular and extracellular Ca2+ and Na+ is further regulated by the plasmalemmal Na+-Ca2+ exchanger.30,31 In its forward mode, the Na+-Ca2+ exchanger promotes Ca2+ extrusion from the cell, whereas in the reverse mode, it promotes Ca2+ entry into the cell.30,32 However, whether physiological increases in extracellular Na+ enhance vascular reactivity and the Ca2+-handling mechanisms of vascular smooth muscle contraction is unclear. Also, the suggested vascular protective effects of estrogen in females with intact gonads and their proposed absence in males and in females with reduced gonadal function imply that the mechanisms of vascular smooth muscle contraction may be modified by gender and by the presence or absence of functional female gonads.1,33 However, little is known about whether the effects of extracellular Na+ on the mechanisms of vascular smooth muscle contraction are affected by gender and by the presence or absence of gonadal hormones.

The purpose of the present study was to test the hypothesis that physiological increases in [Na+], enhance the mechanisms of vascular smooth muscle contraction and that the vascular effects of [Na+], are modified depending on the gender and the presence or absence of gonadal hormones. Experiments were designed to investigate (1) whether small increases in [Na+], cause or enhance vascular smooth muscle contraction, (2) whether the changes in vascular contraction with increasing [Na+] involve changes in Ca2+ release from the intracellular stores or Ca2+ entry from the extracellular space or the plasmalemmal Na+-Ca2+ exchange mechanism, and (3) whether the effects of [Na+], on the mechanisms of vascular smooth muscle contraction exhibit differences depending on the gender and the presence or absence of gonadal hormones.

Methods

Animals

Sprague-Dawley rats (12 weeks, Harlan Sprague Dawley, Indianapolis, Ind) were divided into 4 groups (16 rats each): intact males, intact females, castrated males, and ovariectomized (OVX) females. Gonadectomy was performed in the rats at 8 weeks of age by the vendor. Other OVX females (n = 16) and castrated males (n = 16) were given a subcutaneous timed-release 17α-estradiol or 17β-estradiol implant (30-day release, 0.125 mg per pellet, Innovative Research of America) or 17β-estradiol implant plus daily subcutaneous injection of the estrogen receptor antagonistICI 182.780 (Tocris, 1 mg/kg per day) 3 days after gonadectomy, and the rats were studied 4 weeks later.34 OVX female and castrated male rats were fed a soy-based diet to minimize dietary intake of phytoestrogens. The plasma 17β-estradiol was 77 ± 8 pmol/L in intact female rats, 17 ± 2 pmol/L in OVX female rats, 83 ± 9 pmol/L in OVX female rats with 17β-estradiol implants, 16 ± 2 pmol/L in intact male rats, 18 ± 3 pmol/L in castrated male rats, and 84 ± 6 pmol/L in castrated male rats with 17β-estradiol implants, as previously described.34 All procedures followed the guidelines of the Institutional Animal Care and Use Committee.

Tissue Preparation

The rats were anesthetized by inhalation of isoflurane. The thoracic aorta was excised, placed in Krebs’ solution, and cleaned of connective tissue. The aorta was cut transversely into 3-mm-wide strips. Because changes in [Na+], may affect various types of vascular cells, including endothelial cells, the endothelium was removed by rubbing the vessel interior with wet filter paper.

Isometric Contraction

Aortic strips were transferred to a tissue bath containing Krebs’ solution bubbled with 95% O2/5% CO2 at 37°C. One end of the strip was fixed to a glass hook, and the other end was connected to a force transducer (Grass FT03C, Astro-Med). The strips were stretched to 1.5 the initial unloaded length and allowed to equilibrate for 1 hour while the changes in tension were displayed on a Grass polygraph (model 7D). After two 96 mmol/L KCl contractions followed by washing with normal Krebs’ solution, the tissues were incubated for 30 minutes in either (1) Krebs’ solution containing control [Na+], (146.2 mmol/L) or containing increasing [Na+], by the addition of 1, 3, 6, 10, 20, and 30 mmol/L NaCl, (2) Krebs’ solution containing increasing [Na+], plus 2,4-dichlorobenzamil (10–5 mol/L), inhibitor of the Na–Ca2+ exchange, or KB-R7943 (10–5 mol/L), selective inhibitor of the reverse mode of the Na–Ca2+ exchange, or (3) Krebs’ solution containing control [Na+], plus 1, 3, 6, 20, and 30 mmol/L LiCl or N-methyl-D-glucamine. The vascular strips were stimulated with phenylephrine (Phe), and concentration-contraction curves were constructed.

