Postovariectomy Hypertension Is Linked to Increased Renal $\text{AT}_1$ Receptor and Salt Sensitivity

Lisa M. Harrison-Bernard, Ivonne Hernandez Schulman, Leopoldo Raij

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 ($\text{AT}_1$) receptor expression and to increase the bioavailability of endothelium-derived NO, which decreases $\text{AT}_1$ 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), $\text{AT}_1$ receptor blockade (119±3 mm Hg), or feeding a very low salt diet (0.1% NaCl; 129±4 mm Hg). Renal $\text{AT}_1$ 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 rats, 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 7.5% 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 SHR 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

Baseline Body Weights in SHR, WKY, DR, and DS Rats

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>SHX</th>
<th>OVX</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR, g</td>
<td>154±4</td>
<td>143±6</td>
</tr>
<tr>
<td>WKY, g</td>
<td>126±9</td>
<td>139±7</td>
</tr>
<tr>
<td>DR, g</td>
<td>106±7</td>
<td>106±4</td>
</tr>
<tr>
<td>DS, g</td>
<td>177±6</td>
<td>170±9</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar Kyoto rats; DR, Dahl salt-resistant; DS, Dahl salt-sensitive; SHX, sham surgery; and OVX, ovariectomy.
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).

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 time 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
SBP in DS Rats

SHX, OVX; /H11005 /H11005

rats fed a low salt diet (n = 5, 7; 0.1% NaCl). SBP was significantly lower in DS OVX rats. SBP was significantly

treated with the AT1 receptor blocker candesartan for 14

weeks. Figure 4 reveals that AT1 receptor antagonism com-

pletely 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).

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 com-

pletely 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 post-

menopausal 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 hyper-
tension.3–6 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
al32 demonstrated this anti-natriuretic shift in the pressure-
natriuresis relation in DS rats compared with DR rats. OVX

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 hyperten-
sion 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
al36 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. Consis-
tent with our findings, a higher incidence of LVH in salt-
sensitive hypertensive patients than in salt-resistant hyperten-
sive patients has been reported.27 Three large studies of
patients with essential hypertension demonstrated that pa-
tients who were salt-sensitive more often had LVH, cardio-
vascular 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
substrressor 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.\textsuperscript{17,35–38} 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.\textsuperscript{37,39} This effect was reversed by estrogen replacement. Furthermore, NO has been shown to decrease AT₁ receptor gene expression in vascular smooth muscle cells,\textsuperscript{40} 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,1 disturbed renal sodium handling,2 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. Particular 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, ovarectomy 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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