Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats

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Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. Am J Physiol Heart Circ Physiol 291: H2166–H2172, 2006. First published June 9, 2006; doi:10.1152/ajpheart.00061.2006.—The generation of combination of renin gene into the rat genome founded a model of experimental hypertension characterized by an accelerated form of hypertension more pronounced in male rats than in female rats (1) and accompanied by hypertension-induced cardiac and vascular hypertrophy (2, 3, 26) as well as disturbances in body fluid regulation and renal function (15, 31).

Because the (mRen2)27 model of transgenic hypertension was originally bred from the Wistar-Kyoto and Sprague-Dawley strains, the heterogeneity of the parent strains contributed to the variability found within the transgenic strain. To eliminate this problem, we backcrossed the (mRen2)27 transgenic hypertensive rat with Lewis normotensive rats, creating the Lew.Tg(mRen2) congenic strain. In this new model of hypertension, we have now evaluated whether or not blockade of either the synthesis or activity of angiotensin II (ANG II) is associated with an increase in the actions of the opposing arm of the RAS, comprising the angiotensin-(1–7) (ANG-(1–7))/angiotensin-converting enzyme 2 (ACE2) axis. A significant body of literature from this (10, 13) and another laboratory (28) showed that ANG-(1–7) opposes the vasoconstrictor and growth-promoting actions of ANG II, whereas recent studies (13, 33) demonstrated that ACE2 has a preferential affinity for metabolizing ANG II into ANG-(1–7).

METHODS

Animals. Experiments were conducted in adult male Lew.Tg (mRen2) rats (290–300 g), a new congenic strain developed by our program from the backcross of the original (mRen2)27 transgenic with normotensive Lewis rats obtained from Charles River (Wilmington, MA). In brief, homozygous (mRen2)27 transgenic hypertensive rats were mated with Lewis controls to produce a F1 generation that was hemizygous for the mouse Ren2 gene. Offspring from the F1 generation were mated with Lewis rats to yield a F2 generation. All offspring of the F2 generation and those from successive backcrosses were genotyped to isolate offspring positive for the mouse Ren2 gene. After nine successive backcrosses, male and female rats were mated to each other to obtain rats homozygous for the mouse Ren2 gene. Homozygous litters expressing the mouse Ren2 gene were established as the founder rats of the new Lew.Tg(mRen2) breeder colony. The presence of the mouse Ren2 gene was determined by polymerase chain reaction (PCR) using oligonucleotide primers specific for either the rat Ren1 or the mouse Ren2 gene. An earlier report from this laboratory (4) showed that the hypertension in this new strain was equivalent to that found in (mRen2)27 rats, with the rats exhibiting comparable sex differences in the magnitude of the blood pressure elevation but no evidence of development of malignant hypertension.

For the current series of studies, 27 Lew.Tg(mRen2) congenic rats (age range, 8–10 wk) were fed a powdered rat chow (Purina Mills, the rat genome founded a model of experimental hypertension characterized by an accelerated form of hypertension more pronounced in male rats than in female rats (1) and accompanied by hypertension-induced cardiac and vascular hypertrophy (2, 3, 26) as well as disturbances in body fluid regulation and renal function (15, 31).

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For the current series of studies, 27 Lew.Tg(mRen2) congenic rats (age range, 8–10 wk) were fed a powdered rat chow (Purina Mills,
Richmond, VA) to provide a daily intake of 17 and 28 meq of sodium and potassium per 100 g of body weight, respectively. Rats had full access to tap water and were housed in individual cages within an American Association for Accreditation of Laboratory Animal Care-approved facility. Experiments were approved by our Institutional Animal Care and Use Committee.

Experimental protocol. Congenic hypertensive rats were randomly divided into three treatment groups. The regimen began with a 3-day baseline period during which all groups received tap water in addition to rat chow. On initiating treatment, the control group (vehicle, n = 11) continued to receive tap water, while the other two groups were administered either lisinopril (n = 8) or losartan (n = 8) dissolved in the drinking water. Water intake, food consumption, and urine production were recorded daily. The dosing of the agents was adjusted each day to maintain a daily intake of 10 mg·kg⁻¹·day⁻¹ of either lisinopril or losartan.

Blood pressure was assessed with tail-cuff plethysmography for three consecutive baseline days and thrice weekly through the duration of a 12-day treatment period. After this time, rats were anesthetized with halothane (1.5%; Halocarbon, River Edge, NJ), and a fluid-filled catheter was inserted into the right carotid artery to obtain direct arterial pressure and heart rate measurements, after which 5 ml of arterial blood were withdrawn for biochemical measurements. The heart was removed from the deeply anesthetized rats by cardiopulmonary excision. The atria were removed, and the remaining ventricles were weighed. The cardiac tissue and the kidneys were submerged in liquid N₂ and stored at −80°C.