In other experiments, the vascular strips were incubated in Ca2+-free (2 mmol/L EGTA) Krebs’ solution containing increasing [Na+], for 5 minutes and then stimulated with Phe (10–4 mol/L) or caffeine (25 mmol/L) to stimulate Ca2+ release from the intracellular stores.27,36 and the resulting transient contraction was measured. The developed force was corrected for the cross-sectional area of the vascular strip and expressed as active stress (N/m2) by using the following equation: stress = force/cross-sectional area, where cross-sectional area is wet weight/tissue density × length of the strip), and tissue density is 1.055 g/cm3.37

45Ca2+ Influx

Vascular strips were incubated for 30 minutes in Krebs’ solution containing increasing [Na+], by the addition of 1, 3, 6, 10, 20, and 30 mmol/L NaCl or Krebs’ solution containing increasing [Na+], plus the Na–Ca2+ exchange inhibitor 2,4-dichlorobenzamil or KB-R7943 (10–5 mol/L). The tissues were stimulated with Phe (10–5
Ecolite scintillation cocktail was added, and the \( \text{Ca}^{2+} \) substitution of NaCl with KCl. Stock solution of Phe (10\(^{-5}\) mol/L) was omitted and replaced with 2 mmol/L EGTA. The high KCl depolarized the vascular strips were weighed and placed in 2 mL hypotonic solution for 45 minutes to quench extracellular \( \text{Ca}^{2+} \) label. The vascular strips were weighed and placed in 2 mL hypotonic (5 mmol/L) EDTA for 24 hours at 4°C to disrupt the cell membranes and release the intracellular content of \( \text{Ca}^{2+} \). The next day, 4 mL of Ecolite scintillation cocktail was added, and the \( \text{Ca}^{2+} \) in the samples was counted in a scintillation counter. 28,37

The vascular strips were pretreated with 2,4-dichlorobenzamil (Molecular Probes) and KB-R7943 (Tocris) 5mol/L, selective inhibitor of the \( \text{Na}^{+} \)-Ca\(^{2+} \) exchanger (Figures 2A and 2C), the basal \( \text{Ca}^{2+} \) influx was not significantly affected; however, the increases in Phe-induced contraction and \( \text{Ca}^{2+} \) influx at increasing \( \text{Na}^{+} \), and after the addition of 3 or 6 mmol/L NaCl caused further increases in Phe-induced \( \text{Ca}^{2+} \) influx (Figure 1C).

In vascular strips pretreated with 2,4-dichlorobenzamil (10\(^{-5}\) mol/L), inhibitor of Na\(^{+}\)-Ca\(^{2+} \) exchanger (Figures 2A and 2C), or KB-R7943 (10\(^{-5}\) mol/L), selective inhibitor of the reverse mode of the Na\(^{+}\)-Ca\(^{2+} \) exchanger (Figures 2B and 2C), the basal \( \text{Ca}^{2+} \) influx was not significantly increased. In untreated and untreated rats versus rats treated with estrogen), followed by the Bonferroni post hoc test to compare selected groups or the Dunnett post hoc test to compare all groups with intact male rats. Differences were considered statistically significant at \( P<0.05 \).

