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Ovariectomy is protective against renal injury in the high-salt-fed older mRen2.Lewis rat

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Yamaleyeva LM, Pendergrass KD, Pirro NT, Gallagher PE, Groban L, Chappell MC. Ovariectomy is protective against renal injury in the high-salt-fed older mRen2.Lewis rat. Am J Physiol Heart Circ Physiol 293: H2064–H2071, 2007. First published July 13, 2007; doi:10.1152/ajpheart.00427.2007.—Studies in experimental animals and younger women suggest a protective role for estrogen; however, clinical trials may not substantiate this effect in older females. Therefore, the present study assessed the outcome of ovariectomy in older mRen2.Lewis rats subjected to a high-salt diet for 4 wk. Intact or ovariectomized (OVX, 15 wk of age) mRen2.Lewis rats were aged to 60 wk and then placed on a high-salt (HS, 8% sodium chloride) diet for 4 wk. Systolic blood pressures were similar between groups [OVX 169 ± 6 vs. Intact 182 ± 7 mmHg; P = 0.22] after the 4-wk diet; however, proteinuria [OVX 0.8 ± 0.2 vs. Intact 11.5 ± 2.6 mg/mg creatinine; P < 0.002, n = 6], renal interstitial fibrosis, glomerular sclerosis, and tubular casts were lower in OVX vs. Intact rats. Kidney injury molecule-1 mRNA, a marker of tubular damage, was 53% lower in the OVX HS group. Independent from blood pressure, OVX HS rats exhibited significantly lower cardiac (24%) and renal (32%) hypertrophy as well as lower C-reactive protein (28%). Circulating insulin-like growth factor-I (IGF-I) levels were not different between the Intact and OVX groups; however, renal cortical IGF-I mRNA and protein were attenuated in OVX rats [P < 0.05, n = 6]. We conclude that ovariectomy in the older female mRen2.Lewis rat conveys protection against salt-dependent increase in renal injury.

estrogen; aging; female rat; renal injury; insulin-like growth factor-I; kidney injury molecule-1

A CONGENIC model of tissue renin expression that is derived from the mRen2 (27) Sprague-Dawley strain is the mRen2.Lewis female rat that exhibits both estrogen- and salt-dependent alterations in blood pressure and tissue injury (7, 8, 38). Estrogen depletion through bilateral ovariectomy has a profound influence on the development of hypertension in the congenic rats. Moreover, 17β-estradiol replacement or blockade with an angiotensin II (ANG II) type 1 (AT1) receptor antagonist abolishes the increase in blood pressure following ovariectomy and reduces blood pressure below that of the intact mRen2.Lewis rat (7). Chronic salt loading in the intact strain also induces a substantial and sustained increase in blood pressure as well as cardiac hypertrophy and proteinuria that is not evident in the normotensive Lewis strain (8). The enhanced expression of the renin-angiotensin-aldosterone system (RAAS) likely contributes to the genesis of the salt-sensitive phenotype in the mRen2.Lewis strain; circulating levels of ANG II, as well as angiotensin-converting enzyme (ACE) and renin activities, are either increased or maintained with a chronic high-salt diet (8). Moreover, estrogen depletion exacerbates the salt-dependent increase in both blood pressure and renal injury (proteinuria). Indeed, plasma ANG II, ACE, and renin achieved their highest levels in estrogen-depleted, high-salt-fed mRen2.Lewis rats (8).

Although these studies clearly support the well-accepted view for a protective role of ovarian hormones such as estrogen in mRen2.Lewis hypertensive rats, estrogen depletion and salt loading were begun at a relatively early age of 4–5 wk. Indeed, this time period precedes the rapid development of hypertension that occurs in many experimental models, including the mRen2.Lewis strain (41). Previous studies in Dahl salt-sensitive rats revealed that initiation of a high-salt diet at a young age results in a more profound increase in blood pressure than at later time points, suggesting that salt sensitivity, at least in terms of the blood pressure response, may reflect a critical developmental period (41). In this regard, blockade of the RAAS in young male spontaneously hypertensive rats (SHR) before the development of established hypertension conveys long-lasting actions to reduce blood pressure (15). In the present studies, we began to assess the benefits of ovarian hormones in older female mRen2.Lewis rats with established hypertension. We specifically determined whether ovariectomy in older rats 1) exacerbates the degree of hypertension and renal injury in rats maintained on a normal-salt diet; 2) influences the extent of salt sensitivity in the older mRen2.Lewis rat; and 3) influences the intrarenal RAAS and other systems associated with renal injury.

