Sex differences in circulating and renal angiotensins of hypertensive mRen(2).Lewis but not normotensive Lewis rats

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Pendergrass KD, Pirro NT, Westwood BM, Ferrario CM, Brosnihan KB, Chappell MC. Sex differences in circulating and renal angiotensins of hypertensive mRen(2).Lewis but not normotensive Lewis rats. Am J Physiol Heart Circ Physiol 295: H10–H20, 2008. First published May 2, 2008; doi:10.1152/ajpheart.01277.2007.—Sex differences in blood pressure are evident in experimental models and human subjects, yet the mechanisms underlying this disparity remain equivocal. The current study sought to define the extent of male-female differences in the circulating and tissue renin-angiotensin aldosterone systems (RAASs) of congenic mRen(2).Lewis and control Lewis rats. Male congenics exhibited higher systolic blood pressure than females [200 ± 4 vs. 146 ± 7 mmHg, P < 0.01] or Lewis males and females [113 ± 2 vs. 112 ± 2 mmHg, P > 0.05]. Plasma ANG II levels were twofold higher in male congenics [47 ± 3 vs. 19 ± 3 μM, P < 0.01] and fivefold higher than in male or female Lewis rats [6 ± 1 vs. 6 ± 1 μM]. ANG I levels were also highest in the males; however, plasma ANG-(1-7) was higher in female congenics. Male congenics exhibited greater circulating renin and angiotensin-converting enzyme (ACE) activities, as well as angiotensinogen, than female littermates. Renal cortical and medullary ANG II levels were also higher in male congenics versus the Lewis strain. Male congenics exhibited greater circulating renin and angiotensin-converting enzyme (ACE) activities, as well as angiotensinogen, than female littermates. Renal cortical and medullary ANG II levels were also higher in male congenics versus all the other groups; ANG I was lower in the males. Cortical ACE2 activity was higher in male congenics, yet neprilysin activity and protein were greater in females, which may contribute to reduced renal levels of ANG II. These data reveal that sex differences in both the circulating and renal RAAS are apparent primarily in the hypertensive group. The enhanced activity of the RAAS in male congenics may contribute to the higher pressure and tissue injury evident in the strain.

angiotensin II; angiotensin-converting enzyme; angiotensin-(1-7), estrogen; neprilysin; cardiac hypertrophy; proteinuria; renin-angiotensin aldosterone system

SIMILAR TO OTHER experimental models of high blood pressure, the congenic mRen(2).Lewis strain exhibits significant sex differences in the extent of hypertension and renal injury (21, 42). The mRen(2).Lewis rat was derived from the mRen2.(27) Sprague-Dawley rat, originally developed by Mullins et al. (38) as a model of tissue renin expression, and was backcrossed into the Lewis strain to yield the new congenic model. The hemizygous male mRen(2).Lewis rats exhibit systolic blood pressures 50–60 mmHg higher than their female littermates, although the blockade of the renin-angiotensin-aldosterone system (RAAS) lowers blood pressure to similar levels (17, 32). In contrast to the spontaneously hypertensive rat, estrogen depletion by ovariectomy in young mRen(2).Lewis rats significantly exacerbates the hypertension (21). Moreover, estrogen replacement or treatment with an ANG II type 1 (AT1) receptor antagonist reduces blood pressure to a similar extent and clearly supports a protective role for estrogen in this hypertensive model (17). Indeed, the cardioprotective effects of estrogen replacement in mRen(2).Lewis rats, an ANG II-dependent model of hypertension, are consistent with its actions to directly attenuate key components of the RAAS, including angiotensin-converting enzyme (ACE) and the AT1 receptor (12, 40, 45), as well as the increase of competing components such as ACE2 and the ANG II type 2 (AT2) receptor or influence other signaling pathways (nitric oxide and prostaglandins) that may converge on the RAAS (3, 11, 41, 60, 65). Androgens, however, may also have a significant effect on cardiovascular regulation to increase blood pressure and exacerbate renal injury associated with compensations of the RAAS, including the increased expression of renin, angiotensinogen, and ACE (6, 8, 9, 47). Furthermore, estrogen may exhibit additional effects on the RAAS, including the stimulation of renin and angiotensinogen that in some instances may promote an increase in blood pressure (12).