Results

In vascular strips that were isolated from intact male rats and incubated in Krebs’ solution containing control \([\text{Na}^{+}]\), (146.2 mmol/L), Phe caused concentration-dependent increases in active stress (Figure 1A). Preincubation of the vascular strips in Krebs’ solution containing increasing \([\text{Na}^{+}]\), by the addition of 1, 3, and 6 mmol/L NaCl did not significantly change the basal vascular tone but increased the Phe-induced active stress (Figure 1A). Increasing \([\text{Na}^{+}]\), by the addition of 10 mmol/L NaCl did not significantly change the Phe-induced stress (Figure 1B). Increases in \([\text{Na}^{+}]\), by the addition of 20 and 30 mmol/L NaCl significantly decreased Phe contraction (Figure 1B). When the Phe response was presented as a percentage of maximum Phe contraction, the potency of Phe, as indicated by the concentration that produces half the maximum response (EC\(_{50}\)), was not significantly different in vascular strips of intact male rats incubated in Krebs’ solution containing increasing \([\text{Na}^{+}]\) (Table).

To investigate whether the changes in Phe contraction at increasing \([\text{Na}^{+}]\), reflect increases in \( \text{Ca}^{2+} \) entry from the extracellular space, \( \text{Ca}^{2+} \) influx was measured. In unstimulated vascular strips incubated in Krebs’ solution containing control \([\text{Na}^{+}]\), 146.2 mmol/L, the basal \( \text{Ca}^{2+} \) influx was 13.8±1.6 mmol/kg per minute. Phe (10\(^{-5}\) mol/L) caused a significant increase in \( \text{Ca}^{2+} \) influx (Figure 1C). Preincubation of the vascular strips in Krebs’ solution containing increasing \([\text{Na}^{+}]\), by the addition of 1, 3, and 6 mmol/L NaCl did not change the basal \( \text{Ca}^{2+} \) influx but significantly increased the Phe-induced \( \text{Ca}^{2+} \) influx (Figure 1C).

In vascular strips pretreated with 2,4-dichlorobenzamil (10\(^{-5}\) mol/L), inhibitor of Na\(^{+}\)-Ca\(^{2+} \) exchange (Figures 2A and 2C), or KB-R7943 (10\(^{-5}\) mol/L), selective inhibitor of the reverse mode of the Na\(^{+}\)-Ca\(^{2+} \) exchange (Figures 2B and 2C), the basal \( \text{Ca}^{2+} \) influx was not significantly different from the Phe responses in Krebs’ solution containing control \([\text{Na}^{+}]\). In vascular strips incubated in Krebs’ solution containing control \([\text{Na}^{+}]\), plus 1, 3, or 6 mmol/L LiCl (Figure 3A) or N-methyl-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control [Na(^{+})] mmol/L</th>
<th>[Na(^{+})] +NaCl mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe pEC(_{50}) (–log [mol/L]) in Ca(^{2+})-containing Krebs’ solution</td>
<td>7.1±0.2</td>
<td>7.0±0.1</td>
</tr>
<tr>
<td>Phe-induced stress (×10(^{6}) N/m(^{2})) in Ca(^{2+})-free Krebs’ solution</td>
<td>3.3±0.5</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Caffeine-induced stress (×10(^{6}) N/m(^{2})) in Ca(^{2+})-free Krebs’ solution</td>
<td>1.2±0.2</td>
<td>1.1±0.3</td>
</tr>
</tbody>
</table>

\( \text{pEC}_{50} \) (–log [mol/L]) is the concentration required to produce half-maximal Phe contraction. Values are mean±SEM of measurements in 24 to 32 vascular strips of 6 to 8 intact male rats. *P<0.05 vs measurements at control [Na\(^{+}\)].

Solutions, Drugs, and Chemicals

Normal Krebs’ solution contained the following (mmol/L): NaCl 120, KCl 5.9, NaHCO\(_{3}\) 25, NaH\(_{2}\)PO\(_{4}\) 1.2, dextrose 11.5, MgCl\(_{2}\) 1.2, and CaCl\(_{2}\) 2.5 at pH 7.4. For Ca\(^{2+}\)-free Krebs’ solution, CaCl\(_{2}\) was omitted and replaced with 2 mmol/L EGTA. The high KCl depolarizing solution was prepared as Krebs’ solution but with equimolar substitution of NaCl with KCl. Stock solution of Phe (10\(^{-5}\) mol/L) was prepared in distilled water. Stock solutions (10\(^{-5}\) mol/L) of 2,4-dichlorobenzamil (Molecular Probes) and KB-R7943 (Tocris) were prepared in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in solution was ≤0.1%. Caffeine (25 mmol/L) was directly dissolved in Ca\(^{2+}\)-free Krebs’ solution. All other chemicals were of reagent grade or better.