MATERIALS AND METHODS

Experimental animals. Female mRen2.Lewis rats at the age of 14 wk were obtained from the congenic colony of the Hypertension and Vascular Research Center of Wake Forest University. Animals were housed in a temperature-controlled room with a 12:12-h light-dark cycle (lights on 6:00 AM to 6:00 PM) in an American Association for Accreditation of Laboratory Animal Care-approved facility and were randomly assigned to study groups, six animals per group. Rats were ovariectomized at 15 wk of age by bilateral flank incisions under

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Fig. 1. Systolic blood pressure (SBP) and proteinuria (inset) in older intact and ovariectomized (OVX) female mRen2.Lewis rats. Blood pressures were measured at 15 (before ovariectomy), 30, and 60 wk of age. Data are means ± SE; *P < 0.05 vs. 15-wk-old Intact; #P < 0.05 vs. 15-wk-old OVX; n = 6 for each group. Inset: proteinuria was determined at 60 wk of age. Data (expressed as mg protein/mg creatinine) are means ± SE; n = 6 for each group.

Fig. 2. SBP (A) and proteinuria (B) in 64-wk-old intact or OVX mRen2.Lewis rat after a high-salt (HS) diet. A: SBP in 64-wk-old mRen2.Lewis rats. Data are means ± SE; n = 6 for each group. B: urinary protein data expressed as mg protein/ mg creatinine. Data are means ± SE; *P < 0.05 vs. Intact HS group; n = 6 for each group.
was a standard.

Proteins were determined in the initial tissue homogenate fraction with a Bradford protein assay kit (Bio-Rad) with bovine IgG protein

trointestinal, bronchial, and interstitial areas of the renal cortex of Intact HS (Fig. 3 A, inset). There was moderate to very marked deposition of collagen fibers and analyzed by Experimental Pathology Laboratories (Charlottesville, VA). Each lesion was given a grade with a five-point grading scale: grade 1 = minimal, grade 2 = mild, grade 3 = moderate, grade 4 = marked, grade 5 = very marked. The slides were scored by a grader blind to the individual groups.

Statistical analysis. All measurements were expressed as means ± SE computed from an average of five determinations per rat for SBP or duplicate values for the biochemical data from each rat. Comparisons between groups were evaluated by one-way analysis of variance followed by Tukey’s post hoc test, by unpaired t-test [GraphPad Prism IV plotting and statistical software (San Diego, CA) or Microsoft Excel 2002], or by Aspin-Welch unequal variance test for the analysis of trichrome stain data (NCSS Statistical Software, Kaysville, UT).

RESULTS

Systolic blood pressures and proteinuria. Animals were either ovariectomized (OVX) at 15 wk or left intact and followed to 60 wk of age to investigate whether long-term estrogen depletion influences hypertension and renal injury in the older female mRen2.Lewis rat. As shown in Fig. 1, SBP was similar between the Intact and OVX groups at 15 (before ovariectomy), 30, and 60 wk of age; however, blood pressures were significantly increased at 60 wk vs. 15 wk for both intact and OVX mRen2.Lewis rats (Intact at 60 wk 162 ± 8 mmHg vs. Intact at 15 wk 142 ± 6 mmHg; OVX at 60 wk 164 ± 6 mmHg vs. OVX at 15 wk 147 ± 7 mmHg; P < 0.05, n = 6 for each group). There were no differences in proteinuria between Intact and OVX groups at 60 wk of age (Fig. 1, inset; Intact 0.25 ± 0.03 vs. OVX 0.23 ± 0.02 mg/mg creatinine; n = 6). The 60-wk-old rats were then placed on an 8% sodium chloride diet for 4 wk and SBP and proteinuria were measured at the end of this period. After the high-salt diet, SBP was similar between groups (Fig. 2A; Intact 182 ± 7 vs. OVX 169 ± 6 mmHg; n = 6); however, there was a 14-fold difference in the extent of proteinuria between the two groups (Fig. 2B; Intact 11.5 ± 2.6 vs. OVX 0.8 ± 0.2 mg/mg creatinine; P < 0.002, n = 6).

