Gender Differences in Ca\textsuperscript{2+} Entry Mechanisms of Vasoconstriction in Wistar-Kyoto and Spontaneously Hypertensive Rats

Janice K. Crews, Jason G. Murphy, Raouf A. Khalil

Abstract—We investigated whether putative vascular protection against hypertension in females reflects differences in the Ca\textsuperscript{2+} mobilization mechanisms of vasoconstriction depending on the gender and the status of the gonads. Active stress and \textsuperscript{45}Ca\textsuperscript{2+} influx were measured in aortic strips isolated from intact and gonadectomized male and female Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). In aortic strips of intact male WKY incubated in normal Krebs’ solution (2.5 mmol/L Ca\textsuperscript{2+}), both phenylephrine (10\textsuperscript{-5} mol/L) and membrane depolarization by 96 mmol/L KCl caused significant increases in active stress and \textsuperscript{45}Ca\textsuperscript{2+} influx. In intact female WKY, the phenylephrine- and KCl-induced stress and \textsuperscript{45}Ca\textsuperscript{2+} influx were significantly reduced. In Ca\textsuperscript{2+}-free (2 mmol/L EGTA) Krebs’ solution, stimulation of aortic strips with phenylephrine or caffeine (25 mmol/L) to induce Ca\textsuperscript{2+} release from intracellular stores caused a transient increase in stress that was not significantly different between males and females. In SHR, the phenylephrine- and KCl-induced stress and \textsuperscript{45}Ca\textsuperscript{2+} influx were significantly greater than those in WKY in all groups of rats. The reduction in stress and Ca\textsuperscript{2+} entry in intact females compared with intact males was greater in SHR than in WKY. The contractile responses and Ca\textsuperscript{2+} entry in castrated male and ovariectomized female WKY or SHR were not significantly different from the respective responses in intact males. The contractile responses and Ca\textsuperscript{2+} entry in ovariectomized female WKY or SHR with 17β-estradiol implant were not significantly different from the respective responses in intact females. Thus, the phenylephrine- and depolarization-induced vascular reactivity and Ca\textsuperscript{2+} entry in vascular smooth muscle are dependent on gender and on the presence or absence of functional female gonads. Ca\textsuperscript{2+} release from intracellular stores is not affected by gender or gonadectomy. The gender-specific changes in vascular reactivity and Ca\textsuperscript{2+} entry are augmented in hypertension. (Hypertension. 1999;34[part 2]:931-936.)

Key Words: hormones \ bullet muscle, smooth, vascular \ bullet constriction \ bullet gender \ bullet calcium

Hypertension is a major cardiovascular disease in the industrialized world. The greater incidence of hypertension in men and postmenopausal women than in premenopausal women has suggested vascular protective effects of female sex hormones in premenopausal women. The beneficial effects of estrogen replacement therapy in postmenopausal women have further supported a protective role for estrogen against hypertension.

The beneficial vascular effects of estrogen have been ascribed to a variety of factors, including endothelium-dependent vascular relaxation and endothelium-independent vascular relaxation, that involve direct action on vascular smooth muscle. Vascular smooth muscle contraction is triggered by increases in intracellular [Ca\textsuperscript{2+}] due to Ca\textsuperscript{2+} release from the intracellular stores and Ca\textsuperscript{2+} entry from the extracellular space. We and others have reported that estrogen causes rapid relaxation of isolated segments of vascular smooth muscle and have suggested additional mechanisms independent of the classic genomic pathway of steroid action possibly mediated by an effect on Ca\textsuperscript{2+} mobilization and/or fluxes.

The suggested vascular protective effects of estrogen in females as opposed to their proposed absence in males imply gender-specific differences in vascular smooth muscle contractility. Additionally, the suggested vascular protective effects of estrogen in females with intact gonads as opposed to their proposed absence in females with reduced gonadal functions imply that vascular smooth muscle contractility may be modified by the presence or absence of functional female gonads. However, little is known about the effect of gender and the status of the gonads on the Ca\textsuperscript{2+} mobilization mechanisms of vascular smooth muscle contraction. In addition, since hypertension is often associated with changes in vascular reactivity, it is predicted that the gender-dependent changes in vascular reactivity may be altered in cases of hypertension. However, whether the effects of
gender and the status of the gonads on vascular reactivity and the Ca\(^{2+}\) mobilization mechanisms of vasoconstriction are modified in hypertension is unclear.