Statistical Analysis

The data were analyzed and presented as mean±SEM. In intact males, the Phe contraction and \( \text{Ca}^{2+} \) influx data at increasing [Na\(^{+}\)] in both the untreated and the dichlorobenzamil-treated or KB-R7943-treated vessels were compared by 2-way repeated measures ANOVA followed by the Student-Newman-Keuls post hoc test. The gender difference data were compared by using ANOVA with multiple classification criteria (male versus female rats, intact versus gonadectomized rats, and untreated rats versus rats treated with estrogen), followed by the Bonferroni post hoc test to compare selected groups or the Dunnett post hoc test to compare all groups with intact male rats. Differences were considered statistically significant at \( P<0.05 \).
Krebs' solution containing control [Na\(^+\)]\(_e\), plus 20 or 30 mmol/L LiCl (Figure 3A) or N-methyl-D-glucamine (Figure 3B), the Phe-induced contraction was significantly reduced. In Ca\(^2+\)-free (2 mmol/L EGTA) Krebs' solution containing control [Na\(^+\)]\(_e\), Phe (10\(^{-5}\) mol/L) and caffeine (25 mmol/L) caused a transient increase in active stress (Table). The Phe- and caffeine-induced stress in Ca\(^2+\)-free Krebs' solution containing increasing [Na\(^+\)]\(_e\) by the addition of 1, 3, and 6 mmol/L NaCl was not significantly different from the Phe and caffeine response in Ca\(^2+\)-free Krebs' solution containing control [Na\(^+\)]\(_e\). In Ca\(^2+\)-free Krebs' solution containing increasing [Na\(^+\)]\(_e\) by the addition of 10, 20, and 30 mmol/L NaCl, the Phe- and caffeine-induced contractions were significantly reduced (Table).

In vascular strips of intact female rats incubated in Krebs' solution containing control [Na\(^+\)]\(_e\), the Phe-induced stress and \(^{45}\)Ca\(^{2+}\) influx were less than that in intact male rats (Figure 4). In intact female rats, increasing [Na\(^+\)]\(_e\) by the addition of 6 mmol/L NaCl did not significantly affect the Phe-induced stress or \(^{45}\)Ca\(^{2+}\) influx (Figures 4A and 4C). In intact female rats, larger increases in [Na\(^+\)]\(_e\) by the addition of 20 and 30 mmol/L NaCl reduced the maximal Phe-induced stress to 3.9±0.3×10\(^4\) and 2.8±0.6×10\(^4\) N/m\(^2\), respectively, compared with the maximal Phe stress at control [Na\(^+\)]\(_e\), (5.5±0.6×10\(^4\) N/m\(^2\)). The enhancement of Phe contraction and Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), by the addition of 6 mmol/L NaCl was greater in OVX female rats than in intact female rats (Figures 4A and 4C) but was not significantly different between castrated and intact male rats (Figures 4B and 4D).