Shown in Table 1 are the physiological indexes for Intact and OVX mRen2.Lewis rats on high salt at 64 wk of age. The OVX high-salt rats were 35% heavier than the high-salt-fed Intact group, emphasizing the influence of ovarian hormones on weight gain. Indeed, plasma estradiol levels, although still detectable in the OVX group, were 50% lower than in the
Intact group, while free testosterone was undetectable in both groups. Daily urine volume was 37% higher in the Intact group on high salt compared with the OVX group; however, creatinine clearance was not different between groups. Despite the similar level of blood pressure among the two groups, the OVX high-salt rats exhibited significantly lower cardiac (24%) and renal (32%) hypertrophy (Table 1), although uncorrected heart weights were similar between the two groups. The circulating levels of ANG II and IGF-I were not different between groups; however, CRP, a marker of inflammation, was significantly reduced (28%) in the OVX high-salt-fed rats (Table 1).

Renal histology. In light of the striking differences in the extent of renal hypertrophy and proteinuria, we characterized the renal injury between the Intact and OVX rats at 64 wk of age. As shown by Masson’s trichrome stain (Fig. 3), the most prominent changes in both groups were glomerular sclerosis, interstitial and periglomerular fibrosis, as well as tubular casts, with a higher incidence of these lesions in the kidneys from the Intact high-salt rats (Table 2). Inflammation was also detected in the kidneys from both groups; however, this was evident only in one animal from the OVX group and in all kidneys from the intact group. Finally, vascular smooth muscle cell and tubular hyperplasia were found only in the Intact high-salt group (Table 2).

Renal molecular markers. mRNA of the major podocyte proteins nephrin and podocin was quantified in the renal cortical tissue of Intact and OVX rats fed a high-salt diet to distinguish between glomerular and tubular injury. Neither nephrin (Fig. 4A) nor podocin (Fig. 4B) mRNA values were different between the Intact and OVX groups after a high-salt diet. However, the mRNA levels of kidney injury molecule 1 (KIM-1), a marker of tubular damage, were 44% lower in the OVX high-salt group (Fig. 4C), which is consistent with the histological data suggesting tubular injury.

Intrarenal peptides. The mRen2.Lewis rat is a model of tissue renin expression that contributes to the development of hypertension and organ injury (7). Blockade of the RAAS is known to attenuate the progression of age-associated pathologies (20). As shown in Fig. 5, renal cortical and medullary ANG II tissue levels were not different between the Intact and OVX groups (Fig. 5A). ANG-(1-7), an alternative product of the RAAS, has opposing actions to ANG II and reduces the extent of tissue injury in various models (3, 6). Although ANG-(1-7) was detectable in the kidney, the levels of heptapeptide were not different between groups. Interestingly, there were significant differences between renal cortical and medullary concentrations of the peptide, with higher medullary ANG-(1-7) levels evident in both the Intact and OVX groups (Fig. 5B).

Since IGF-I expression is associated with renal injury in old rats, we assessed both circulating and renal tissue IGF-I levels. The circulating IGF-I levels were not different between the two groups (Table 1). The kidney contains a local or tissue IGF-I system similar to the RAAS and other peptidergic systems (28). Therefore, we assessed intrarenal IGF-I content in the cortical and medullary tissues of the Intact and OVX high-salt