The purpose of this study was as follows: (1) to determine whether vascular smooth muscle contractility is modified by gender and by the presence or absence of gender-specific gonads; (2) to determine whether the gender-specific changes in vascular reactivity reflect changes in the Ca\(^{2+}\) mobilization mechanisms of vascular smooth muscle contraction, ie, Ca\(^{2+}\) release from the intracellular stores and Ca\(^{2+}\) entry from the extracellular space; and (3) to determine whether the gender-specific changes in vascular contractility and Ca\(^{2+}\) mobilization mechanisms are modified in animal models of hypertension.

### Methods

#### Animals

Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) (aged 12 weeks, Harlan) were housed in the animal facility and maintained on ad libitum standard rat chow and tap water in a 12-hour light/12-hour dark cycle. Both WKY and SHR were divided into 4 groups: intact males (n = 12), intact females (n = 12), castrated males (n = 12), and ovariectomized (OVX) females (n = 12). Gonadectomy was performed and verified by the vendor at 8 weeks of age. Some OVX female WKY (n = 8) and SHR (n = 8) were given subcutaneous timed-release 17β-estradiol implants (30-day release, 0.125 mg per pellet, Innovative Research of America) 3 days after ovariectomy and were studied 4 weeks later. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Mississippi Medical Center and the American Physiological Society.

#### Blood Samples

On the day of the experiment, the rats were anesthetized by inhalation of isoflurane. Blood was collected for measurement of plasma 17β-estradiol by radioimmunoassay with the use of a 17β-estradiol kit (ICN Biomedicals). In WKY, plasma 17β-estradiol was 16.2 ± 2 pmol/L (n = 12) in intact males, 77.7 ± 8 pmol/L (n = 12) in intact females, 18.3 ± 3 pmol/L (n = 12) in castrated males, 17.2 ± 2 pmol/L (n = 12) in OVX females, and 83.9 ± 9 pmol/L (n = 8) in OVX females with 17β-estradiol implants. The plasma 17β-estradiol in SHR was not significantly different from that in WKY in each group of rats.

#### Isometric Tension

The thoracic aorta was excised, placed in oxygenated Krebs’ solution, and cleaned of connective tissue. The aorta was cut transversely into 3-mm-wide rings. The endothelium was removed by rubbing the vessel interior with forceps. Aortic rings were cut open into strips. One end of the strip was attached to a glass hook with a thread loop, and the other end was connected to a Grass force transducer (FT03, Astro-Med). Aortic strips were stretched to maximum length (1.5 initial unloaded length) and allowed to equilibrate for 1 hour in a water-jacketed, temperature-controlled tissue bath filled with 50 mL Krebs’ solution continuously bubbled with 95% O\(_2\)/5% CO\(_2\) at 37°C. The changes in isometric tension were recorded on a Grass polygraph (model 7D, Astro-Med). Removal of the endothelium was routinely verified by the absence of acetylcholine (10\(^{-6}\) mol/L)-induced vasorelaxation in aortic strips precontracted with phenylephrine (3X10\(^{-7}\) mol/L).

Three different agonists were used. The α-adrenergic agonist phenylephrine was used to stimulate both Ca\(^{2+}\) release from the intracellular Ca\(^{2+}\) stores and Ca\(^{2+}\) entry from the extracellular space.\(^6\) Caffeine was used to activate Ca\(^{2+}\)-induced Ca\(^{2+}\) release in Ca\(^{2+}\)-free solution.\(^9\) Membrane depolarization by high KCl solution was used to activate Ca\(^{2+}\) entry from the extracellular space.\(^9\)

Three protocols were followed in the present study. In the first protocol, the tissues were incubated in normal Krebs’ solution (2.5 mmol/L Ca\(^{2+}\)), and a maximal control contraction to phenylephrine (10\(^{-5}\) mol/L) was elicited. In some strips, when maximal phenylephrine contraction or contraction to the ED\(_{50}\) of phenylephrine (3X10\(^{-5}\) mol/L) reached a plateau, 17β-estradiol was added at increasing concentrations, and the changes in tension were observed. In the second protocol, the bathing solution was changed to 96 mmol/L KCl solution to elicit a maximal contraction. In the third protocol, the tissues were incubated in normal Krebs’ solution (2.5 mmol/L Ca\(^{2+}\)) for 1 hour, transferred to Ca\(^{2+}\)-free (2 mmol/L EGTA) Krebs’ solution for 10 minutes, then stimulated with phenylephrine (10\(^{-3}\) mol/L) or caffeine (25 mmol/L) for 2 minutes or until the transient contraction returned to baseline.