In OVX females or castrated male rats treated with the inactive 17\(\alpha\)-estradiol subcutaneous implants, significant increases in Phe contraction and Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), obtained by the addition of 6 mmol/L NaCl could still be observed (Figure 5). In OVX female rats or castrated male rats treated with 17\(\beta\)-estradiol subcutaneous implants, no significant changes in Phe contraction or Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), were observed (Figure 5). In OVX female rats or castrated male rats simultaneously treated with 17\(\beta\)-estradiol subcutaneous implants, no significant changes in Phe contraction or Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), were observed (Figure 5). In OVX female rats or castrated male rats simultaneously treated with 17\(\beta\)-estradiol subcutaneous implants, significant increases in Phe contraction and Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), obtained by the addition of 6 mmol/L NaCl could still be observed (Figure 5). In OVX female rats or castrated male rats treated with the inactive 17\(\alpha\)-estradiol subcutaneous implants, significant increases in Phe contraction and Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), obtained by the addition of 6 mmol/L NaCl could still be observed (Figure 5). In OVX female rats or castrated male rats simultaneously treated with 17\(\beta\)-estradiol subcutaneous implants, no significant changes in Phe contraction or Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), were observed (Figure 5). In OVX female rats or castrated male rats simultaneously treated with 17\(\beta\)-estradiol subcutaneous implants, no significant changes in Phe contraction or Ca\(^{2+}\) influx with increases in [Na\(^+\)]\(_e\), were observed (Figure 5).
Vascular smooth muscle contraction is triggered by increases in intracellular Ca\(^{2+}\) that are due to Ca\(^{2+}\) release from the intracellular stores and Ca\(^{2+}\) entry from the extracellular space.\(^{27-29}\) The observation that small increases in [Na\(^+\)], by the addition of 1, 3, and 6 mmol/L NaCl did not affect the Phe- or caffeine-induced contraction in Ca\(^{2+}\)-free solution suggests that the increases in vascular contraction with increases in [Na\(^+\)], are not due to the enhancement of Ca\(^{2+}\) release from the intracellular stores. On the other hand, the increases in Phe-induced Ca\(^{2+}\) influx at increasing [Na\(^+\)], suggest an enhancement of Ca\(^{2+}\) entry from the extracellular space. The effects of increases in [Na\(^+\)], with the use of 1, 3, and 6 mmol/L NaCl on vascular contraction and Ca\(^{2+}\) influx appear to be Na\(^+\) specific, inasmuch as similar increases in extracellular salt with the use of LiCl or N-methyl-D-glucamine did not increase Phe contraction or Ca\(^{2+}\) influx.

An important question is how the increases in [Na\(^+\)], may increase Ca\(^{2+}\) entry. The increases in [Na\(^+\)], are expected to increase the electrochemical gradient for Na\(^+\) and stimulate Na\(^+\) entry into vascular smooth muscle via nonspecific cation entry pathways, Na\(^+\) channels, or Ca\(^{2+}\) channels.\(^{31,38-40}\) Although the increases in intracellular Na\(^+\) could be extruded via the Na\(^+\)-K\(^+\) pump, the Na\(^+\)-Ca\(^{2+}\) exchanger has also been suggested to maintain the balance between extracellular and intracellular Na\(^+\) and Ca\(^{2+}\) ions.\(^{30,31}\) The observation that the increases in Phe contraction and Ca\(^{2+}\) influx are inhibited by 2,4-dichlorobenzamil, an inhibitor of the Na\(^+\)-Ca\(^{2+}\) exchanger, suggests that the Na\(^+\)-Ca\(^{2+}\) exchanger is involved. In its forward mode, the Na\(^+\)-Ca\(^{2+}\) exchanger is known to play a role in Ca\(^{2+}\) extrusion. On the other hand, the reverse mode of the Na\(^+\)-Ca\(^{2+}\) exchanger facilitates Na\(^+\) extrusion and Ca\(^{2+}\) entry and thereby causes more Na\(^+\) to leave the cell and more Ca\(^{2+}\) to enter the cell.\(^{30,32}\) The observation that the increases in Phe contraction and Ca\(^{2+}\) influx with increases in [Na\(^+\)], are inhibited by KB-R7943, selective inhibitor of the reverse mode of the Na\(^+\)-Ca\(^{2+}\) exchanger,\(^{35}\) further suggests the involvement of the Na\(^+\)-Ca\(^{2+}\) exchanger. Taken together, the present data suggest that small increases in [Na\(^+\)], could stimulate small increases in Na\(^+\) entry and intracellular Na\(^+\), which, in turn, activate the reverse mode of the Na\(^+\)-Ca\(^{2+}\) exchanger and thereby increase Ca\(^{2+}\) entry. This is supported by reports that small increases in intracellular Na\(^+\) concentration are sufficient to generate significant Ca\(^{2+}\) influx in smooth muscle.\(^{32}\)