### Table 2. Histological evaluation of trichrome-stained kidney sections

<table>
<thead>
<tr>
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<th>Intact HS</th>
<th>OVX HS</th>
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<tr>
<td></td>
<td>Severity</td>
<td>Incidence rate, %</td>
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<tr>
<td>Interstitial fibrosis</td>
<td>2 ± 0</td>
<td>100</td>
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<tr>
<td>Glomerular sclerosis</td>
<td>2 ± 0</td>
<td>100</td>
</tr>
<tr>
<td>Vascular smooth muscle hyperplasia</td>
<td>1.3 ± 0.3</td>
<td>75</td>
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<tr>
<td>Tubular casts</td>
<td>2.5 ± 0.3</td>
<td>100</td>
</tr>
<tr>
<td>Tubular hyperplasia</td>
<td>1.38 ± 0.2</td>
<td>100</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1 ± 0</td>
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Values are means ± SE, n = 4 for each group. Severity was defined as 1 (minimal)–5 (very marked). ND, not detectable.

Fig. 4. Markers for glomerular (nephrin and podocin) and tubular (kidney injury molecule-1, KIM-1) damage in 64-wk-old Intact HS and OVX HS female mRen2.Lewis rats. A and B: mRNA levels of the podocyte-comprising proteins nephrin (A) and podocin (B). C: mRNA of KIM-1. mRNA data are expressed as ratio of the target gene to 18 S rRNA. Data are means ± SE. *P < 0.05 vs. Intact HS group; n = 6 for each group.
groups (Fig. 6). Renal expression of cortical IGF-I (Fig. 6B) was significantly lower in the OVX high-salt group compared with the Intact group (Intact 1.5 ± 0.2 vs. OVX 0.7 ± 0.1 ng/mg protein; P < 0.05; n = 6). In contrast, the medullary levels of IGF-I were not statistically different between groups, although the Intact group tended to exhibit higher IGF-I content (Fig. 6B). Consistent with the cortical tissue IGF-I expression, the Intact group exhibited significantly higher expression of IGF-I mRNA (Fig. 6A), suggesting that translational regulation may contribute to the differences in peptide levels (Intact 1.0 ± 0.1 vs. OVX 0.55 ± 0.1 units; P < 0.01, n = 6). Finally, we assessed the distribution of immunoreactive IGF-I in the renal cortex of the Intact high-salt rats at 64 wk of age (Fig. 7). The staining for IGF-I (Fig. 7, A and B) was predominantly localized to periglomerular area, cortex, and collecting tubules; absence of the primary antibody abolished any staining in adjacent sections (Fig. 7, C and D). A similar pattern for IGF-I staining was also observed in the kidney of the OVX high-salt rats (data not shown).

**DISCUSSION**

The influence of estrogen and other reproductive hormones on cardiovascular function remains an important and timely issue in the aging female. In the present study, we demonstrate that in 64-wk-old female mRen2.Lewis rats long-term ovariectomy conveyed protective actions on salt-dependent renal injury compared with age-matched intact rats. A 4-wk course of a high-salt diet did not increase SBP in the older rats but clearly revealed a beneficial effect of ovariectomy on the extent of proteinuria. Indeed, the intact older rats maintained on a high-salt diet exhibited a 14-fold increase in urinary excretion of protein. The intact mRen2.Lewis group on high salt also exhibited higher circulating levels of the inflammatory marker CRP and increased renal expression of KIM-1 and IGF-I compared with the estrogen-depleted group.

The present results demonstrating a protective role of ovariectomy on the extent of salt-dependent renal injury are an unexpected finding, particularly given the number of studies that demonstrate beneficial actions of estrogen within the kidney (1, 24, 25). Our own studies in younger ovariectomized mRen2.Lewis rats clearly showed that exogenous 17β-estradiol reduced blood pressure to approximately the same extent as treatment with the AT1 receptor antagonist olmesartan (7). Although the latter findings support the view that endogenous estrogens participate in cardioprotection of premenopausal women, the role of estrogen remains controversial. Two large clinical studies—the Women’s Health Initiative (WHI) and the Heart and Estrogen/Progestin Replacement Study (HERS)—found no overall benefit, and in certain instances estrogen-progesterin or estrogen-alone therapies exacerbated underlying cardiovascular events (11). Changes in renal injury or function, however, were not evaluated in WHI or HERS. The beneficial effects of ovariectomy in our study may reflect a specific response in older hypertensive mRen2.Lewis rats. For example, estrogen has differential effects on inflammatory markers in younger compared with older rats. Nordell and colleagues (26, 30) found that the neuroprotective effects of estrogen were age specific; estrogen replacement decreased interleukin-1β in young adult female rats but enhanced its expression in reproductive senescent females. Oestreicher et al. (27) also reported

