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Differential Effects of Sex Steroids in Young and Aged Female mRen2.Lewis Rats: A Model of Estrogen and Salt-Sensitive Hypertension

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Abstract

Background—Male–female differences in the expression of hypertension and in end-organ damage are evident in both experimental models and human subjects, with males exhibiting a more rapid onset of cardiovascular disease and mortality than do females. The basis for these male–female differences is probably the balance of the complex effects of sex steroids (androgens, estrogen, progesterone) and their metabolites on the multiple regulatory systems that influence blood pressure (BP). A key target of estrogen and other steroids is likely to be the different components of the renin-angiotensin-aldosterone system (RAAS).

Objective—The aim of this study was to review the current experimental evidence on the protective effects of estrogen in hypertensive models.

Methods—The search terms *estrogen*, *renin-angiotensin-aldosterone system*, *renin receptor*, *salt-sensitivity*, *end-organ damage*, *hypertension*, *kidney*, *mRen2.Lewis*, and *injury markers* were used to identify relevant publications in the PubMed database (restricted to the English language) from January 1990 to October 2007.

Results—In a new congenic model that expresses the mouse *renin 2* gene (mRen2.Lewis), estrogen depletion (via ovariectomy [OVX]) in young rats was found to have a marked stimulatory effect on the progression of increased BP and cardiac dysfunction. Moreover, estrogen depletion exacerbated salt-sensitive hypertension and the extent of salt-induced cardiac and renal injury in young mRen2.Lewis rats, which probably reflected the inability to appropriately regulate various components of the RAAS. However, OVX in aged mRen2.Lewis rats conveyed renal protective effects from a high-salt diet compared with intact hypertensive littermates (64 weeks), and these effects were independent of changes in BP.

Conclusion—These studies in hypertensive mRen2.Lewis rats underscored the influence of ovarian hormones on BP and tissue injury, as well as the plasticity of this response, apparently due to age and salt status.

Keywords

mRen2.Lewis; renin-angiotensin-aldosterone system; salt sensitivity; proteinuria; estrogen

INTRODUCTION

Male–female differences in the development and progression of hypertension and in end-organ damage are evident in both experimental models and human subjects.^{1,2} In general, males exhibit a more rapid onset of cardiovascular disease that may result in a greater degree of tissue injury than in their female counterparts. The basis for this sex difference is primarily believed to reflect the powerful and complex effects of sex steroids (androgens, estrogen, progesterone) and their metabolites on the multiple regulatory systems that influence blood pressure (BP). Estrogen, for example, is known to have protective effects on vascular endothelium that are probably due to its positive influence on endothelial nitric oxide synthase (eNOS) and the subsequent production of nitric oxide (NO).³

The beneficial actions of estrogen may also reflect its influence on various components of the renin-angiotensin-aldosterone system (RAAS). Estrogen's role in the regulation of this hormonal system, however, is complicated by the existence of both circulating and multiple-tissue RAASs, including the brain, heart, kidney, adrenal gland, pancreas, and reproductive organs. Each of these tissue systems may potentially exhibit different responses to sex hormones. Moreover, multiple pathways within the RAAS that may oppose the classic constrictor and proliferative effects of the angiotensin II–angiotensin-converting enzyme–angiotensin II type 1 (Ang II-ACE-AT1) receptor axis have been identified in the past several years.⁴ The discovery of these buffering components, which include the peptide Ang-(1–7), the Ang-(1–7)/Mas receptor, ACE2, and the Ang II type 2 (AT2) receptor, not only reveals a more diverse functionality of the RAAS than previously imagined, but also expands the number of components that are potential targets of estrogen and other sex steroids.⁵

The beneficial actions of estrogen have been questioned because of the negative outcomes of hormone replacement therapy (monotherapy or progestin combination therapy) in several large clinical trials comprising older women with established cardiovascular disease.^{6–8} The outcomes of these trials raise the issue of whether aging may compromise the cardiovascular benefits of estrogen.

In this review, we examine the influence of estrogen on the RAAS in terms of the development of elevated BP and tissue injury in young and older female rats of a new congenic hypertensive model.

METHODS

We identified relevant publications for the current review using the search terms *estrogen*, *renin-angiotensin-aldosterone system*, *renin receptor*, *salt-sensitivity*, *end-organ damage*, *hypertension*, *kidney*, *mRen2.Lewis*, and *injury markers* in the PubMed database (restricted to the English language) from January 1990 to October 2007.