Typically, the characterization of sex-based differences in experimental models of hypertension has focused on the components of the circulating RAAS, such as ANG II, ANG-(1-7), and ACE (10), or the receptor expression in various target tissues (28, 63). Current evidence strongly supports the existence of local or tissue RAASs in male organs that may have a significant if not greater impact than the circulating system. However, to our knowledge, no studies have evaluated sex-dependent differences in the tissue expression of angiotensin peptides, particularly ANG II and ANG-(1-7) in the mRen2(Lewis or its founder strain, the transgenic mRen2(27) Sprague-Dawley rat. Moreover, the balance of these two peptides, which is likely influenced by the post-renin processing enzymes ACE, ACE2, and neprilysin within various tissues, may well contribute to the development and progression of hypertension and tissue damage (10, 19). Therefore, we tested the hypothesis as to whether there is an...
imbalance in the expression of ANG II and ANG-(1-7) within the circulation, heart, and renal compartments of the mRen(2).Lewis rat that is associated with the markedly higher blood pressure and organ injury particularly evident in the male hypertensive strain.

**MATERIALS AND METHODS**

**Experimental animals.** Hemizygous male and female mRen(2).Lewis (congenic) rats were obtained from the Hypertension and Vascular Disease Center Transgenic colony (21, 42) at 14 to 15 wk of age. Normotensive male and female Lewis rats were purchased from Charles River (Raleigh, NC) and were age matched with the congenic rats. Animals were fed a powdered rat chow (Purina Mills, Richmond, VA) to provide a daily intake of 17 and 28 meq/100 g body wt of sodium and potassium, respectively, had full access to water, and were housed in an American Association of Laboratory Animal Care-approved facility in rooms maintained on a 12-h:12-h light-dark cycle (lights on 6:00 AM to 6:00 PM). Animals were housed in metabolic cages (Harvard Bioscience, South Natick, MA) for a 24-h collection period, and systolic blood pressure was measured with a Narco Biosystems device (Houston, TX) (42). These procedures were approved by the Wake Forest University School of Medicine Institutional Animal Care and Use Committee.

**Plasma and renal tissue hormone assays.** Rats were decapitated without anesthesia. Trunk blood (3 to 5 ml) was collected into chilled Vacutainer blood collection tubes (Becton Dickinson, Sandy, UT) for plasma renin or in tubes containing peptidase inhibitors and processed for direct radioimmunoassay (RIA) of angiotensin peptides (1, 42). Trunk blood was also collected in separate tubes without inhibitors and allowed to clot to obtain the serum. Blood was spun at 1,800 g, and the plasma or serum was stored at −80°C. Following blood collection, tissues were rapidly collected. For hearts and kidneys, the tissue was blotted, weighed, and snap frozen on dry ice. The cardiac and renal weight indexes were expressed as milligrams of organ tissue was blotted, weighed, and snap frozen on dry ice. The cardiac and renal weight indexes were expressed as milligrams of organ tissue.

**Immunoblot analysis.** Circulating angiotensinogen were measured by immunoblot assay with an antibody directed against an epitope on the carboxy (residues 428-441) terminus of the protein. The antibody was produced in rabbits by coupling the COOH-terminus of either Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Tyr-Tyr-Ser-Cys* via an added Cys residue to keyhole limpet hemocyanin (21). For protein analysis, 0.38 μl of plasma were separated on 10% SDS polyacrylamide gels for 1 h at 120 V in Tris-Glycine SDS, transferred onto PVDF membranes, and subsequently blocked for 1 h with 5% Bio-Rad dry milk and TBS plus Tween before incubation with the COOH-(A2504, I2,000) terminus-directed antibody (21). Neprilysin expression was determined in the solubilized membranes of the renal cortex (10 μg) with an antibody raised to rat neprilysin (Lot No. 0702053058, Chemicon, Temecula, CA).

