Postovariectomy Hypertension Is Linked to Increased Renal AT$_1$ Receptor and Salt Sensitivity

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Abstract—The functional balance between angiotensin II (Ang II) and nitric oxide (NO) plays a key role in modulating salt sensitivity. Estrogen has been shown to downregulate angiotensin type 1 (AT$_1$) receptor expression and to increase the bioavailability of endothelium-derived NO, which decreases AT$_1$ receptor expression. The present study tests the hypothesis that in the presence of genetic salt sensitivity, deficiency of endogenous estrogens after ovariectomy (OVX) fosters an upregulation of Ang II. Female Dahl salt-resistant (DR), Dahl salt-sensitive (DS), Wistar-Kyoto (WKY), and spontaneously hypertensive (SHR) rats underwent bilateral OVX or sham surgery (SHX) and were fed a normal salt diet (0.5% NaCl) for 14 weeks. Systolic blood pressures were measured every 2 weeks and were not significantly different between OVX and SHX for DR, WKY, and SHR groups. However, at the end of 14 weeks of normal salt diet, hypertension developed in DS OVX but not SHX rats (160±3 versus 136±3 mm Hg; P<0.05). Hypertension also developed in DS OVX rats pair-fed a normal salt diet (166±7 mm Hg). Development of hypertension in DS OVX rats was prevented by estrogen replacement (132±3 mm Hg), AT$_1$ receptor blockade (119±3 mm Hg), or feeding a very low salt diet (0.1% NaCl; 129±4 mm Hg). Renal AT$_1$ receptor protein expression was significantly elevated 2-fold in DS OVX relative to SHX rats and was prevented by estrogen replacement. These data strongly suggest that after OVX in salt-sensitive rats there is a lower threshold for the hypertensinogenic effect of salt that is linked to an activation of Ang II. (Hypertension. 2003;42:1157-1163.)

Key Words: rats, Dahl □ rats, spontaneously hypertensive □ sodium, dietary □ angiotensin II □ estrogen □ receptors, angiotensin

Cardiovascular diseases are the leading cause of death in women and claim the lives of more than half a million women every year. The incidence of cardiovascular disease is 4-fold higher in postmenopausal women than in women of the same age who are premenopausal. Hypertension is a major risk factor for cardiovascular disease. It has been shown that after adjustment for age and body mass index, postmenopausal women are more than twice as likely to be hypertensive as premenopausal women. Thus, after menopause, hypertension may contribute to the increase in cardiovascular risk of postmenopausal women.

Accumulating evidence also suggests that postmenopausal women are more salt-sensitive than premenopausal women. A study conducted in postmenopausal Japanese women showed that salt sensitivity correlated inversely with levels of circulating hormones, suggesting that decreases in ovarian hormone levels and increased sensitivity to dietary sodium may be important factors in the genesis of postmenopausal hypertension. Moreover, it has been recently reported that in contrast to women studied during the different phases of the menstrual cycle, menopausal women are characterized by a blunted suppression of renin by salt, which could contribute to the development of salt-sensitive hypertension.

These findings suggest that endogenous estrogens participate in the protection afforded to premenopausal women. It has been demonstrated that the cardiovascular protective effects of endogenous estrogens involve direct effects on blood vessels through modulation of endogenous vasoconstrictors such as Angiotensin II (Ang II) and vasodilators such as nitric oxide (NO), as well as reductions in serum lipoproteins and cholesterol levels. After the onset of menopause, deficiency of endogenous estrogens may unmask a population of women particularly prone to salt-sensitive hypertension.

Current data indicate that in hypertension, salt sensitivity is a marker for a disproportionate susceptibility to cardiovascular and renovascular injury. Similar to populations of humans, Dahl salt-sensitive (DS) rats, a well-established animal model of salt-sensitive hypertension, remain normotensive on a normal salt (0.5% NaCl) diet but become hypertensive when given high dietary salt. We have previously shown that despite a similarly elevated systolic blood pressure, hypertensive DS rats have significantly more left ventricular hypertrophy (LVH), vascular hypertrophy, and renal injury than spontaneously hypertensive (SHR) rats, an animal model of salt-resistant hypertension.
The renin-angiotensin system (RAS) and the NO pathway play a central role in blood pressure regulation and electrolyte balance and are involved in the phenomenon of salt sensitivity. Studies indicate that 17β-estradiol and inhibitors of the RAS reduce blood pressure. Estrogen has been shown to increase the bioavailability of NO and oppose the blood pressure–elevating and cell growth–promoting effects of Ang II. The present study tests the hypothesis that in the presence of genetic salt sensitivity, deficiency of endogenous estrogens after OVX is linked to a functional upregulation of Ang II action that lowers the threshold for the hypertensinogenic effect of salt.