ESTROGEN-SENSITIVE HYPERTENSION

The mRen2.Lewis strain, which expresses the mouse *renin 2* (mRen2) gene, is a new congenic model of Ang II-dependent hypertension produced by the successive backcross of the mRen(2).27 rat onto the Lewis background.⁹ Similar to other models of hypertension, including the original mRen(2).27 transgenic rats, the hemizygous mRen2.Lewis congenic rats exhibit striking differences in BP, with males having systolic BP 50 to 60 mm Hg higher than their female littermates.^{10–12}

Studies in spontaneously hypertensive rats (SHRs) and mRen(2).27 transgenic rats found that orchietomy or treatment with the androgen antagonist flutamide markedly attenuated

the development of hypertension, supporting a predominant role of androgens in these models.^{13–15} Moreover, ovariectomy (OVX) had little effect on BP in SHR and mRen2.27 rats maintained on a normal chow diet.¹⁵ In contrast, our research group found that OVX of young mRen2.Lewis rats (4–5 weeks of age) induced a marked increase in the development of hypertension that almost eliminated the sex differences in BP in this strain. Importantly, estrogen depletion in the Lewis control group did not increase BP. Estrogen replacement with 17 β -estradiol reversed the trend toward higher BP by 8 weeks of age and ultimately reduced BP below that of the intact female mRen2.Lewis rats.¹⁶ It is not clear whether the absence of progesterone or the sustained noncyclical administration of 17 β -estradiol accounted for the lower BP level achieved in the treated group; serum estradiol concentrations were 2- to 3-fold higher than endogenous concentrations in the intact rats.¹⁶ Using the potent and selective AT1 receptor antagonist olmesartan, BP in OVX mRen2.Lewis rats was also reduced to a similar extent as to that achieved with exogenous 17 β -estradiol. The BP-lowering effects of olmesartan were evident for 2 months after discontinuation of the antagonist. In general, RAAS blockade with an ACE inhibitor or an AT1 antagonist during the early developmental phase of hypertension (at 3–5 weeks of age) in Ang II-dependent hypertension conveys long-term protective effects in rats.^{17,18} However, our study in OVX mRen2.Lewis rats showed this effect in adult females.

The comparable BP-lowering actions of exogenous estrogen and olmesartan indicate that estrogen status may exert a significant influence on the RAAS. Our research group found that circulating Ang II, ACE, and plasma renin concentrations were substantially increased, whereas plasma concentrations of Ang-(1–7) were reduced, in estrogen-depleted mRen2.Lewis rats.¹⁶ Similar effects were observed both in the kidneys, with higher tissue Ang II concentration and higher urinary excretion of Ang II, and in downstream factors, including endothelin-1 and the oxidative marker 8-isoprostane. Moreover, estrogen replacement restored the circulating, tissue, and urinary concentrations of these components to those of intact mRen2.Lewis rats.¹⁴ Reversal of these components with estrogen replacement to concentrations found in intact rats may explain most of the BP-lowering actions of the steroid; however, that BP was lower in the estrogen replete group compared with the intact group suggests that estrogen may have additional actions on the RAAS. Studies have shown that estrogen may downregulate the AT1 receptor or attenuate AT1 receptor-mediated signaling pathways,^{19,20} although stimulatory effects of estrogen on this receptor in an Ang II-N(ω)-nitro-L-arginine-methyl-ester (L-NAME) model of hypertension and renal injury were recently reported.²¹

In addition to the influence of the RAAS, we observed differential effects on eNOS and neuronal NOS (nNOS) expression in the kidney after estrogen depletion in mRen2.Lewis rats. Both cortical and medullary mRNA concentrations of eNOS were markedly reduced and were negatively associated with the change in BP.²² The reduction in eNOS may directly contribute to the increase in BP in mRen2.Lewis rats as well as to the activation of the RAAS components. Although the inducible NOS isoform was not detected in the kidneys, nNOS mRNA expression was increased in both cortical and medullary areas after estrogen depletion. Immunostaining for nNOS revealed the expected expression in the macula densa of both intact and OVX mRen2.Lewis rats; however, tubular staining was more prominent in the estrogen-depleted group.