**Peptidase activities.** Frozen renal cortex and cardiac tissues were homogenized in enzyme reaction buffer, containing 10 mM HEPES, 125 mM NaCl, and 10 μM ZnCl2 (pH 7.4), with Qiagen Tissue Lyser for 1 min at 25 Hz. The homogenate was spun at 28,000 g for 10 min at 4°C, the pellet was resuspended, and the centrifuge step was repeated. The supernatant was stored at −80°C until assayed for renin concentration. The resultant pellet was resuspended in 0.5% Triton X-100 overnight at 4°C on ice to solubilize proteins. Following the same centrifugation step, the solubilized supernatants were used as the source of peptidase activity. Either the substrate 125I-ANG I or 125I-ANG II (2 × 106 counts/min, 2,200 curies/mmol) was added to the supernatant with various inhibitors and incubated at 37°C for up to 2 h. The reaction was terminated with 1.0% phosphoric acid and centrifuged at 16,000 g, and the supernatant was stored at 4°C. Samples were then filtered and the enzymatic products quantified on HPLC as previously described (54). The following inhibitors comprised the inhibitor cocktail in the assay: amastatin (200 μM), bestatin (1 μM), chymostatin (1 μM), benzyl succinate (1 μM), and parachloromercuribenzoic acid (0.5 mM). Lisinopril was added to inhibit ACE activity, SCH-39370 to block neprilysin activity, and MLN-4760 to attenuate ACE2 activity (all at a final concentration of 10 μM) (54). Renal ACE activity was too low to measure by this method, particu-
Fig. 2. Circulating renin-angiotensin aldosterone system hormones in Lewis and mRen(2).Lewis rats. Plasma angiotensins and aldosterone were measured by separate radioimmunoassays for ANG II (A), ANG I (B), ANG-(1-7) (C), and aldosterone (D). Peptide values are expressed as picomolar concentration. Plasma aldosterone is expressed as nanograms per deciliter. Values are means ± SE. *P < 0.001 between sex differences; **P < 0.001 between strains; †P < 0.05 between strains (n = 5 to 6 rats/group).

RESULTS

Circulation. The systolic blood pressure was markedly higher in 15-wk-old congenic rats compared with age-matched normotensive Lewis rats for both sexes (Fig. 1A). The congenic males also exhibited a greater degree of hypertension than the female congenics (200 ± 4 vs. 146 ± 7 mmHg, P < 0.001), whereas there was no sex difference in systolic blood pressure for the Lewis strain. The males exhibited no weight difference between strains; however, the female mRen(2).Lewis rats were 11% heavier than the female Lewis rats (261 ± 5 vs. 233 ± 2 g, P < 0.01, Fig. 1B). CRP was determined in the serum as a circulating marker of inflammation. Male mRen(2).Lewis rats exhibited the highest serum CRP levels among these groups, consistent with the greater extent of blood pressure and plasma ANG II (see discussion on Fig. 2 below). However, we also observed a significant sex difference in serum CRP for both hypertensive and normotensive strains (Fig. 1C).

We assessed plasma peptide concentrations in trunk blood collected from unanesthetized rats. As shown in Fig. 2A, plasma ANG II levels were similar for the normotensive male and female Lewis rats (P > 0.05). However, the male mRen(2).Lewis rats exhibited higher-circulating ANG II than the female congenics (47.1 ± 2.5 vs. 18.6 ± 3.2 pM, P < 0.001). Moreover, the congenic strain exhibited significantly higher levels of plasma ANG II compared with the Lewis strain (P < 0.01). Consistent with the higher ANG II levels, the male mRen(2).Lewis rats had higher circulating levels of ANG I than both the females congenics (201 ± 21 vs. 56.8 ± 3.8 pM, P < 0.001, Fig. 2B) and the Lewis rats (Fig. 2B). However, plasma levels of ANG-(1-7) were highest in the female congenics (Fig. 2C). Aldosterone levels were similar among the groups, although there was a trend for higher plasma levels of this steroid in the male versus the female mRen(2).Lewis rats (Fig. 2D). Plasma angiotensinogen was measured by Western blot analysis using an antibody that recognizes a distinct epitope on the COOH-terminal domain of the protein (Fig. 3).
The immunoblot revealed two bands for angiotensinogen at 55/60 kDa; however, protein expression was significantly lower in plasma extracts of female mRen(2).Lewis rats compared with female Lewis rats (Fig. 3). Quantification of the band densities revealed a trend for lower levels of angiotensinogen for the female versus male congenics. When compared with the male Lewis rats, the male congenics also exhibited a lower expression of angiotensinogen. In addition, plasma renin concentrations were assessed at a pH level of 6.5 and 8.5, using an excess of exogenous rat angiotensinogen. The male congenics exhibited a fourfold higher level of renin at a pH of 6.5 than either the female congenics or the Lewis rat strain (Fig. 4B). However, renin concentration at pH 8.5 in Lewis rats were at the assay’s detection limit (Fig. 4A). Renin concentration assessed at a pH of 8.5 was fivefold higher in the male versus female congenics or the Lewis rat strain (Fig. 4A). Protein expression was significantly higher in the male mRen(2).Lewis rats by 22% and 60% compared with the male Lewis and female mRen(2).Lewis rats, respectively (Fig. 4C).

