Ovariectomy Augments Hypertension in Aging Female Dahl Salt-Sensitive Rats

Carmen Hinojosa-Laborde, Teresa Craig, Wei Zheng, Hong Ji, Joseph R. Haywood, Kathryn Sandberg

Abstract—The ovariectomized (OVX) Dahl salt-sensitive (DS) rat fed a low-salt diet is a model of postmenopausal hypertension. In addition to estrogen loss, aging can also contribute to postmenopausal hypertension. We hypothesized that: (1) female DS rats on a low-salt diet become hypertensive with age; (2) ovariectomy accelerates age-dependent hypertension in the DS rat caused by estrogen depletion; and (3) this hypertension correlates with increased type 1 angiotensin receptor (AT1 R) number (Bmax). Blood pressure was monitored by telemetry from 3 to 12 months and AT1R Bmax was determined by Scatchard analysis in glomeruli and adrenal cortex. Three groups of DS rats were studied: intact, OVX, and 17β-estradiol–replaced OVX (OVX+E). In intact rats, aging to 12 months resulted in hypertension (159±6 mm Hg) and an 82% decrease in estrogen. Blood pressure in OVX was significantly higher than OVX+E through 12 months of age (173±4 versus 150±8 mm Hg). At 4 months, OVX increased AT1R Bmax compared with intact and OVX+E in both glomeruli and adrenal cortex. Aging also increased AT1R Bmax in these tissues in intact rats. In summary, female DS rats fed a low-salt diet have hypertension develop with age, that is accelerated by OVX and attenuated by estrogen replacement. Concurrently, AT1Rs are upregulated by age and OVX, which is prevented by estrogen replacement. This study suggests that an increased activity of the renin angiotensin system contributes to the development of hypertension, and estrogen protects against this process. (Hypertension. 2004; 44:405-409.)

Key Words: aging • Dahl rats • hypertension, sodium-dependent • estrogen • renin-angiotensin system

The prevalence of hypertension in premenopausal women is lower compared with men of the same age.1 After menopause, the incidence of hypertension in women doubles and becomes similar to men, suggesting that the loss of female gonadal steroids caused by menopause are a risk factor for postmenopausal hypertension.2 In addition, there is evidence that salt-sensitivity also contributes to postmenopausal hypertension.3 The mechanisms of postmenopausal hypertension are not well-understood and few animal models exist to study this phenomenon. We have shown that adult (4 months) female normotensive Dahl salt-sensitive (DS) rats maintained on a low-salt (LS) diet become hypertensive after ovariectomy (OVX).4 Thus, the ovariectomized DS rat serves as an experimental model of postmenopausal hypertension. Furthermore, this is the first animal model in which OVX results in spontaneous hypertension. These studies suggest that salt-sensitive premenopausal women may be protected against the development of hypertension because of the presence of female gonadal steroids and that after menopause, the protective effects of these hormones are lost, thus contributing to the development of hypertension. Although these observations implicate a role for estrogen (E), the effects of aging on the regulation of blood pressure could also contribute to the development of salt-sensitive hypertension in postmenopausal women.

The purpose of the present study was to determine the effect of aging and E loss on the development of hypertension in DS rats. We tested 3 hypotheses. First, DS rats maintained on LS diet will become hypertensive as they age. Second, the age-induced increase in blood pressure will be accelerated in ovariectomized rats and delayed in E-replaced ovariectomized animals. Third, the mechanism by which E loss accelerates the development of hypertension is via upregulation of angiotensin receptors (AT1R) in key angiotensin II (Ang II) target tissues. Thus, in this study we investigated the effects of aging and E loss on blood pressure regulation and AT1R binding and affinity in glomeruli and the adrenal cortex.

Methods

Experimental Protocol

DS (Rapp strain) female rats were purchased from Harlan Sprague-Dawley (Indianapolis, Ind) at 6 to 7 weeks of age and were
maintained on a phytoestrogen-free, sodium-deficient diet (0.1% NaCl) for the duration of the study. Soon after arrival, the rats were divided into 3 groups: sham surgery (intact), ovariectomy surgery (OVX), and ovariectomy surgery with implantation of a silastic implant containing 17β-estradiol (OVX+E). Two weeks later, when the rats were 8 to 9 weeks old, they were implanted with a radiofrequency transmitter (Data Sciences, Inc) to monitor arterial pressure by telemetry receivers (Data Sciences, Inc) as previously described.4 Beginning at 3 months of age, blood pressure was recorded for 2 to 3 consecutive days per week until the rats reached 12 months of age. The blood pressure measurements obtained during a 10-second sampling period (500 Hz) were averaged and recorded every 10 minutes during the recording periods. The data were then reduced to 24-hour averages. All rats remained in their home cage throughout the study. At 12 months of age, the animals were euthanized by decapitation and trunk blood was collected for the measurement of plasma concentrations of estradiol and progesterone.