#### 45\(^{Ca}\) Influx

Aortic strips were incubated in normal Krebs’ solution for 1 hour, then stimulated with phenylephrine (10\(^{-5}\) mol/L) or 96 mmol/L KCl for 15 minutes. In some experiments, the tissues were pretreated with 17β-estradiol (10\(^{-7}\) mol/L) for 30 minutes before stimulation with phenylephrine. The tissues were transferred to the respective radioactively labeled (ICN Radiochemical) solution (specific activity, 2 μCi/mL) for 90 seconds, then transferred to ice-cold Ca\(^{2+}\)-free (2 mmol/L EGTA) Krebs’ solution for 45 minutes to quench extracellular 45Ca\(^{2+}\) label.\(^7\) The tissue samples 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 45Ca\(^{2+}\). The next day, 4 mL of Ecolite scintillation cocktail was added, and the samples were counted in a scintillation counter (Beckman LS 6500).

#### Solutions, Drugs, and Chemicals

Normal Krebs’ solution contained the following (in mmol/L): NaCl 120, KCl 5.9, NaHCO\(_3\) 25, NaHPO\(_4\) 1.2, dextrose 11.5, MgCl\(_2\) 1.2, 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’ but with equimolar substitution of NaCl with KCl. Stock solution of phenylephrine (i-phenylephrine HCl; Sigma) was prepared as 10\(^{-7}\) mol/L in distilled water. Caffeine (Sigma) was prepared as 25 mmol/L in Ca\(^{2+}\)-free Krebs’. All other chemicals were of reagent grade or better.

#### Statistical Analysis

The developed force was normalized for the cross-sectional area of each individual strip and expressed as active stress (N/m\(^2\)) according to the equation Stress = Force/Cross-Sectional Area, where cross-sectional area = wet weight/(tissue density x length of the strip) and tissue density = 1.055 g/cm\(^3\). Data were analyzed and expressed as mean ± SEM. Data were compared by ANOVA with 3 classification criteria (strain, gender, and treatment [gonadectomized versus intact]). Scheffe’s F test was used for comparison of multiple means. Student’s t test for unpaired data was used for comparison of 2 means. Differences were considered statistically significant at P < 0.05.

#### Results

In normal Krebs’ solution (2.5 mmol/L Ca\(^{2+}\)), phenylephrine (10\(^{-5}\) mol/L) caused contraction of aortic strips that reached a plateau at ~15 minutes, and therefore the phenylephrine response was compared at this time in all groups of rats. Phenylephrine increased active stress to 15.92 ± 0.65 × 10\(^4\) N/m\(^2\) (n = 8) in intact male WKY (Figure 1A). The phenylephrine-induced stress was significantly reduced by 20% in intact female WKY. The phenylephrine-induced stress was not significantly different between intact and castrated males but was significantly greater in OVX females than in intact females. There was no significant difference in the phenylephrine-induced stress between OVX females and castrated
In intact male SHR, the KCl-induced stress was increased by 10% and 21% compared to that in WKY, which was slightly but not significantly greater than or lower than that in intact female, castrated male, or OVX female WKY. Phenylephrine increased Ca$^{2+}$ influx to 24.2±1.2 μmol/kg per minute (n=20) in intact male WKY (Figure 1B). The phenylephrine-induced increases in Ca$^{2+}$ influx were significantly reduced by 20% in intact female WKY. The phenylephrine-induced Ca$^{2+}$ influx was not significantly different between intact males and castrated males but was significantly greater in OVX females than in intact females. There was no significant difference in the phenylephrine-stimulated Ca$^{2+}$ influx between intact female WKY and intact female SHR with 17β-estradiol implants. Data bars represent the mean±SEM of measurements in 10 to 20 individual aortic strips from 5 to 6 rats of each group. *SHR are significantly different (P<0.05) from WKY. †Intact female WKY and OVX female WKY with 17β-estradiol implants are significantly different from other groups of WKY. ‡Intact female SHR and OVX female SHR with 17β-estradiol implants are significantly different from other groups of SHR.