Kidney. Renal hypertrophy was significantly greater in the male mRen(2).Lewis rats compared with their female littermates or the male Lewis rats (Fig. 5A). Proteinuria and urinary creatinine were also significantly higher in the male congenic strain compared with all other groups (P < 0.01, Fig. 5, B and C, respectively). A sex difference in the urinary excretion of the oxidative stress marker 8-OH-dG was evident in the congenic strain (male, 10.8 ± 1.1 vs. female, 3.7 ± 0.7 μg·kg−1·day−1, P < 0.001, Fig. 5D), whereas there was no difference in the Lewis rats. The urinary levels of 8-OH-dG in the male mRen(2).Lewis strain were also significantly higher than in the Lewis strain (P < 0.05).

As shown in Fig. 6A, ANG II content in the renal cortex was highest in the male mRen(2).Lewis rats with ANG II levels twofold higher than the female congenics. Cortical levels of ANG II were similar between the female mRen(2).Lewis rats and both male and female Lewis rats. In contrast, ANG I was 50% lower in the male versus female mRen(2).Lewis rats (2.0 ± 0.2 vs. 4.7 ± 0.6 fmol/mg protein, P < 0.01, Fig. 6B). ANG(1-7) was not significantly different between the four groups, although the female mRen(2).Lewis rats tended to exhibit higher peptide levels (Fig. 6C). The cortical renin concentration measured at pH 6.5 was significantly lower in both sexes of the mRen(2).Lewis strain compared with the Lewis rats (Fig. 7A). The female rats also exhibited higher renin levels than male littermates, irrespective of strain. Renal renin concentration at pH 8.5 was significantly higher in the female versus male mRen(2).Lewis rats (Fig. 7B). Cortical ACE activity was not different among all four groups, whereas ACE2 activity was 70% higher in male compared with female mRen(2).Lewis rats (Fig. 8, A and B, respectively). A strain difference was also evident for ACE2 activity in the male rats (P < 0.01). As shown in Fig. 8, C and D, respectively, neprilysin activity was significantly higher (threefold) in the female mRen(2).Lewis rats and Lewis rats compared with their male littermates, regardless of whether ANG I or ANG II was used as the substrate. Neprilysin protein expression in the renal cortex of the male and female mRen(2).Lewis was also assessed by Western blot analysis. The immunoblot revealed a predominant band at 88 kDa, identical to the molecular mass of the standard (lane 7), and neprilysin expression was noticeably more predominant in the female renal extracts (Fig. 9A). A more diffuse band at 65 kDa was also evident on the full-length gel. Quantification of the gel staining revealed 10- and 3-fold higher density of the 88- and 65-kDa bands, respectively, for the female mRen(2).Lewis rats (Fig. 9, B and C, respectively).

As shown in Fig. 10A, the renal medullary ANG II content was significantly higher in the male mRen(2).Lewis rats than for all other groups (P < 0.001). Moreover, there was no sex difference for ANG II in the Lewis strain. Medullary ANG I was significantly higher in the female Lewis rat compared with the male Lewis rat (Fig. 10B). ANG(1-7) levels were lower in the mRen(2).Lewis rats, independent of sex difference (Fig. 10C).