Small increases in [Na\(^+\)], did not enhance Phe contraction or Ca\(^{2+}\) influx in vascular strips of intact female rats, which is in contrast to the effect in intact male rats. Chronic studies in Dahl salt-sensitive rats and DOCA-salt hypertensive rats have suggested gender differences in sensitivity to high dietary Na\(^+\).\(^{11-14}\) and have suggested possible alterations in the vascular reactivity in male rats compared with female rats.\(^{12-17}\) The present data are in agreement with these reports and provide evidence that small increases in [Na\(^+\)], enhance the Ca\(^{2+}\)-entry mechanisms of vascular smooth muscle contraction in a gender-specific manner. The lack of enhancement of Phe contraction or Ca\(^{2+}\) influx with small increases in [Na\(^+\)], in intact female compared with intact male Sprague-Dawley rats is consistent with previous reports that the Ca\(^{2+}\)-entry mechanisms are reduced in female compared with

estradiol plus the estrogen receptor antagonist ICI 182,780, the Phe-induced contraction and Ca\(^{2+}\) influx were significantly enhanced with increases in [Na\(^+\)], obtained by the addition of 6 mmol/L NaCl (Figure 5).

**Discussion**

The main findings of the present study are as follows: (1) in vascular strips of intact male rats, small increases in [Na\(^+\)], enhance Phe-induced contraction and Ca\(^{2+}\) influx from the extracellular space but not Ca\(^{2+}\) release from the intracellular stores, (2) the enhancement of Phe contraction and Ca\(^{2+}\) influx by small increases in [Na\(^+\)], is blocked by inhibitors of the Na\(^+\)-Ca\(^{2+}\) exchanger, and (3) the enhancement of Phe contraction and Ca\(^{2+}\) influx with small increases in [Na\(^+\)], could be observed in castrated male rats and OVX female rats.

In vascular strips of intact male rats, small increases in [Na\(^+\)], by the addition of 1, 3, and 6 mmol/L NaCl enhanced the vascular contraction to Phe. Phe interacts with \(\alpha\)-adrenergic receptors in vascular smooth muscle.\(^{36}\) The observation that the Phe EC\(_{50}\) did not change with increases in [Na\(^+\)], suggests that the increases in Phe contraction are not likely due to changes in the apparent \(\alpha\)-adrenergic receptor affinity. On the other hand, the increases in the magnitude of Phe contraction with small increases in [Na\(^+\)], suggest possible enhancement of signaling mechanisms downstream from receptor activation.
male Wistar-Kyoto and spontaneously hypertensive rats. The present results suggest that the reduction in Ca$^{2+}$ entry in blood vessels of intact female rats may be, at least in part, related to reduction in the reverse mode of the Na$^{+}$-Ca$^{2+}$ exchanger.

Chronic studies in DOCA-salt hypertensive rats have shown that the arterial pressure is greater in male rats than in female rats and that the hypertension is exacerbated by gonadectomy in female rats, suggesting that the gender differences in salt sensitivity are related to endogenous gonadal hormones. The present acute studies agree with these reports and suggest that the gender differences in the sensitivity to increasing [Na$^+$] may be related to estrogen, because the enhancements of Phe contraction and Ca$^{2+}$ influx with increases in [Na$^+$] were (1) not significantly different between castrated male rats and intact male rats, (2) significantly greater in OVX female rats than in intact female rats, and (3) not observed in OVX female rats or castrated male rats treated with 17β-estradiol subcutaneous implants. Also, the inhibitory effects of estrogen appear to be specific, because the enhancements of Phe contraction and Ca$^{2+}$ influx with increases in [Na$^+$] could still be observed in OVX female rats or castrated male rats treated with the inactive 17α-estradiol subcutaneous implants and in OVX female rats treated with the estrogen receptor antagonist ICI 182,780 (ICI).

Figure 4. Effect of increases in [Na$^+$] on Phe contraction and 45Ca$^{2+}$ influx in vascular strips of intact and gonadectomized (OVX) female (A and C) and gonadectomized (Cast) male (B and D) rats. Data points represent the mean±SEM of measurements in 24 to 32 vascular strips from 6 to 8 rats of each group. *P<0.05 for measurements at increasing [Na$^+$] by addition of 6 mmol/L NaCl vs control [Na$^+$].