Methods

Experimental Animals
Female DS and Dahl salt-resistant (DR) rats from the Brookhaven strain and SHR and Wistar-Kyoto (WKY) rats were purchased from Harlan Sprague Dawley Inc. Four- to 5-week-old rats underwent bilateral ovariectomy (OVX) or sham (SHX) operation under sodium pentobarbital (30 mg/kg body wt IP) anesthesia. After surgery, animals were fed standard rat chow that contained either normal salt (0.5% NaCl; Harlan Teklad Diet 8604) or low salt (0.1% NaCl diet; Harlan Teklad Diet TD 92238) for a period of 14 weeks. OVX animals were pair-fed according to the food intake of the SHX on the respective NaCl diets to rule out the possible effects of elevated body weight to increase blood pressure. Specifically, all animals were housed in individual cages, and the food intake of the SHX animals was determined daily. OVX animals were given the least amount of food consumed by a rat in the SHX group on the respective low or normal salt diet. Body weight and systolic blood pressure (SBP) of all rats were measured by tail-cuff method at 2-week intervals. Kidney tissues were collected at the end of the 14-week period for protein extraction. Hearts were removed and left ventricles were weighed. Adequacy of OVX was confirmed by the absence of ovarian tissue and marked atrophy of the uterus. A 73% reduction in uterine wet weight has previously been reported for OVX rats. All rats had free access to water and were housed in facilities accredited by AAALAC. The Institutional Animal Care and Use Committee approved the animal studies.

Experimental Groups
The following groups of rats were fed the 0.5% NaCl diet: (1) SHR that underwent SHX (n = 8) or OVX (n = 6), (2) WKY SHX (n = 6), OVX (n = 6); (3) DR SHX (n = 5), OVX (n = 5); and (4) DS SHX (n = 8), OVX (n = 15). The following groups of rats were pair-fed the 0.5% NaCl diet: (5) DS SHX (n = 7), DS OVX (n = 6); (6) DS OVX treated with estrogen replacement therapy (n = 5; 1.7 mg 17β-estradiol, 90-day subcutaneous pellet; Innovative Research of America); and (7) DS OVX administered an AT1 receptor blocker, candesartan (n = 6; 10 mg/kg body wt, gavage). This group of rats was pair-fed the 0.1% NaCl diet: (8) DS SHX (n = 5), DS OVX (n = 7). A recent study has shown that plasma 17β-estradiol levels of intact female Sprague-Dawley rats and OVX plus estrogen replacement (2.5 mg/90 d SQ 17β-estradiol pellet) were not significantly different over the time period of 3 to 21 months of age.

Western Blot Analysis of Whole-Kidney AT1 Receptor Protein
Proteins were extracted from kidneys obtained from pair-fed DS OVX, SHX, and OVX treated with estrogen after homogenization as described previously and measured by the method of Lowry et al. Kidney (10 to 50 μg) protein extracts were separated by gel electrophoresis, transferred to nitrocellulose membrane, blocked, and incubated with antipeptide AT1 polyclonal antibody (1:200; SC-1173, Santa Cruz) as previously described. Duplicate gels were prepared and stained with 0.1% Coomassie blue R250 and then destained in 7% acetic acid/5% methanol to visualize protein bands for total protein quantification and confirmation of equal protein loading between the groups. Alternatively, blots were stripped and reprobed with a monoclonal anti-β-actin antibody (1:20,000; Clone AC-15, Sigma-Aldrich). Signals were detected through the use of enhanced chemiluminescence, and the blots were exposed to x-ray film. The films and stained gels were scanned with the use of Digital Imaging and Analysis Systems (Alpha Innotech Corp).

Data Analysis
Systolic blood pressures over the 14-week period were analyzed by 1-way repeated ANOVA followed by the Dunnett test, using Sigma Stat Statistical Software. Comparisons between SHX and OVX SBP for a given rat model for a given time point were made by means of an unpaired t test, using the Bonferroni correction for multiple comparisons. Systolic blood pressures between the 6 pair-fed groups at 14 weeks were analyzed by 1-way ANOVA followed by multiple comparisons, using the Bonferroni t test. Other comparisons between groups were performed by means of a t test. A probability value <0.05 was considered statistically significant. All data are presented as mean±SEM.