Heart. As shown in Fig. 11A, the male mRen(2).Lewis rats exhibited the highest extent of cardiac hypertrophy among all four groups with a difference in strain present only in the males (4.0 ± 0.05 vs. 2.4 ± 0.05 mg/g, P < 0.001). Cardiac hypertrophy was strongly correlated to the systolic blood pressure among all four groups (r² = 0.85, P < 0.001, Fig. 11B). Neither ANG II nor ANG(1-7) content in the ventricular tissues were different among the four groups of rats (Fig. 11, C and D, respectively). ANG I

Fig. 4. Circulating renin and angiotensin-converting enzyme (ACE) activities in the Lewis and mRen2.Lewis rats. A: plasma renin concentration (PRC) was measured at the pH optima for rat renin (pH of 6.5). B: PRC was measured at the pH optima of mouse renin (pH of 8.5). C: serum ACE activity was measured using the synthetic substrate [3H]-[Hip-His-Leu]. PRCs and ACE activity were expressed as nanograms per milliliters per hour and nanomoles per milliliter per minute, respectively. Values are means ± SE. #P < 0.001 between sex differences; *P < 0.001 between strains; **P < 0.001 compared with male Lewis rats (n = 5 rats/group).
values were below the minimum detectable limit of the ANG I RIA (<0.33 fmol/mg protein). A sex difference was not present in cardiac ACE activity among the Lewis and mRen(2).Lewis rats, although there was a trend for higher activity in the male and female congenics (Fig. 11E). Sex differences for ACE2 activity were apparent only in the mRen(2).Lewis rats with significantly higher activity (35%) in the male congenics (Fig. 11F).

Fig. 5. Renal indexes in the Lewis and mRen(2).Lewis rats. A: renal hypertrophy was expressed as the ratio of left kidney to body weight (in mg/g). B: proteinuria was measured from a 24-h collection of urine (in mg protein·kg⁻¹·day⁻¹). C: urinary creatinine excretion (in mg·kg⁻¹·day⁻¹). D: urinary 8-hydroxy-2′-deoxyguanosine (8-OH-dG) excretion (in μg·kg⁻¹·day⁻¹). Values are means ± SE. #P < 0.001 between sexes; *P < 0.001 between strains (n = 5 rats/group).

Fig. 6. Renal cortical angiotensins in the Lewis and mRen(2).Lewis rats. Angiotensins were measured by separate radioimmunoassays of cortical extracts and expressed (in fmol/mg protein) for ANG II (A), ANG I (B), and ANG-(1-7) (C). Values are means ± SE. #P < 0.001 between sex differences; *P < 0.05 between strains (n = 4–6 rats/group).

Fig. 7. Renal renin concentrations in the Lewis and mRen(2).Lewis rats. A: renal renin concentration was measured at the pH optima for rat renin (pH of 6.5). B: renal renin concentration was measured at the pH optima of mouse renin (pH of 8.5). Renal renin concentrations were expressed (in μg·mg protein⁻¹·h⁻¹). Values are means ± SE. #P < 0.05 between sex differences; *P < 0.01 between strains (n = 3 to 4 rats/group).
DISCUSSION

The congenic mRen(2).Lewis hypertensive strain exhibits marked sex differences regarding the extent of the hypertension, cardiac hypertrophy, and proteinuria that are not evident in the normotensive Lewis rats. Indeed, the sex difference in the mRen(2).Lewis rats is similar to that observed in other hypertensive models (12, 13, 27, 46) and may reflect the inequality of sex difference in the progression of cardiovascular disease observed in the human population (4). Although we previously reported that the female mRen(2).Lewis rat is an estrogen-sensitive model whereby ovariectomy significantly exacerbates the degree of hypertension and that estrogen replacement normalizes blood pressure, the status of the RAAS that may contribute to the sex differences in this hypertensive strain is not known (17). Moreover, the assessment of the RAAS in the Lewis normotensive rats provides an appropriate control for the influence of sex alone on the regulation of these components. In this regard, our findings reveal that the male mRen(2).Lewis rats exhibited the highest circulating and renal tissue levels of ANG II among the four groups, consistent with the greater degree of increased blood pressure, inflammation, cardiac hypertrophy, and proteinuria. The female mRen(2).Lewis rat, which exhibits moderate hypertension compared with their male littermates, also expressed significantly higher levels of circulating ANG II than the female or male Lewis rats. Interestingly, the female mRen(2).Lewis rats displayed higher levels of circulating ANG-(1-7) than either the male congenics or the Lewis control strain. The higher expression of ANG-(1-7), a peptide with vasodilatory and anti-inflammatory actions, may provide an effective compensatory mechanism to attenuate the extent of hypertension and renal injury in the female hypertensive strain (10).