Renal nNOS is believed to facilitate the regulation of juxtaglomerular renin and to attenuate tubuloglomerular feedback,^{23–25} yet chronic administration of the selective and irreversible nNOS inhibitor N5-(1-imino-3-butenyl)-L-ornithine (L-VNIO) resulted in a sustained reduction in BP for 4 weeks in OVX mRen2.Lewis rats.²² Although the role of renal nNOS is far from established, particularly in the hypertensive phenotype, these studies emphasized that nNOS may be an important target for ovarian hormones. Our preliminary data also

indicated that estrogen depletion increased the expression of cyclooxygenase 2 (COX-2) mRNA, but not COX-1 mRNA, in the kidneys.²⁶ Similar to nNOS, an increase in immunostaining for COX-2 was evident in the macula densa and the proximal tubules of the renal cortex, thus supporting a role for COX-2 in the downstream regulation of nNOS in the macula densa, as well as potential regulation in the tubular epithelium of the kidneys.²⁴

SALT SENSITIVITY

Clinical studies have suggested that estrogen, particularly oral contraceptives, may promote sodium retention.²⁷ However, lower concentrations of endogenous estrogen may have natriuretic effects, perhaps through the inhibition of the RAAS.²⁸ Schulman et al²⁹ found that OVX in young women increased the incidence of salt-sensitive hypertension, as assessed in the same group of pre- and post-OVX women. In experimental studies, OVX enhanced the BP response to a high-salt diet in both SHR and Dahl sensitive (S) rats, and augmented the response in the Dahl S rats fed a chronic low-salt diet.^{10,15,19,30} The enhanced response after OVX in Dahl S rats fed a high-salt diet was completely reversed by treatment with the AT1 antagonist losartan, confirming the link between estrogen and the regulation of the RAAS with increased sodium uptake.¹⁹

Our studies in female mRen2.Lewis rats also found that estrogen depletion exacerbated the salt-dependent increase in BP.¹² Moreover, this response was associated with increased concentrations of circulating Ang II, ACE, and renin, supporting a regulatory effect of estrogen on the RAAS in salt-sensitive rats. In contrast to the mRen2.Lewis rats maintained on a normal-salt diet, estrogen depletion also markedly exacerbated the degree of both proteinuria and albuminuria in the group fed a high-salt diet, and tended to further reduce creatinine clearance.

With regard to cardiac function, estrogen depletion further impaired diastolic dysfunction in mRen2.Lewis rats maintained on a high-salt diet.³¹ The reduction in function was associated with a marked increase in the extent of cardiac tissue fibrosis.³⁰ We have not performed a comparable assessment of the influence of salt on the circulating or renal RAAS components in male mRen2.Lewis rats to determine whether sex differences exist in this strain; however, the BP response to a high-salt diet was markedly greater in the females.³²

The increasing number of measurable indices, in part reflected by the greater number of RAAS components, combined with the varying conditions of estrogen and salt prompted the application of new data-mining techniques to attempt to identify high degrees of correlation among our data sets with multiple groups.³³ Figure 1A shows the regression heat map from the correlate summation analysis of the data from 4 groups of female mRen2.Lewis rats.^{12,30,31} The figure conveys both the clustering of the data and the correlations used for this analysis. Further analysis of these data revealed nonlinear relationships between proteinuria and Ang II (Figure 1B), ACE (Figure 1C), renin (Figure 1D), and cardiac hypertrophy (Figure 1E, red line). These curves indicate that the effects on proteinuria of estrogen depletion and a high-salt diet were not simply additive, suggesting that this condition may promote a relationship of positive feedback among the RAAS components and increasing proteinuria. Moreover, the nonlinear relationship between proteinuria and the heart-weight to body-weight index may imply that the kidneys have a role in the progression of cardiac hypertrophy and possibly diastolic dysfunction that was evident in the OVX mRen2.Lewis rats fed a high-salt diet.³² Our research group contrasted the proteinuria–cardiac hypertrophy curve to the linear relationship between BP and the extent of hypertrophy (Figure 1E). Although we have not determined the outcome of RAAS blockade on diastolic dysfunction, various components, including Ang II, aldosterone, and renin (or prorenin) through its interaction with the renin receptor,^{34,35} may contribute to impaired cardiac function in female mRen2.Lewis rats.