Results
Effect of OVX on Body Weight and Heart Weight in DS, DR, SHR, and WKY Rats
Body weights were not different between SHX and OVX animals at baseline (Table). All animals gained weight in a similar pattern that was independent of the diets or treatments. Importantly, OVX rats in the DS, DR, and SHR groups, maintained on a normal salt diet, weighed more than the SHX females in their respective groups at the end of the study (P<0.01 versus SHX) (Figures 1A and 1B). Although the OVX rats in the WKY group also showed a tendency toward greater weight gain than the SHX females, the difference was not statistically significant. Surprisingly, DS OVX animals that were pair-fed throughout the study also showed a significantly higher body weight when compared with the pair-fed DS SHX (Figure 1B).

Left ventricular weight was measured at the end of the study period in DR, DS, pair-fed DS, WKY, and SHX groups maintained on a normal salt diet (Figure 2). Compared with SHX females, OVX rats in the salt-sensitive DS group exhibited a greater left ventricular weight (P<0.05). There was no significant difference in left ventricular weight between OVX and SHX females in the salt-resistant DR, SHR, and WKY groups.

Effect of OVX on SBP in DS, DR, SHR, and WKY Rats
As the animals matured, SBP increased significantly in all animals over the 14-week period (Figure 3, P<0.05). However, SBP in the DS OVX rats was significantly higher than...
SHX females in the DS group at 2, 8, 10, 12, and 14 weeks (Figure 3A). The OVX and SHX females in the DR (Figure 3B) and WKY (Figure 3C) groups demonstrated a similar, gradual increase in SBP during the time course of the study but remained normotensive. The SHR females were hypertensive at the beginning of the study and exhibited a gradual elevation in SBP that did not differ between OVX and SHX (Figure 3C).

To assess whether the development of hypertension in DS OVX rats was independent of the amount of food intake, DS OVX and SHX females were pair-fed a normal salt diet. The pair-fed DS OVX rats also had a significant rise in SBP as compared with DS SHX rats at the end of the study ($P<0.05$; Figure 4).

Figure 1. Body weight at 14 weeks in SHX (hatched bars) and OVX (solid bars) DR (n=5, 5), WKY (n=6, 6), and SHR (n=8, 6) (A). Body weight was significantly higher in ovariectomized DR and SHR rats. Similarly, DS (n=8) OVX rats gained significantly more weight compared with DS SHX (n=8) (B). Pair-feeding (PF) DS SHX (n=8) and DS OVX (n=9) animals did not normalize body weight at 14 weeks. n=SHX, OVX; *$P<0.05$ vs SHX.

Figure 2. Left ventricular weight in SHX (hatched bar) and OVX (solid bar) DR (n=5, 5), DS (n=8, 8), pair-fed DS (PF/DS) (n=7, 9), WKY (n=6, 8), and SHR (n=8, 7) rats (A). Only DS OVX animals with hypertension showed elevated left ventricular weight. n=SHX, OVX; *$P<0.05$ vs SHX.

Figure 3. SBP measured at 2-week intervals during 14-week period in SHX (open symbols) and OVX (closed symbols) rats. All rats showed significant rise in SBP over the 14-week period (A). DS OVX (n=15) demonstrated a significantly elevated SBP compared with DS SHX (n=8) rats. Hypertension was observed as early as 2 weeks after OVX. SBP of DR SHX (n=5) and DR OVX (n=5) were not different (B). SBP of WKY SHX (n=6) and WKY OVX (n=6) or SHR SHX (n=8) and SHR OVX (n=6) rats were not different (C). *$P<0.05$ vs baseline; +$P<0.05$ vs SHX.

**Effect of Low Salt Diet After OVX on SBP in DS Rats**

Hypertension developed in salt-sensitive DS rats fed a normal salt diet after OVX. To assess the contribution of dietary salt to SBP after the loss of ovarian hormones, we tested the effect of a low salt diet on SBP for the same period after OVX or SHX. Figure 4 shows that pair-feeding a low salt diet prevented the rise in SBP in DS OVX rats ($P<0.05$ versus DS OVX rats on a normal salt diet). Moreover, there was no significant difference in SBP between DS SHX and DS OVX rats maintained on a low salt diet throughout the study period.