**Fig. 5. Renal angiotensin II (ANG II) (A) and ANG-(1-7) (B) in 64-wk-old Intact and OVX female mRen2.Lewis rats after HS diet. Filled bars, Intact HS group; open bars, OVX HS group. Data [expressed as fmol ANG II or ANG-(1-7)/mg tissue protein] are means ± SE. *P < 0.05 vs. cortical ANG-(1-7) in both Intact and OVX groups; n = 6 for each group.**

**Fig. 6. Insulin-like growth factor-I (IGF-I) expression in 64-wk-old Intact and OVX female mRen2.Lewis rats after HS diet. A: IGF-I mRNA levels in renal cortex of mRen2.Lewis rat. Real-time PCR data (expressed as ratio of IGF-I to 18S rRNA) are means ± SE. *P < 0.05 vs. Intact HS group; n = 6 for each group. B: renal cortical and medullary IGF-I protein measured by ELISA. Filled bars, Intact HS group; open bars, OVX HS group. Data (expressed as ng IGF-I/mg tissue protein) are means ± SE. *P < 0.05 vs. cortical Intact HS group; n = 6 for each group.**
that ovariectomy reduced the extent of glomerular damage, renal vascular injury, and fibrinoid necrosis in the L-NAME-ANG II rat model. Interestingly, estrogen increased triglyceride-rich lipoproteins and thereby accelerated the development of glomerular damage in obese Zucker rats (13, 32). Moreover, ovariectomy of these animals attenuated renal injury by lowering hypertriglyceridemia and urinary albumin to levels below those of the intact group (32). The status of circulating lipoproteins was not determined in the present study, and we are not aware of any evidence that a reduction in lipid levels may ameliorate salt-dependent renal injury in the mRen2.Lewis rat or other salt-sensitive models. Finally, we did not ascertain the levels of progesterone in the present study, and the decline in this steroid may contribute to the renoprotective actions of ovariectomy in older mRen2.Lewis rats.

As to other potential mechanisms that may exacerbate the renal injury in older high-salt-fed mRen2.Lewis rats, we found reduced circulating levels of CRP in the ovariectomized group. Several clinical trials reported a concomitant increase in circulating CRP after hormone replacement therapy, and there is evidence linking CRP to cardiovascular and renal disease, although whether CRP is a marker of inflammation or plays a direct role in tissue injury is equivocal at this time (23). The higher content of circulating CRP may reflect the proinflammatory effects of estrogen. CRP may enhance injury by activating complement, facilitating the uptake of low-density lipoproteins by macrophages, increasing plasminogen activator inhibitor-1 expression, as well as stimulating AT1 receptor levels (12, 17, 33, 36, 42). Trachtman et al. (34) found that CRP both attenuated nitric oxide concentrations and increased the levels of superoxide, most likely via stimulation of NADPH in rat mesangial cells. In human distal tubular cells, Baer et al. (2) reported that CRP stimulated protein secretion of both MAP kinase and the chemokine RANTES. Although it may be argued that a 30% change in circulating CRP would be insufficient to induce renal injury, local tissue levels of CRP within the kidney may be increased as well to promote damage, particularly in the presence of a high-salt diet (19).