The basis for sex differences in cardiovascular disease is generally thought to involve the overexpression of various components of the RAAS, including ACE, the AT1 receptor, and angiotensinogen (5, 15, 19, 44). Estrogen is known to downregulate both ACE and the AT1 receptor, as well as increase the expression of the AT2 receptor and ACE2, which may further attenuate the actions of the ACE-ANG II-AT1 axis (3, 10, 17, 27, 33, 56, 59). In contrast, testosterone may increase ACE and the AT1 receptor as well as angiotensinogen (9, 27). To our knowledge, the current studies are the first to document the sex-based differential expression of both circulating and tissue ANG II and ANG-(1-7) in any hypertensive strain. Importantly, we find no differences in ANG II or ANG-(1-7) between the male and female Lewis rats, suggesting that sex difference alone does not account for the differential expression of these peptides. Circulating ANG II was fivefold higher in the male mRen(2).Lewis strain than in either the male or female Lewis strain, as well as twofold higher than the female mRen(2).Lewis strain. The plasma level of ANG I was also highest in the male mRen(2).Lewis strain, approximately fourfold greater than the three other groups. Consistent with the greater ANG I, both plasma renin concentrations at a pH of 6.5 and 8.5 were highest in the male mRen(2).Lewis strain and likely contribute to the greater expression of ANG I and ANG II in the male hypertensives. Although studies suggest an androgen-dependent regulation of mouse and rat renin in the mRen2.(27) rats (8, 9), ovariectomy of the female mRen(2).Lewis rats results in a twofold increase in plasma renin and ANG II, as well as a marked increase in blood pressure (17). Thus the sex difference in circulating renin may reflect the positive influence of androgens in males, as well as the inhibitory effects of ovarian hormones in the female mRen(2).Lewis rats. The higher renin concentration at pH 8.5 is not unexpected in the male mRen(2).Lewis rats; however, the increase in renin concentration at pH 6.5 is surprising, particularly given the greater level of blood pressure and plasma ANG II in the male congenics. The inability to effectively downregulate rat renin may be an additional factor that contributes to the sustained elevation of blood pressure in the mRen(2).Lewis rats, although the exact mechanism(s) for the disinhibition of renin release remains to be defined. In addition to the sex differences in renin concentration, we find that the female mRen(2).Lewis rats express significantly lower levels...
of plasma angiotensinogen. For this analysis, we used an antibody that recognizes the COOH-terminal domain of the protein. We can exclude an overall effect of sex difference due to no difference in plasma angiotensinogen in the Lewis rat strain. The lower levels of the precursor could contribute to the differences in plasma ANG I and ANG II between the male and female mRen(2).Lewis strain. The lower levels of angiotensinogen in the female mRen(2).Lewis rats contrast with the positive influence of estrogen or estrogen agonists on angiotensinogen expression (52). It is possible that the female congenics may exhibit greater feedback control of angiotensinogen than their hypertensive male littermates that may lead to reduced circulating levels of the precursor. We also find both sex and strain differences in circulating ACE activity. The male congenics exhibited higher ACE activity than the male Lewis and the female congenics. Again, the higher levels of ACE activity suggest that the dysfunctional regulation of the endogenous RAAS likely contributes to the elevated blood pressure in the male mRen(2).Lewis rat. The lower levels of serum ACE activity, however, may also contribute to the reduced level of blood pressure in the female congenics through lower ANG II and higher levels of ANG-(1-7). ACE is the major pathway for the metabolism of ANG-(1-7) in the circulation, cleaving the dipeptide His-Pro to form ANG-(1-5) (20, 64). In contrast to ANG II, ANG-(1-7) exhibits vasodilatory properties most likely by stimulating the release of prostaglandins and nitric oxide (31, 51). Indeed, we have shown that the blockade of ANG-(1-7) attenuates the blood pressure-lowering actions of ACE inhibitors (18, 30).