The time of decapitation, kidney and adrenal cortex tissues were harvested to measure tissue AT1 R binding and affinity. Parallel groups of intact, OVX, and OVX+E (without telemetry transmitters) were euthanized at 4 months of age for blood and tissue collection. The experiments were performed according to the guidelines recommended by the National Institutes of Health and approved by the University of Texas Health Science Center and Georgetown University Animal Care and Use Committees.

Ovariectomy and Estrogen Replacement
Rats underwent OVX surgery as previously described.4 In those rats receiving E replacement therapy, 17β-estradiol (5 mg; Sigma) filled silastic tubes 1 cm in length were implanted subcutaneously at the time of OVX surgery. Estradiol silastic implants were removed and replaced every 12 weeks.

AT1 R Binding Studies
Kidney glomeruli were isolated and membranes from adrenal cortex were prepared for AT1 R binding studies as previously described.4 Protein concentrations of isolated glomeruli and adrenal cortex membranes were determined by the Bradford method6 using bovine serum albumin as the standard (Bio-Rad Laboratories).

Plasma Estradiol and Progesterone
Plasma estradiol levels were analyzed by radioimmunoassay according to manufacturer instructions (Ultra-Sensitive Estradiol Kit; Diagnostic Systems Laboratories). Plasma progesterone levels were also analyzed by radioimmunoassay according to manufacturer instructions (Progesterone Kit; Diagnostic Systems Laboratories). No extraction or sample pretreatment was required. The minimum detection limits for estradiol and progesterone were 2.2 pg/mL and 0.10 ng/mL, respectively.

Statistical Analysis
Data are expressed as mean±SEM. Blood pressures were measured on 2 to 3 sequential days each week until the animals reached 12 months of age. Based on the animal’s date of birth, the 24-hour averages of blood pressure during the weeks that marked 3, 4, 6, 8, 10, and 12 months of age were averaged over the 2- to 3-day recording period to obtain an average blood pressure recording for each week. The weekly averages of blood pressure corresponding to 3, 4, 6, 8, 10, and 12 months of age in the experimental groups were analyzed by 2-way analysis of variance (ANOVA) with repeated measures. Measurements of plasma hormones and tissue AT1 R binding were compared by 2-way ANOVA, nonrepeated measures. With significance, subsequent 1-way ANOVAs were performed. Multiple range tests were applied to assess individual differences. Significance is defined at P<0.05.

Results
Development of Hypertension
Mean arterial pressure (MAP) was monitored in intact, OVX, and OVX+E rats from 3 months to 12 months of age (Figure 1). At 3 months of age, MAP was similar in all 3 groups of rats (MAP [mm Hg]: intact, 119±2; OVX, 124±3; OVX+E, 118±2). As the animals aged, MAP increased steadily in all 3 animal groups; however, the rate of the MAP increase was significantly greater in the OVX group followed by the intact and, lastly, the OVX+E group (slope [MAP/age]: intact, 4.38±0.53; OVX, 6.04±0.41; OVX+E, 3.28±0.57). MAP increased in intact rats from 3 months of age (119±2 mm Hg) to 12 months of age (159±6 mm Hg). Significant increases in MAP in intact (compared with 3 months) were observed beginning at 6 months of age. Significant increases in MAP in OVX were observed beginning at 4 months of age, whereas significant increases in MAP in OVX+E were observed beginning at 8 months of age. Estrogen replacement in the OVX animals markedly attenuated the OVX-induced increase in MAP resulting in significantly lower levels of MAP in OVX+E throughout the study. MAP in OVX+E showed an upward trend at 12 months of age but remained significantly lower than OVX (150±8 versus 173±4 mm Hg, respectively).

Body Weight and Uterine Weight
All animals gained weight with age as indicated by the 30% to 50% increases in body weight at 12 months compared with 4 months of age in each animal group (Table 1). Comparing OVX and OVX+E at 12 months, the OVX rats were 40% heavier than the OVX+E group (P<0.05). Even at 4 months of age, the OVX rats were 40% heavier than the OVX+E group (P<0.05). Ovariectomy markedly reduced uterine weight in both the 4-month-old and 12-month-old OVX rats. Uterine atrophy was prevented by E replacement in OVX+E rats. Uterine weights in 4-month-old and 12-month-old intact and OVX+E rats were not different.