Figure 5. Effect of increases in [Na$^+$] on Phe contraction and 45Ca$^{2+}$ influx in vascular strips of OVX female (A and C) and Cast male (B and D) rats treated with 17α-estradiol (17α-Est) or 17β-estradiol (17β-Est) subcutaneous implants or with 17β-Est plus the estrogen receptor antagonist ICI 182,780 (ICI). Data points represent the mean±SEM of measurements in 24 to 32 vascular strips from 6 to 8 rats of each group. *P<0.05 vs respective measurements in gonadectomized rats treated with 17α-Est.
or castrated male rats simultaneously treated with 17β-estradiol plus the estrogen receptor antagonist ICI 182,780. However, whether treatment of intact females with ICI 182,780 can unveil the enhancing effects of small increases in [Na⁺], on vascular reactivity and Ca²⁺ influx remains to be investigated. Also, the mechanisms by which estrogen might affect the Na⁺-Ca²⁺ exchanger are unclear at the present time but may be related to decreased expression and/or activity of the exchanger.

In the present study, larger increases in [Na⁺], by the addition of 20 and 30 mmol/L NaCl were associated with significant reduction in Phe contraction despite increases in Ca²⁺ influx. This can be explained by the possibility that the enhancements of vascular contraction and Ca²⁺ influx by the increases in [Na⁺], are counterbalanced by inhibitory effects due to increases in extracellular ionic strength. It has been reported that increases in extracellular ionic strength reduce muscle contraction, possibly because of changes in the actual spacing between the actin and myosin filaments. This is supported by the present observations that (1) increases in [Na⁺], by the addition of 20 and 30 mmol/L NaCl caused significant reduction in Phe contraction in both male and female rats, (2) increases in [Na⁺], by the addition of 10, 20, and 30 mmol/L NaCl caused significant reduction in Phe- and caffeine-induced vascular contraction in Ca²⁺-free solution, and (3) increases in ionic strength with the use of 20 and 30 mmol/L LiCl or N-methyl-d-glucamine caused significant reduction in Phe contraction, providing further evidence that the inhibition of contraction with large increases in [Na⁺], is possibly due to excessive increases in extracellular ionic strength and may mask significant vasoconstrictor effects of physiological increases in [Na⁺].

The present study highlights the importance of studying the effects of small and physiological increases in [Na⁺], on vascular function in both males and females. Although small increases in [Na⁺], enhance vascular contraction and intracellular Ca²⁺ entry in male rats, these effects appear to be reduced in female rats. We should emphasize that the present study examined the effect of brief exposures to increases in [Na⁺], on vascular contraction in ex vivo conditions. Whether these effects occur in vivo is unclear; however, frequent brief exposures to small increases in [Na⁺], may have long-term cumulative vasoconstrictive effects on the vasculature. Also, in the present study, [Na⁺], was increased relative to a control [Na⁺], of 146.2 mmol/L. Because the control [Na⁺], may vary in different animal species and in humans, it is important to take into consideration the species differences in the control physiological [Na⁺], in studies involving the effect of increases in [Na⁺], on vascular function. Finally, in the present study, the extracellular concentration of not only Na⁺ but also Cl⁻ has been changed; thus, it would be difficult to rule out an effect of extracellular Cl⁻ under these conditions. Future studies should test the effects of increasing extracellular Na⁺ while keeping Cl⁻ constant and vice versa.

Thus, in intact male rats, small physiological increases in [Na⁺], enhance vascular smooth muscle contraction to Phe by a mechanism possibly involving Ca²⁺ entry via the reverse mode of the Na⁺-Ca²⁺ exchanger. This mechanism appears to be reduced in the presence of endogenous or exogenous estrogen and thereby protects females against excessive increases in vascular reactivity during high dietary Na⁺ intake.

Acknowledgments
This work was supported by grants from the American Heart Association (Grant-in-Aid, Southeast Affiliate) and the National Heart, Lung, and Blood Institute (HL-51971 and HL-52969).

References


Gender Differences in Vascular Smooth Muscle Reactivity to Increases in Extracellular Sodium Salt
Laura A. Barron, GaChavis M. Green and Raouf A. Khalil

Hypertension. 2002;39:425-432
doi: 10.1161/hy02t2.102779

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/39/2/425

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/