In addition to the greater circulating levels of ANG II, male and female mRen(2).Lewis rats exhibit higher serum levels of CRP than their respective normotensive controls. These findings are consistent with the proinflammatory events following the chronic administration of exogenous ANG II in the normotensive models (43) and the anti-inflammatory effects of RAS blockade in hypertensive rats and humans (49, 53). Sex differences in circulating CRP were evident for both mRen(2).Lewis and Lewis strains, with
the males exhibiting higher levels of this inflammatory marker. The differences in CRP between the male and female Lewis rats, however, were apparently not associated with increased circulating ANG II or systolic blood pressure. Several clinical studies also find that males exhibit higher circulating levels of CRP than females (25, 50), although others report the opposite (36). We found that ovariectomy in older female mRen(2).Lewis rats (12 mo of age) fed a high-salt diet was associated with lower circulating CRP, as well reduced proteinuria and other indexes of renal injury (65). Yang et al. (66) also reported that ovariectomy reduced CRP levels in older Fisher 344 rats and that estrogen replacement restores CRP to that of the sham-operated rats. The latter study, however, found no association between circulating CRP and the extent of complement activation, suggesting that altered levels of CRP may reflect the hepatic effects of estrogen rather than an increase in inflammation per se. Although ovariectomy markedly exacerbates hypertension in young mRen(2).Lewis rats and that estrogen replacement restores CRP to that of the sham-operated rats. The latter study, however, found no association between circulating CRP and the extent of complement activation, suggesting that altered levels of CRP may reflect the hepatic effects of estrogen rather than an increase in inflammation per se. Although ovariectomy markedly exacerbates hypertension in young mRen(2).Lewis rats and that estrogen replacement reverses this effect (17), additional studies are required to elucidate the degree of chronic inflammation and the contribution of this response to hypertension in the congenic strain.

In the kidney, the cortical levels of ANG II were significantly higher in the male mRen(2).Lewis rats compared with all other groups. The higher renal content of ANG II was associated with increased blood pressure, proteinuria, and urinary levels of the oxidative marker 8-OH-dG in the male congenics. In contrast to the circulatory levels of ANG I, cortical ANG I levels were significantly reduced compared with the female congenics and the Lewis rats. The ANG II-to-ANG I ratio (>4) suggests an enhanced conversion of ANG I to ANG II in the renal cortex rather than primarily an increase in renal renin activity or angiotensinogen. However, renal renin activity (at pH 6.5) was markedly reduced in both the male and female mRen(2).Lewis rats compared with Lewis controls and likely reflects the response to the sustained increase in blood pressure and ANG II levels in the congenics. The suppressed renal renin supports earlier studies that found reduced renin mRNA and protein in the kidney of the male transgenic mRen2.(27) rats (7, 61). In contrast to plasma renin, both Lewis and mRen2.Lewis females exhibited higher cortical renin activity (at pH 6.5 and 8.5) than their male littermates. In this regard, it is conceivable that renin from multiple organs (kidney, adrenal, etc.) may be regulated differently by estrogen or androgens, which may account for the sex differences in plasma and renal renin in the mRen(2).Lewis strain.

Cortical ACE activity was similar among all four groups; however, ACE2 activity was significantly higher in the male versus female mRen(2).Lewis rat and somewhat lower than that of the male Lewis rat as previously reported (22). Indeed, there were significant sex differences in both normotensive and hypertensive strains with higher ACE2 activity in the males. This finding is surprising given that the ACE2 gene is located on the X chromosome and that females should have an additional copy of this gene that may contribute to higher activity (22). Thus our data do not support the concept that higher ACE2 activity in the intact
female may contribute to increased metabolism of ANG II to ANG-(1-7) at least in the mRen(2).Lewis strain. However, cortical neprilysin activity and protein content were markedly higher in the female hypertensives than in males. Neprilysin is an endopeptidase that directly converts ANG I to ANG-(1-7) but metabolizes ANG II to ANG-(1-4) in addition to the hydrolysis of other vasoactive peptides, including endothelin, bradykinin, and natriuretic peptides (2, 54, 58, 62). A higher expression of neprilysin may contribute to the lower levels of ANG II in the renal cortex of the female mRen(2).Lewis rat, as well as to their lower pressure and proteinuria. However, neprilysin activity was also significantly higher in the female Lewis strain, and angiotensin content and blood pressure were similar between the male and female Lewis rats. Moreover, neprilysin inhibitors are generally thought to lower blood pressure, although these agents are more effective when combined with an ACE blocker (14, 34, 37). To our knowledge, experimental studies with neprilysin inhibitors have predominantly, if not exclusively, used male hypertensive strains. In the male mRen2.(27) transgenic rat, chronic neprilysin inhibition reduced the progression of hypertension although the exact mechanism for this effect was not defined (57). Thus studies are needed to determine the influence of neprilysin inhibition in the female mRen(2).Lewis rat on angiotensin expression and blood pressure.