Our research group's preliminary studies in mRen2.Lewis rats have begun to address the extent to which an activated RAAS contributes to increased BP in the estrogen-depleted, high-salt diet state. As shown in Figure 2, we found that the ACE inhibitor lisinopril substantially reduced BP in OVX mRen2.Lewis rats fed a high-salt diet, confirming the participation of the RAAS in this condition. Chronic treatment with the superoxide dismutase mimetic tempol did not lower BP; however, additional treatment of the tempol group with lisinopril subsequently lowered BP to approximately the same level as lisinopril alone (Figure 2). Although we predicted that estrogen depletion in mRen2.Lewis rats fed a high-salt diet would result in enhanced expression of reactive oxygen species (ROS), the inability of tempol to reduce BP suggested that oxidative stress may not play a significant role in this hypertensive model. These data clearly contrast with the prevailing view of the predominant influence of ROS in the development and maintenance of high BP.^{36,37} Moreover, we did not determine whether tempol treatment improved vascular dysfunction or other indices of cardiac or renal injury. However, our data support earlier findings by Fortepiani and Reckelhoff³⁸ that chronic tempol treatment reduced BP in adult male but not adult female SHR. Other investigators also found that the nicotinamide adenine dinucleotide phosphate (NADPH) inhibitor apocyanin effectively reduced BP only in male SHR.³⁹ In addition, the laboratories of Touyz⁴⁰ and Martin⁴¹ reported that vascular NADPH oxidase was increased after chronic Ang II infusion in male but not in female normotensive animals. This greater response in NADPH oxidase with Ang II was associated with a higher BP level in the males. These studies suggest that sex differences in BP may reflect the attenuated increase in oxidative stress in females. However, in addition to the effects of OVX and a high-salt diet, other mechanisms that contribute to the sustained increase in BP in female hypertensive mRen2.Lewis rats remain to be established.

ESTROGEN IN AGED MREN2.LEWIS RATS

The protective effects of estrogen are well documented in experimental models, including the mRen2.Lewis strain, but were not evident in recent clinical trials.^{6,7,42} Although there were myriad issues in the Women's Health Initiative regarding the deleterious effects of hormone replacement therapy, including the extent of underlying cardiovascular disease in the treated group, the aspect of differential responses to estrogen in younger versus older women may be important.⁴²

Our studies support the protective role of estrogen in young hypertensive mRen2.Lewis rats, because both estrogen depletion and salt loading were initiated at the relatively early age of 4 to 5 weeks. This time period precedes the rapid development of hypertension that occurs in many experimental models, including the mRen2.Lewis strain.^{16,43,44} In Dahl S rats, initiation of a high-salt diet at a young age resulted in a more profound increase in BP than at a later age, suggesting that salt sensitivity is associated with a critical period in development.⁴⁵ Moreover, Fortepiani et al⁴⁶ reported that OVX of 8-month-old SHR resulted in a reduction in BP at 18 months compared with the age-matched intact group.

We assessed the effects of OVX in female mRen2.Lewis rats at 3 months and followed this group for more than a year. Although the older rats (60 weeks) exhibited substantially higher BP than did the younger rats (15 and 30 weeks of age), we found no difference in BP between the intact and OVX groups, despite the fact that the latter was 35% heavier.⁴⁷ Both groups were then placed on a high-salt diet for an additional month to discern whether estrogen depletion would reveal salt-dependent changes in BP. Although BPs were again similar on the high-salt diet, urinary protein concentration was markedly higher (>15-fold) in the intact group.

As shown in Figure 3,⁴⁷ histologic analysis of the kidneys revealed a greater incidence and severity of injury in intact compared with OVX mRen2.Lewis rats. In addition, circulating

concentrations of complement reactive protein, renal hypertrophy, and the renal mRNA expression of kidney injury molecule 1 (Kim-1, a marker of tubular damage)^{48–50} were higher in the intact versus the OVX group.⁴⁷ The finding of an elevated marker for tubular injury and/or repair supports the histologic findings of greater tubulointerstitial damage in the intact mRen2.Lewis group.

Although we did not assess the effects of RAAS blockade, the renal cortical and medullary concentrations of Ang II and Ang-(1–7) were similar in the 2 groups. Moreover, creatinine clearance and podocin and nephrin mRNA (2 key glomerular proteins) expression were similar in the 2 groups. Renal cortical insulin-like growth factor (IGF)-1 mRNA and peptide concentrations were substantially higher in the intact group after a high-salt diet; however, there were no alterations in the circulating peptide. IGF-1 is a pluripotent peptide that may influence various functions within the kidney, including renal hypertrophy, glomerular filtration, release of prostaglandin E₂ and NO, sodium reabsorption, and mesangial cell apoptosis and proliferation.^{51–54} All components of the IGF-1 system (IGF-1 and IGF-1 binding proteins and receptors) are present in the kidneys, and IGF-1 was localized primarily to the periglomerular area and the proximal, distal, and collecting tubules of older mRen2.Lewis rats.⁴⁷ A recent study also found that suppression of the growth hormone–IGF-1 axis may afford protection from renal injury in aging rats maintained on a normal-salt diet.⁵⁵

We do not know if estrogen (or progesterone) replacement in older OVX mRen2.Lewis rats on a high-salt diet will mimic the extent of renal damage apparent in intact rats. Moreover, the exact mechanism that accounts for the protective effect of OVX has not been resolved; however, the mRen2.Lewis strain may be an appropriate model to define the role of estrogen in aging and hypertension.