Plasma Estradiol and Progesterone
By 12 months of age, plasma estradiol levels in intact animals significantly declined by 82% compared with the 4-month-
old animals \( (P<0.05) \) and were at levels similar to 12-month-old OVX (Table 1). Plasma estradiol was decreased by 3-fold in the OVX compared with OVX +E \( (P<0.05) \) at both 4 and 12 months. The estradiol levels in the 4-month-old OVX +E animals were similar to the 4-month-old intact group; however, at 12 months, the OVX +E had 3.3-fold higher levels than the intact.

Plasma progesterone was markedly higher in intact rats \( (P<0.05) \) compared with OVX and OVX +E groups at both 4 and 12 months of age (Table 1). Aging did not have a significant effect on plasma progesterone levels in the intact or OVX groups; however, there was a significant decline in progesterone levels in the 12-month-old compared with the 4-month-old OVX +E group.

### AT\(_1\)R Binding

Radioligand saturation curves were performed on isolated glomeruli and membranes prepared from the adrenal cortex in each animal group using \(^{125}\text{I}-\text{[Sar}^1\text{,Ile}^8\text{]}\text{Ang II} \) as the radioligand. AT\(_1\)R binding sites (Bmax) and receptor affinity (Kd) were determined by Scatchard analysis. No significant differences in the receptor dissociation constant (Kd) were observed in isolated glomeruli or adrenal cortex membranes in all animal groups at 4 months and 12 months of age (Table 2). In contrast, there were significant effects of E status and aging on AT\(_1\)R numbers in these tissues.

Aging increased AT\(_1\)R Bmax by 1.3-fold in glomeruli (Bmax [fmol/mg]: 4 months, 512±21 versus 12 months, 675±24; \( P<0.001 \)) (Figure 2) and by 1.8-fold in adrenal cortex (Bmax [fmol/mg]: 4 months, 210±18 versus 12 months, 381±22; \( P<0.001 \)) (Figure 3) in the intact animals. This effect of aging on AT\(_1\)R expression was attenuated in the OVX +E animals; aging did not increase AT\(_1\)R Bmax in glomeruli (Bmax [fmol/mg]: 4 months, 505±47 versus 12 months, 603±23) (Figure 2) and only by 1.5-fold in the adrenal cortex (Bmax [fmol/mg]: 4 months, 144±12 versus 12 months, 227±12, \( P<0.01 \)) (Figure 3).

Compared with OVX +E, ovariectomy without estrogen replacement markedly increased AT\(_1\)R Bmax by 1.6-fold in glomeruli (Bmax [fmol/mg]: OVX, 836±38 versus OVX +E, 505±47, \( P<0.001 \)) (Figure 2) and by 2.4-fold in the adrenal cortex (Bmax [fmol/mg]: OVX, 351±13 versus OVX +E, 144±12, \( P<0.001 \)) (Figure 3) in 4-month-old animals. This effect of ovariectomy was also observed at 12 months in the glomeruli (Bmax [fmol/mg]: OVX, 814±30 versus OVX +E, 603±23, \( P<0.001 \)) (Figure 2) and in the adrenal cortex (Bmax [fmol/mg]: OVX, 347±45 versus OVX +E, 227±12, \( P<0.01 \)) (Figure 3). In OVX animals, aging did not further increase AT\(_1\)R expression in either the glomeruli (Figure 2) or the adrenal cortex (Figure 3).

### Discussion

We previously showed that ovariectomy of adult (4 months) normotensive DS female rats induced spontaneous hypertension despite the fact that the animals were maintained on LS diet. These findings suggest that the ovarioctomized DS rat is a useful experimental model of postmenopausal hypertension.\(^3\) Because menopause is associated with both gonadal steroid loss and aging, the current study was designed to investigate the effects of aging and estrogen loss on blood pressure regulation in DS rats. We hypothesized that blood pressure would increase with aging in DS females despite being maintained on LS diet, and that the development of hypertension would be accelerated in OVX rats and delayed in OVX +E rats. In addition, we hypothesized that the increase in blood pressure would involve activation of the renin-angiotensin system (RAS).
suggests that the OVX-induced increase in MAP is associated with decreased levels of plasma estrogen because estradiol replacement was able to prevent the OVX-induced hypertension. The results of this study indicate that estradiol replacement significantly reduced blood pressure in OVX rats through 12 months of age. Thus, this study extends the findings of our first report showing that OVX induces hypertension in normotensive DS rats by demonstrating that the ovarian hormone, estrogen, plays a key role in this OVX-induced hypertension.