The medullary content of ANG II was also significantly higher in the male mRen(2).Lewis rats versus their female littermates and the normotensive groups. Similar to levels in the cortex, medullary levels of ANG I and ANG-(1-7) tended to be lower, again suggesting either an enhanced conversion from ANG I by ACE or the reduced metabolism of ANG II. In this regard, Neves et al. (39) reported higher medullary levels of neprilysin activity in female Sprague-Dawley and transgenic mRen2.(27) rats than the male strains using a synthetic substrate to measure enzyme levels. Alternatively, the increased tissue content of ANG II in the medulla and possibly the cortex as well may reflect the enhanced uptake of circulating ANG II delivered to the kidney (29), particularly given the markedly lower expression of renal renin in the congenics. Indeed, the fourfold difference in medullary ANG II content between the male and female mRen(2).Lewis rats may arise from the higher circulating levels of ANG II in males and the potentially lower AT1 receptor expression in females, as well as the reduced levels of neprilysin. Estrogen is known to negatively influence AT1 receptor density in the kidney and other tissues (28, 63). Moreover, ovariectomy markedly increased blood pressure in the female mRen(2).Lewis rat, and subsequent treatment with either estrogen or the AT1 antagonist olmesartan normalized pressure in this strain (17). The current studies lacked sufficient tissue to determine the levels of ANG receptors or whether there are differences in the processing enzymes for ANG I and ANG II in the medullary region. The renal medulla is an important target tissue for ANG II, and the assessment of the mechanisms leading to sex differences in angiotensin expression is a needed focus for future studies, particularly when compared with the renal cortex.

In contrast to both the circulation and kidney, the cardiac levels of ANG II and ANG-(1-7) were not different between the male and female hypertensive rats or the normotensive groups. The male mRen(2).Lewis hearts exhibited significant hypertrophy compared with those of their female littermates or male Lewis rats. In this case, cardiac hypertrophy was highly associated with blood pressure, suggesting that the increased afterload contributes to the marked hypertrophy in males. We did not determine cardiac function or other markers of cardiac damage, such as the extent of fibrosis in the male and female mRen(2).Lewis rats. Consistent with the peptide data, cardiac ACE activity was not different in the male mRen(2).Lewis rats compared with the female rats but tended to be higher than the male and female Lewis rats. Although ACE2 likely constitutes the major pathway for the metabolism of ANG II in the heart, the relatively small difference in activity between the male and female mRen(2).Lewis rats may not significantly influence tissue peptide levels in this strain (26). Cardiac ANG I content was not detected in any of the four groups, and the lack of ANG I raises the issue of whether the heart contains a complete RAAS (23, 24, 48). In this regard, receptor-mediated uptake of ANG II may account for the cardiac levels of the peptide, and the subsequent processing by ACE2 may contribute to cardiac ANG-(1-7). Although the current studies were not designed to investigate this issue in depth, they clearly reveal tissue-dependent differences in the expression of angiotensin peptides in the Lewis and mRen(2).Lewis rats.

In summary, the present studies using the congenic mRen(2).Lewis model find significant differences in the expression of circulating and renal angiotensin peptides between the males and females that were not evident in the Lewis normotensive rats. Indeed, the differential expression of ANG II and ANG-(1-7) may contribute to sex-based differences in the extent of hypertension and renal injury in the mRen(2).Lewis rat. Although, an ongoing study of the additional components, including ACE2, ANG-(1-7) and its receptor, as well as the renin receptor, has redefined the regulation and functional aspects of this important hormonal system (16). Moreover, both experimental and clinical evidence show that males and females clearly exhibit cardiovascular differences, particularly in the setting of hypertension, clearly mandates the assessment of these components as a basis for sex differences and toward the continued development of effective therapeutic interventions. Finally, these data support the overall concept that sex steroids are of significant importance for both hypertensive females and males regarding the regulation of the RAAS, as well as the development and progression of cardiovascular disease.

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