Biochemistry. Arterial blood samples were collected and processed as previously documented (11, 12). Angiotensin peptide concentrations in plasma and urine were measured by radioimmunoassay as described elsewhere (11, 12). The minimum detectable levels of these assays were 0.9 fmol for ANG II and 1.39 fmol for ANG-(1–7). The intra-assay coefficient of variation was 12% for ANG II and 8% for ANG-(1–7). Serum samples were incubated with radiolabeled [¹²⁵I]Hip-Gly-Gly (pH 8.0) for 1 h at 37°C to determine the activity of ACE (11, 12).

Figure 1. Time course of tail-cuff systolic blood pressure in Lew.Tg(mRen2) congenic rats given either vehicle, lisinopril, or losartan for 12 days. Values are means ± SE; *P < 0.05 vehicle vs. all other groups; †P < 0.05 vs. lisinopril.

ACE2 activity in isolated cardiac and renal cortical membranes was quantified by determining the rate of [¹²⁵I]labeled ANG II conversion to [¹²⁵I]labeled ANG-(1–7) as previously described by us (11, 12). [¹²⁵I]labeled ANG II was iodinated by the chloramine T method and purified by HPLC. ACE2 activity was expressed as the amount of ANG-(1–7) generated per minute per milligram protein that was inhibited by addition of 10 μM of the ACE2 inhibitor MLN-4760 (Millennium Pharmaceuticals, Cambridge, MA).

RNA isolation and reverse transcriptase/real-time PCR. Isolation of RNA and reverse transcriptase real-time PCR were performed as previously described elsewhere (11, 12). Angiotensin-(1–7) concentrations in plasma and urine were measured by radioimmunoassay as described elsewhere (11, 12). The minimum detectable levels of these assays were 0.9 fmol for ANG II and 1.39 fmol for ANG-(1–7). The intra-assay coefficient of variation was 12% for ANG II and 8% for ANG-(1–7). Serum samples were incubated with radiolabeled [¹²⁵I]Hip-Gly-Gly (pH 8.0) for 1 h at 37°C to determine the activity of ACE (11, 12).

Figure 2. Plasma concentrations of angiotensin II (ANG II) and angiotensin-(1–7) [ANG-(1–7)] in Lew.Tg(mRen2) rats given either vehicle or medications for 12 days. Values are means ± SE; *P < 0.05 vs. vehicle; †P < 0.05 vs. lisinopril.

Table 1. Effects of drug treatments on renal function variables

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Lisinopril</th>
<th>Losartan</th>
</tr>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>347.88±9.32</td>
<td>353.88±6.12</td>
<td>360.13±6.48</td>
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<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>3.90±1.28</td>
<td>3.07±0.79</td>
<td>3.21±0.48</td>
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<td>P value</td>
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<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Water intake, ml</td>
<td>39.09±2.01</td>
<td>33.66±2.30</td>
<td>28.36±1.77</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>&lt;0.001*</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary output, ml</td>
<td>18.21±1.61</td>
<td>13.71±2.12</td>
<td>14.98±0.96</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Urine osmolality, mosmol/l</td>
<td>1570±126</td>
<td>1954±110</td>
<td>2318±123</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
<td>&lt;0.01*</td>
<td>NS</td>
</tr>
<tr>
<td>Sodium excretion, meq/24 h</td>
<td>1.63±0.19</td>
<td>2.09±0.32</td>
<td>2.85±0.15</td>
</tr>
<tr>
<td>P value</td>
<td>NS</td>
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<tr>
<td>Potassium excretion, meq/24 h</td>
<td>3.59±0.31</td>
<td>4.13±0.50</td>
<td>5.31±0.33</td>
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<tr>
<td>P value</td>
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<td>NS</td>
</tr>
<tr>
<td>Creatinine excretion, mg/24 h</td>
<td>17.33±1.53</td>
<td>14.91±1.74</td>
<td>18.22±0.86</td>
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<tr>
<td>P value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary protein, mg/24 h</td>
<td>51.56±4.49</td>
<td>22.28±1.67</td>
<td>35.56±2.62</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Data are means ± SE; NS, no significant difference. *Statistical difference vs. rats medicated with vehicle; †P < 0.05 vs. lisinopril.

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previously described (11, 12). The results were quantified as \( C_T \) values, where \( C_T \) is defined as the threshold cycle of PCR at which amplified product is first detected and expressed as relative gene expression (the ratio of target to 18S rRNA control).

**Statistical analysis.** All values are expressed as means ± SE. One-way analysis of variance followed by either the Tukey or the two-tailed Student's \( t \)-tests was used for comparing the differences between treatment groups at a \( P \) value < 0.05, whereas a two-way analysis of variance was used for comparing within and between treatment group differences over time.

**RESULTS**

**Hemodynamic effects of chronic inhibition of ACE and ANG II receptors.** Grouped baseline systolic blood pressures of congeneric male Lew.Tg(mRen2) rats before the initiation of treatment averaged 200 ± 2 mmHg, and their heart rates were 237 ± 4 beats/min. The hypertension was associated with cardiac hypertrophy and augmented excretion of protein in urine (Table 1). Figure 1 shows that both lisinopril and losartan produced significant decreases in systolic blood pressure of essentially the same magnitude during the first 8 days of therapy. However, between days 10 and 12, the systolic blood pressure of rats treated with lisinopril was below the values recorded in rats given losartan. Mean arterial pressure, obtained in anesthetized rats before termination of the experiments, was 90 ± 4 mmHg in rats receiving lisinopril and 107 ± 6 mmHg in those given losartan (\( P > 0.05 \)). These values were significantly less than those obtained in anesthetized Lew.Tg(mRen2) rats given the vehicle (150 ± 6 mmHg, \( P < 0.001 \)). None of the treatments had an effect on heart rate.