**Effect of 17β-Estradiol Treatment After OVX on SBP in DS Rats**

To determine the contribution of 17β estradiol to SBP, we treated DS OVX rats with estrogen replacement therapy. Figure 4 demonstrates that treatment with estrogen for 14 weeks prevented the development of hypertension in DS
Expression in DS Rats

Rats fed a low salt diet (n = SHX, OVX; /H11005) had significantly lower blood pressure in DS OVX rats. SBP was significantly elevated in pair-fed DS OVX to similar levels as animals that ate ad libitum. Candesartan and E2 treatment normalized blood pressure in DS OVX rats administered estrogen (E2; n = 5). SBP was significantly decreased in DS OVX rats fed a low salt diet (n = 5, 7, 0.1% NaCl), n = SHX, OVX; *P<0.05 vs DS OVX on 0.5% NaCl.

OVX rats pair-fed a normal salt diet (SBP 132±3.4 mm Hg; n = 5; P<0.05 versus control DS OVX rats).

Effect of OVX on Renal AT1 Receptor Protein Expression in DS Rats

In DS rats fed a normal salt diet for 14 weeks, the rise in SBP after OVX is accompanied by an increase in renal AT1 receptor protein expression that is reduced by estrogen replacement therapy. Densitometric analysis of renal AT1 receptor protein expression showed a 2-fold increase in DS OVX rats relative to DS SHX rats (P<0.05; Figure 5A). This effect was not observed 3 days after OVX in DS rats (Figure 5B). Treatment of DS OVX rats with estrogen significantly decreased renal AT1 receptor protein expression relative to control DS OVX (P<0.05; Figure 5C) and DS SHX (P<0.05; Figure 5D). Equal protein loading was observed for each of the group comparisons (data not shown).

Effect of AT1 Receptor Antagonist After OVX on SBP in DS Rats

Estrogen deficiency of DS females fed a normal salt diet caused a significant elevation in SBP accompanied by an increase in AT1 receptor protein expression. Both effects were reduced by estrogen replacement therapy. To further support the notion that AT1 receptor upregulation contributes to the development of post-OVX hypertension, DS OVX rats were treated with the AT1 receptor antagonist candesartan for 14 weeks. Figure 4 reveals that AT1 receptor antagonism completely prevents the rise in SBP in DS OVX rats maintained on a normal salt diet (SBP 119±3 mm Hg; n = 6; P<0.05 versus control DS OVX rats).

Discussion

Studies done in different ethnic groups indicate that postmenopausal women are more salt-sensitive than premeno-

pausal women, suggesting that decreases in ovarian hormone levels and increased sensitivity to dietary sodium may be important factors in the genesis of postmenopausal hypertension.1–6 The DS and DR rat strains developed by Dahl have been useful as an animal model for the study of salt-sensitive hypertension. One of the defects characterized in this form of hypertension is a blunted pressure-natriuresis relation, so that a higher blood pressure is needed to achieve the same level of sodium excretion. Short-term studies performed by Otsuka et al20 demonstrated this antinatriuretic shift in the pressure-natriuresis relation in DS rats compared with DR rats. O VX further impaired the pressure-natriuresis response in DS but not DR rats. These investigators, however, did not study whether OVX fostered hypertension.

In the present study, we have shown that in salt-sensitive DS rats, despite a normal salt diet, OVX promotes hypertension accompanied by LVH. In normotensive salt-resistant DR and WKY and in hypertensive salt-resistant SHR rats, OVX did not significantly affect systolic blood pressure or left ventricular mass in the setting of a normal salt diet. Fang et al26 demonstrated the lack of an effect of OVX to increase blood pressure in SHR fed a normal salt diet, although the combination of OVX plus high salt diet feeding did elevate blood pressure in this strain. LVH is known to be a powerful predictor of cardiovascular morbidity and mortality. Consistent with our findings, a higher incidence of LVH in salt-sensitive hypertensive patients than in salt-resistant hypertensive patients has been reported.27 Three large studies of patients with essential hypertension demonstrated that patients who were salt-sensitive more often had LVH, cardiovascular events, and/or endothelial dysfunction than non–salt-sensitive hypertensive patients.28–30 These findings highlight the important link between salt-sensitive hypertension and cardiovascular injury.