Comparing estradiol levels with MAP in the 4- and 12-month-old intact animals suggests that the age-associated loss in plasma estradiol contributes to the age-associated increase in MAP; compared with 4 months of age, estradiol levels at 12 months were 82% lower (Table 1). This correlation between low estradiol and increased MAP was also observed in the OVX animals; estradiol levels (Table 1) were markedly lower and MAP (Figure 1) was significantly higher in the OVX animals compared with the OVX+E group at both 4 and 12 months. Age-dependent changes in vascular compliance, cardiac contractility, and baroreflex function have been well-documented and are likely to contribute the development of hypertension in the elderly. Interestingly, the uterine weights in 12-month-old intact rats were similar to that observed in 12-month-old OVX+E rats even though estradiol levels had declined by >2-fold in the intact rats. This observation suggests that the 12-month-old intact DS female serves as an experimental model of estrogen loss because of perimenopause rather than estrogen loss caused by full menopause.

Naturally, ovariectomy decreased progesterone levels and estradiol supplementation did not markedly alter circulating progesterone levels in both age groups. Furthermore, progesterone levels did not correlate with the difference in MAP between the OVX and OVX+E groups. This lack of effect of progesterone supports previous reports in deoxycorticosterone salt hypertension showing that progesterone had no effect on the development of hypertension in OVX rats.

Estrogen has been shown to suppress the renin-angiotensin system (RAS) by decreasing angiotensin-converting enzyme activity in several tissues, and we have found that estrogen decreases Ang II levels in the adrenal. Estrogen also reduces the number of AT1 receptors in the adrenal cortex, pituitary gland, and kidney. Furthermore, we and others have shown that estrogen reduces tissue responsiveness to Ang II. Although some reports indicate that estrogen activates angiotensinogen and increases circulating levels of angiotensin I, the overall effect of the hormone is to limit the production of the vasoconstrictor, Ang II, and to downregulate the AT1R. These inhibitory effects of estrogen on the RAS may protect against the development of hypertension.

Our results suggest that estrogen loss can amplify the activity of the RAS by increasing AT1R density in Ang II target tissues including the kidney and adrenal gland. We observed significantly higher AT1R densities in glomeruli and the adrenal cortex of 4-month-old estrogen-depleted OVX rats compared with the 4-month-old estrogen-replete OVX+E and intact groups (Figures 2 and 3). A recent study by Harrison-Bernard et al in young DS rats fed a normal-
salt diet has also demonstrated this effect of estrogen on AT1R densities in whole kidneys. In the present study, the age-associated loss in estrogen was also associated with higher AT1R densities in these tissues. The density of AT1Rs was markedly increased in glomeruli and the adrenal cortex in 12 month-old intact rats compared with 4-month-old intact animals, whereas no significant differences in glomerular and adrenal cortical AT1R densities were observed between 4- and 12-month-old OVX animals (Figures 2 and 3).

The positive correlation between increases in MAP and increases in AT1R densities in the adrenal and kidney from estrogen-depleted animals and the attenuation of both these effects by estrogen replacement suggests that the loss of estrogen modulation of the RAS plays a role in postmenopausal hypertension. In the kidney, AT1Rs modulate glomerular blood flow and transport processes within the renal tubules. Thus, the overactivity of the RAS induced by increased AT1R expression could lead to impaired sodium homeostasis. These studies also suggest that women prone to salt-sensitivity are more likely to have postmenopausal hypertension. In the adrenal cortex, Ang II stimulates aldosterone secretion to regulate sodium reabsorption. Thus, upregulation of AT1 receptors in the adrenal cortex is likely to result in an increase in sodium retention, which could contribute to the onset of the hypertension in the OVX animals. Estrogen replacement in intact DS rats was not investigated in this study; however, we speculate that estrogen treatment in intact rats at the age when estrogen declines would have attenuated the increase in blood pressure and AT1R expression observed in 12-month-old intact rats.

Perspectives
This study demonstrates that normotensive female DS rats maintained on LS diet develop hypertension as they age. Age-induced estrogen loss contributes to this increase in MAP, possibly by activating the RAS through increasing AT1R densities in the kidney and adrenal. These findings also demonstrate that the female DS rat is an excellent model to elucidate the role of sex steroids in estrogen modulation of the RAS in salt-sensitive postmenopausal hypertension. Further studies are planned to elucidate the effect of an activation of the RAS on sodium homeostasis in aging DS female rats. The effects of interfering with the activation of the RAS with an angiotensin receptor blocker are also a focus of ongoing studies.

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References
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