CONCLUSIONS

The negative findings from large clinical trials of estrogen replacement therapy have spurred a new wave of interest in investigating sex differences and the role of sex steroids in cardiovascular function and tissue injury. These reports have not only revealed significant sex differences in BP control and organ damage, but also have begun to address the mechanisms that may contribute to the complex effects of estrogen, particularly with respect to aging. The current studies in congenic mRen2.Lewis rats indicated sex differences in the development and progression of hypertension and tissue injury, as well as the importance of the regulation of the RAAS by estrogen. Moreover, the beneficial actions attributed to estrogen in young animals may not be evident in older mRen2.Lewis rats. The concept that estrogen may be protective at one age and exacerbate pathologic conditions at a different age is not completely unexpected, given the complexity of the interaction of steroids with other systems that may directly or indirectly influence overall cardiovascular response. The idea that estrogen depletion only exacerbates an existing condition may be simplistic. It is likely that estrogen deficiency represents a different phenotype than does an intact female or male, and age or salt status may further influence this phenotype. For example, our group's preliminary data indicated that COX-2 inhibition increased proteinuria in intact male and female mRen2.Lewis rats on a high-salt diet, but reduced proteinuria in estrogen-depleted rats on the same diet.⁵⁶ Continuing research is clearly necessary not only to define the mechanisms that contribute to sex differences, but also to understand these different phenotypes in estrogen-replete and estrogen-depleted models.

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Figure 1.

(A) Regression map of the correlate summation analysis for the descriptive data on the effects of ovariectomy and a high-salt diet in mRen2.Lewis rats. The graph is overlaid with the descriptive statistics (mean and SEM for each group, as reduced by the mean of all animals for each variable). The differently shaded P values correspond to the linear regression of each group with the indicated measurement on the X axis. The lower panels show separate analyses for proteinuria and (B) Ang II, (C) ACE, (D) renin (r), and (E) cardiac hypertrophy (heart-weight to body-weight index) and its relationship to blood pressure. SBP = systolic blood pressure; Ang II = angiotensin II; renin (r) = rat renin; renin (m) = mouse renin; ACE = angiotensin-converting enzyme; HW/BW = heart weight to body weight; CR/EX = creatinine excretion; Aldo/CR = aldosterone-creatinine; OVX HS = ovariectomized high salt; INT HS = intact high salt. Data adapted from Chappell et al¹² and Westwood and Chappell.³³

Figure 2.

Effects of chronic lisinopril and tempol treatment on systolic blood pressure (SBP) in ovariectomized mRen2.Lewis rats fed a high-salt diet (OVX HS). OVX HS mRen2.Lewis rats were treated with the angiotensin-converting enzyme inhibitor lisinopril (10 mg/kg · d) or the superoxide dismutase mimetic tempol (1 mM) in drinking water for 2 to 8 weeks. The control and tempol groups additionally received lisinopril from weeks 6 to 8. SBPs were determined in conscious rats by tail-cuff plethysmography (5 determinations/measure). Rats were ovari-ectomized and placed on a high-salt diet (8% sodium chloride) at 5 weeks of age; the lisinopril and tempol regimens began at 12 weeks of age. Values are shown as mean (SEM); n = 5 to 6 rats/group. * $P < 0.01$ versus control age-matched group; † $P < 0.05$ versus tempol-treated age-matched group.

Figure 3.

Extent of renal injury in intact and ovariectomized mRen2.Lewis rats fed a high-salt diet (8% sodium chloride) for 4 weeks. (A) Y-axis indicates the cumulative injury score as assessed by renal histology. (B) Example of Masson trichrome-stained renal sections from 64-week-old female rats that were fed a high-salt diet (aged HS) and ovariectomized (aged OVX HS). Incidence of tubular and vascular smooth muscle (VSM) hyperplasia was not evident in the aged OVX HS group. Note the tubular cast above the glomerulus in the aged HS female rat. Data adapted from Yamaleyeva et al.⁴⁷