Whereas a normal salt diet promoted hypertension in DS OVX rats, feeding a diet very low in salt prevented post-OVX hypertension. These findings suggest that OVX lowers the threshold for the hypertensinogenic effect of salt in salt-sensitive hypertension. A recent study by Hinojosa-LaBorde et al31 similarly showed that OVX results in the development of hypertension in DS rats, but maintenance on a 0.15% NaCl diet was not preventive. The reasons for the discrepancy may be related to differences between the two DS rat strains (Rapp versus Brookhaven strains) and the lower salt diet (0.1% NaCl) in our study.

Salt-sensitive hypertension has been linked to decreased renal NO production, inappropriate activation of the RAS, or both. The NO and RAS are key systems for controlling pressure-natriuresis, and there is substantial evidence from human and animal studies that estrogen modulates these systems. Estrogen, through estrogen receptor–dependent and estrogen receptor–independent mechanisms, has been shown to increase the bioavailability of endothelium-derived NO.32,33 A defect exists in the ability of DS rats to increase renal medullary NO concentrations in response to a low subpressor infusion of Ang II as compared with salt-resistant Brown-Norway rats, thus making DS rats more susceptible to the hypertensive actions of small elevations of Ang II.34

Taken together, these data imply that in the presence of
genetic salt sensitivity, a loss of estrogen may further impair the bioavailability of NO.

Estrogen has been shown to inhibit circulating renin and ACE, decrease circulating Ang II levels, and downregulate AT₁ receptor expression in adrenal cortex, hypothalamus, and vascular smooth muscle cells. Despite a lack of effect on blood pressure, WKY OVX and SHR OVX rats showed an increase in Ang II–mediated aortic vasoconstriction that was mediated by an increase in AT₁ receptor gene expression. This effect was reversed by estrogen replacement. Furthermore, NO has been shown to decrease AT₁ receptor gene expression in vascular smooth muscle cells, providing evidence of the interrelation between the two systems.

On the basis of these findings, we hypothesized that OVX lowers the threshold for the hypertensinogenic effect of salt in salt-sensitive subjects through AT₁ receptor upregulation. We found that post-OVX hypertension in DS rats is correlated with an increase in renal AT₁ receptor protein expression. Furthermore, treatment with estrogen replacement or an AT₁
receptor antagonist prevented the development of post-OVX hypertension in DS rats maintained on a normal salt diet.

Salt sensitivity has been proposed as a marker for susceptibility to cardiovascular and renovascular injury. After menopause, the loss of the ovarian hormones may unmask a population of women prone to salt-sensitive hypertension that would be at higher risk for cardiovascular morbidity and mortality. Clinically, we infer that after menopause, estrogen deficiency promotes an overexpression of renal AT1 receptors resulting in oxidative stress, disturbed renal sodium handling, and hypertension, particularly in women genetically prone to salt sensitivity. Our studies demonstrate an important interaction between estrogen and the Ang II system and therefore may provide an insight into preventive and/or therapeutic strategies. Indeed, 2 recent studies have demonstrated that AT1 receptor blockade significantly reduces blood pressure in hypertensive postmenopausal women. Particularly in light of the disappointing cardioprotective results obtained in several clinical trials with hormone replacement therapy, the mechanisms underlying the estrogen/Ang II system interaction merit further study for the treatment of hypertension in postmenopausal women.

Perspectives

Clinically, it has been shown that salt-sensitive individuals exhibit endothelial dysfunction and have a greater susceptibility to cardiovascular injury than salt-resistant individuals. After the loss of ovarian function, the incidence of salt sensitivity and hypertension increases in women and the incidence of cardiovascular disease equalizes between men and women, suggesting that endogenous estrogens participate in the protection afforded to premenopausal women. Studies have demonstrated that estrogen increases the bioavailability of NO and antagonizes the actions of Ang II. The homeostatic balance of these vasoactive agents plays an important role in modulating salt sensitivity as well as hypertensive end-organ injury. Our study demonstrates that in Dahl salt-sensitive rats, despite a normal salt diet, ovariectomy results in a functional upregulation of Ang II that fosters hypertension accompanied by LVH. These findings reveal an important interaction between estrogen and the Ang II system and suggest that inhibition of the Ang II system may be particularly beneficial in the treatment of postmenopausal hypertension and its complications, especially since hormone replacement therapy, in its current form, has failed to provide cardioprotection in several clinical trials.

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References


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