In OVX female WKY with 17β-estradiol implants, the phenylephrine-induced stress was not significantly different from other groups of WKY and SHR intact male, intact female, castrated male, OVX female, and OVX female rats with 17β-estradiol implants. Data bars represent the mean±SEM of measurements in 10 to 20 individual aortic strips from 5 to 6 rats of each group. *SHR are significantly different (P<0.05) from WKY. †Intact female WKY and OVX female WKY with 17β-estradiol implants are significantly different from other groups of WKY. ‡Intact female SHR and OVX female SHR with 17β-estradiol implants are significantly different from other groups of SHR.

We investigated whether the observed gender differences in active stress reflect changes in Ca$^{2+}$ entry from the extracellular space. In unstimulated tissues, the basal Ca$^{2+}$ influx was 13.1±1.2 μmol/kg per minute (n=20) in the aorta of intact male WKY, which was slightly but not significantly greater than or lower than that in intact female, castrated male, or OVX female WKY. Phenylephrine increased Ca$^{2+}$ influx to 24.2±1.2 μmol/kg per minute (n=20) in intact male WKY (Figure 1B). The phenylephrine-induced increases in Ca$^{2+}$ influx were significantly reduced by 20% in intact female WKY. The phenylephrine-induced Ca$^{2+}$ influx was not significantly different between intact males and castrated males but was significantly greater in OVX females than in intact females. There was no significant difference in the phenylephrine-stimulated Ca$^{2+}$ influx between intact female WKY and intact female SHR with 17β-estradiol implants. Data bars represent the mean±SEM of measurements in 10 to 20 individual aortic strips from 5 to 6 rats of each group. *SHR are significantly different (P<0.05) from WKY. †Intact female WKY and OVX female WKY with 17β-estradiol implants are significantly different from other groups of WKY. ‡Intact female SHR and OVX female SHR with 17β-estradiol implants are significantly different from other groups of SHR.

To investigate the possible Ca$^{2+}$ entry pathway involved, we measured active stress and Ca$^{2+}$ influx in the presence of 96 mmol/L KCl. Membrane depolarization by 96 mmol/L KCl is known to stimulate Ca$^{2+}$ entry through voltage-gated Ca$^{2+}$ channels. KCl caused a contraction that reached a plateau at ≈15 minutes, and therefore the KCl response was compared at this time in all groups of rats. KCl increased active stress to 13.9±0.62×10$^{-3}$ N/m$^2$ (n=12) (Figure 1A) and Ca$^{2+}$ influx to 28.2±1.5 μmol/kg per minute (n=20) (Figure 2B) in the aorta of intact male WKY. The KCl-induced stress and Ca$^{2+}$ influx were significantly reduced by 19% and 21%, respectively, in intact female WKY. The KCl-induced responses were not significantly different between intact and castrated males but were significantly greater in OVX females than in intact females. There was no significant difference in KCl-induced stress or Ca$^{2+}$ influx between intact female WKY and intact female SHR with 17β-estradiol implants. Data bars represent the mean±SEM of measurements in 10 to 20 individual aortic strips from 5 to 6 rats of each group. *SHR are significantly different (P<0.05) from WKY. †Intact female WKY and OVX female WKY with 17β-estradiol implants are significantly different from other groups of WKY. ‡Intact female SHR and OVX female SHR with 17β-estradiol implants are significantly different from other groups of SHR.

Active stress ($\times 10^3$ N/m$^2$) induced by phenylephrine ($10^{-5}$ mol/L) and caffeine (25 mmol/L) in rat aortic strips incubated in Ca$^{2+}$-free (2 mmol/L EGTA) Krebs’ solution.
induced active stress and Ca\(^{2+}\) influx were significantly greater than those of WKY in all groups of rats. The KCl-induced active stress and Ca\(^{2+}\) influx in intact female SHR were reduced by 28% and 27%, respectively, compared with those in intact male SHR.