The excretion of urinary protein was markedly increased in the Intact high-salt group; however, we did not detect altered expression of the glomerular proteins nephrin and podocin. Podocytes in the kidney regulate the glomerulosclerosis barrier, and mutations in major podocyte-associated proteins, including nephrin and podocin, lead to progressive renal disease (5, 22). Moreover, creatinine clearance was similar between the two groups, suggesting that structural alterations in the glomerulus may not be the sole cause for the increase in urinary protein excretion. In this regard, we found increased expression of KIM-1 mRNA in the renal cortex, a putative marker for tubular damage. Previous studies demonstrated the upregulation of KIM-1 in injured proximal tubular cells in polycystic kidney disease, ischemic acute tubular necrosis, and protein-overload nephropathy as well as in the male hypertensive Ren2(27) rat, the founder strain of the mRen2.Lewis rat (10, 18, 21, 35). These data and the histological evidence for increased tubular casts in the intact group suggest that the proteinuria may reflect damage to the renal tubule and a reduced capacity to absorb albumin and other proteins. Moreover, renal cortical IGF-I mRNA and peptide levels were elevated approximately twofold in the Intact group after a high-salt diet without alterations in the circulating peptide. IGF-I is a pleuripotent factor that may play a role in various functions including kidney hypertrophy, glomerular filtration, release of prostaglandin E2 and nitric oxide, sodium reabsorption, as well as mesangial cell apoptosis and proliferation (4, 9, 28, 37). All components of the IGF-I system (IGF-I, IGF-I binding proteins and receptors) are present in the kidney, and, consistent with other reports, IGF-I was localized primarily to the periglomerular area and the proximal, distal, and collecting tubules of older mRen2.Lewis rats (14, 37). Recent studies

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**Fig. 7.** Immunocytochemical localization of IGF-I in the kidney of the intact mRen2.Lewis rat fed HS diet. A and B: staining with primary antibody in the renal cortex (A) and medulla (B). C and D: absence of staining without addition of primary IGF-I antibody. Magnification: ×40.
sugest that suppression of the growth hormone-IGF-I axis may be renoprotective in the aging rat maintained on a normal-salt diet (31, 40). It remains to be determined whether the increase in renal IGF-I is a compensatory effect or an initiating event that may lead to tubular damage and hypertrophy in the intact older mRen2.Lewis rat maintained on a high-salt diet. Studies are in progress to determine whether an IGF-I receptor antagonist has an ameliorating effect on proteinuria in the mRen2.Lewis rat.

Finally, the mRen2.Lewis strain is a model of tissue renin expression, and in younger rats ovariectomy and a high-salt diet were associated with increased expression of circulating ANG II, ACE, and renin, suggesting that dysregulation of the RAAS may contribute to the estrogen and salt sensitivity in these rats (7, 8). Our preliminary studies in 15-wk-old female mRen2.Lewis rats demonstrate that high salt increased AT1 mRNA levels threefold and that the AT1 antagonist losartan reduced both the salt-dependent increase in blood pressure and proteinuria (Ref. 39; Yamaleyeva and Chappell, unpublished observations). In the older mRen2.Lewis rat, we found no difference in the circulating levels of ANG II between the intact and ovariectomized groups. Furthermore, both cortical and medullary levels of ANG II and ANG-(1–7) were similar between the two groups, although the medullary content of ANG-(1–7) was significantly higher than the cortical content in both intact and ovariectomized groups. These results suggest that alterations in the renal content of ANG II or ANG-(1–7) do not contribute to the salt-dependent renal injury in the intact mRen2.Lewis rat, although we cannot dismiss the possibility of greater AT1 expression in the kidneys of the ovariectomized group. In general, the lack of estrogen is associated with increased expression of the AT1 receptor, which is routinely invoked as a mechanism for the protective actions of estrogen (16); however, Adler and colleagues (27) demonstrated that estrogen increased renal AT1 expression in an l-NAMe-ANG II model of renal injury. Additional studies are required to address this issue, as well as the question of whether estrogen replacement in the older mRen2.Lewis rat exacerbates either blood pressure or renal injury in high-salt-fed rats.

In summary, ovariectomy in the older female mRen2.Lewis rat conveys protection against salt-dependent increase in urinary proteinuria and is associated with a reduction in renal IGF-I and KIM-1 expression, as well as lower circulating levels of the acute-phase inflammatory marker CRP. Although the exact mechanisms that underlie the protective actions of ovariectomy remain to be resolved, we conclude that the hypertensive mRen2.Lewis rat may be an appropriate experimental model to ascertain whether estrogen has beneficial or detrimental actions in the kidneys of older female animals.

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REFERENCES