Since the gender difference could involve a multitude of factors in vivo, we tested the direct effect of exogenous application of 17β-estradiol on maximal phenylephrine- and KCl-induced contraction and Ca\(^{2+}\) influx. 17β-Estradiol caused concentration-dependent relaxation of phenylephrine-induced contraction. As shown in Figure 3A, 17β-estradiol was more potent in inhibiting the Ca\(^{2+}\) influx in OVX female SHR (23%) than in OVX female WKY (19%) (Figure 3D). 17β-Estradiol (10\(^{-6}\) mol/L) also caused greater inhibition of KCl-induced Ca\(^{2+}\) entry in OVX female SHR (34%) than in OVX female WKY (23%) (Figure 3C).

Discussion

The main findings of the present study are as follows: (1) Vascular reactivity due to Ca\(^{2+}\) entry into vascular smooth muscle, but not Ca\(^{2+}\) release from intracellular stores, is reduced in the presence and enhanced in the absence of female gonads. (2) Estrogen replacement in OVX female WKY reduces vascular reactivity and Ca\(^{2+}\) entry to levels similar to those in intact female WKY. (3) Exogenous application of estrogen to vascular strips reduces vascular reactivity and Ca\(^{2+}\) entry in OVX female WKY. (4) The reduction in vascular reactivity and Ca\(^{2+}\) entry in intact females, in OVX females with estrogen implants, or in response to exogenous application of estrogen on isolated vascular strips of OVX females is greater in SHR than in WKY.

The present study showed that the maximum aortic contraction to the α-adrenergic agonist phenylephrine was greater in intact male than in intact female WKY. These results are consistent with other studies that have shown a greater contraction to vasopressin in the aorta of intact male than intact female rats. The observation that the vascular contractility was not significantly different in castrated males compared with intact males but was significantly enhanced in OVX females compared with intact females suggests that the gender differences are less likely related to androgens and more likely related to estrogens. Since the expression of sex hormone receptors in arterial smooth muscle may vary depending on the gender and the status of the gonads, the observed gender differences in the vascular reactivity to estrogen may well be related to the relative abundance of estrogen receptors. This is supported by reports that estrogen receptors have been identified in the rat aorta and that females have higher levels of estrogen receptors in their arteries than males. However, the gender differences may also be related to differences in the signaling mechanisms downstream from receptor activation. We investigated whether the gender differences in vascular reactivity reflect differences in the mechanisms of Ca\(^{2+}\) mobilization in smooth muscle. Phenylephrine-induced contraction is triggered by an initial inositol 1,4,5-trisphosphate (IP\(_3\))-induced Ca\(^{2+}\) release from intracellular stores and maintained Ca\(^{2+}\) entry from the extracellular space. We found that the transient phenylephrine contraction in Ca\(^{2+}\)-free medium was not significantly different among the different groups of rats, suggesting that the IP\(_3\)-induced Ca\(^{2+}\) release mechanism is not affected by gender. Furthermore, caffeine, which stimulates the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism, caused a transient contraction that was similar in magnitude in the different groups of rats, suggesting that the observed gender differences are likely related to other factors.
differences in vascular reactivity are not related to the Ca\(^{2+}\)-induced Ca\(^{2+}\) release mechanism.

Membrane depolarization by high KCl is known to mainly stimulate Ca\(^{2+}\) entry from the extracellular space.\(^9,17\) The observation that the KCl-induced contraction was greater in intact males than in intact females suggested gender differences in Ca\(^{2+}\) entry mechanisms. In addition, the enhanced KCl-induced contraction in OVX females compared with that in intact females lends support to the contention that the gender differences are more likely related to endogenous estrogens. This is also supported by the observations that the phenylephrine- and KCl-induced increases in aortic Ca\(^{2+}\) entry were reduced in the presence and enhanced in the absence of female gonads. The causes of the gender differences in Ca\(^{2+}\) entry are not clear but may be related to the plasmalemmal density and/or the permeability of the Ca\(^{2+}\) entry pathways, among other factors. This is supported by reports that the expression of the L-type Ca\(^{2+}\) channels in cardiac muscle is substantially increased in estrogen receptor–deficient mice.\(^25\)

We found that the vascular reactivity and Ca\(^{2+}\) entry in OVX female WKY with estrogen implant were not significantly different from those in intact female WKY, providing further evidence that endogenous estrogens may be involved in the observed gender differences. However, the observed gender differences in the mechanisms of Ca\(^{2+}\) mobilization in vascular smooth muscle could be due to a multitude of effects of sex hormones in vivo. On the other hand, we found that exogenous application of estrogen to vascular strips of OVX females caused significant inhibition of vascular reactivity and Ca\(^{2+}\) entry. These results are consistent with the reduced vascular reactivity observed in aortic strips of intact female rats and are in agreement with reports that estrogen causes vascular relaxation in preconstricted rabbit and porcine coronary artery.\(^7,8,10\)

However, on the basis of these results we do not wish to draw conclusions on whether estrogen inhibits Ca\(^{2+}\) entry by direct or indirect action on plasmalemmal Ca\(^{2+}\) channels. Other studies have shown that estrogen blocks Ca\(^{2+}\) channels in cultured A7r5\(^26\) and rat aortic smooth muscle cells.\(^27\) Although the properties of Ca\(^{2+}\) channels may be different in cultured cells, our measurements of Ca\(^{2+}\) entry in aortic smooth muscle are consistent with these reports.

In the present study, aortic strips of SHR showed greater vascular reactivity and Ca\(^{2+}\) entry than those of WKY in all groups of rats. These results are consistent with other studies that have shown increased vascular tone in various blood vessels of SHR.\(^13–16\) The greater reduction in vascular reactivity and Ca\(^{2+}\) entry in intact female SHR and OVX female SHR with estrogen implants or in response to exogenous application of estrogen on isolated vascular strips of OVX female SHR compared with those in WKY could be due to differences in the number of estrogen receptors or in the number or permeability of the Ca\(^{2+}\) channels. This is supported by reports that the activity of the L-type Ca\(^{2+}\) channels is enhanced in vascular smooth muscle cells of SHR.\(^28,29\)

The present study showed gender differences in the vascular reactivity and \(^{45}\)Ca\(^{2+}\) influx to maximal concentrations of phenylephrine and KCl. However, on the basis of these results, we do not wish to draw a general conclusion that similar gender differences also occur at all concentrations of phenylephrine and KCl. Comparison of the whole concentration-response curves to phenylephrine or KCl in rats of different gender should, therefore, represent an interesting area for future experiments. It is also important to note that exogenous estrogen caused vascular relaxation at concentrations several-fold higher than those observed in the plasma of intact females. Although both exogenous application of estrogen and the endogenous presence of estrogen were associated with reduction in vascular reactivity and Ca\(^{2+}\) entry, we do not wish to make a definite conclusion that the cellular mechanisms of estrogen-induced relaxation in vascular strips and the possible vasorelaxant effects of estrogen in...
vivo are identical. The effects of estrogen on target tissues have been classically thought of as arising from genomic actions mediated through interaction with cytoplasmic receptors and translocation of the hormone-receptor complex to the nucleus. Although a genomic action of estrogen on the expression of the Ca$^{2+}$ channels might underlie the decreased responsiveness of aortic strips of intact females, it is less likely to account for the acute vasorelaxant effects of exogenous 17β-estradiol. The acute nature of the vasorelaxant effects of exogenous estrogen may represent additional non-genomic effects of estrogen on the mechanisms of Ca$^{2+}$ entry into vascular smooth muscle.

Finally, since the present study was performed on strips of thoracic aorta, we cannot make a definite conclusion on whether the observed gender differences in the Ca$^{2+}$ entry mechanisms of arterial vasoconstriction also occur in resistance vessels, which should represent an important area for future investigation.

Thus, the vascular reactivity due to Ca$^{2+}$ entry into vascular smooth muscle, but not Ca$^{2+}$ release from intracellular stores, is reduced in the presence and enhanced in the absence of female gonads. The gender-specific changes in vascular reactivity and Ca$^{2+}$ entry are possibly related to endogenous estrogen. The gender-specific changes in vascular reactivity and Ca$^{2+}$ entry are enhanced in hypertension.

Acknowledgments

This work was supported by grants from the American Heart Association (Grant-in-Aid, Mississippi Affiliate) and the National Institutes of Health (HL-52696, HL-51971).

References

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Hypertension. 1999;34:931-936
doi: 10.1161/01.HYP.34.4.931

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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