**Effect of RAS blockade on renal excretory function.** Under baseline conditions and before initiation of treatment, both water intake and urinary output (Table 1) were greater in Lew.Tg(mRen2) rats than those previously reported by us in normotensive Lewis rats (11, 12) by an average of 105% and 52%, respectively. As shown in Table 1, water intake, urinary output, osmolality, and electrolyte excretion did not change in congeneric hypertensive rats medicated with lisinopril. In contrast, losartan caused a decrease in daily water consumption associated with hyperosmolar urine, increased urinary \( \text{Na}^+ \) and
K⁺ excretion, but no differences in urinary output (Table 1). Urinary creatinine was not significantly altered by any of the treatments.

**Plasma and urinary angiotensins response to treatments.** Plasma concentrations of ANG II and ANG-(1–7) averaged 64 ± 12 fmol/ml and 28 ± 7 fmol/ml, respectively, in congenic Lew.Tg(mRen2) rats receiving vehicle (Fig. 2). Administration of lisinopril was accompanied by a large fall in plasma ANG II concentrations, whereas the opposite was true for plasma ANG-(1–7) levels. In contrast, losartan did not significantly alter plasma ANG II or ANG-(1–7) levels. Plasma ACE activity averaged 58 ± 3 nmol·min⁻¹·ml⁻¹ in vehicle-treated rats, 5 ± 1 nmol·min⁻¹·ml in rats given lisinopril (P < 0.05), and 65 ± 2 nmol·min⁻¹·ml in those assigned to losartan.

Urinary excretion rates of ANG II decreased in congenic rats given lisinopril (−37 ± 11%), whereas no changes were found in rats medicated with losartan. In contrast, urinary excretion rates of ANG-(1–7) showed a transient decrease during the first week of lisinopril therapy, whereas losartan induced a sustained reduction in urinary ANG-(1–7) (Fig. 3).

**Changes in gene expression during blockade of RAS.** The effects of the medications on the expression of ACE2 and ACE mRNAs in the left ventricle and kidney of Lew.Tg(mRen2) congenic rats are illustrated in Fig. 4. Both forms of RAS blockade were associated with significant increases in cardiac and renal cortex ACE2 and ACE mRNAs. In addition, ACE2 activity analyzed by assessment of the direct conversion of ANG II into ANG-(1–7) in membranes obtained from both cardiac and renal cortical tissues was significantly augmented in rats medicated with either lisinopril or losartan when compared with vehicle-treated rats (Fig. 5).

These changes were accompanied by increases in cardiac ANG II type 1 (AT₁) receptor mRNA and no changes in cardiac mas receptor mRNA (Fig. 6). A clear dissociation in both AT₁ and mas receptor mRNAs was found between heart and renal cortex tissue. In the kidney, lisinopril induced a small, but significant decrease in AT₁ receptor mRNA while the reduction in mas receptor mRNA was significantly different in both forms of treatment (Fig. 6). Losartan induced mas and renin mRNAs (Fig. 7), whereas losartan significantly increased renin mRNA in the kidney, but to a much lesser extent than did lisinopril. The level of angiotensinogen mRNA in the kidney was elevated in Lew.Tg(mRen2) rats medicated with either lisinopril or losartan (Fig. 7).

**DISCUSSION**

Transfection of the mouse Ren-2 gene into a fertilized ovum of female Sprague-Dawley rats that were mated with a Wistar-Kyoto rat created the first transgenic rat model of hypertension mediated by overexpression of the RAS (22, 23). Over the following decade, experimentation in this species provided new and important information showing that chronic activation of the RAS contributes significantly to the etiopathogenesis of hypertension and the mechanisms of hypertension-related end-organ damage (19, 21, 34). Because the outbred background of (mRen2)27 transgenic rats raised questions as to whether some of the phenotypes of this model may be influenced by an interaction of the Ren-2 gene with the combined diverse genetic background of the Wistar-Kyoto and Sprague-Dawley rats, we generated a new congenic line incorporating the mouse Ren-2 gene into the homogenous genetic background of the Lewis rat. The Lewis rat was chosen because this strain has no reported cardiovascular abnormalities (20).

In keeping with the findings in (mRen2)27 transgenic rats, we now show that the congenic model of hypertension maintains the key traits of the parent strain in terms of development of hypertension, presence of cardiac hypertrophy, sensitivity to blockade of the RAS, and changes in renal function. To this background, we now show that the normalization of blood pressure in Lew.Tg(mRen2) rats after blockade of ANG II synthesis or activity alters plasma concentrations of ANG II and ANG-(1–7) in a manner consistent with what would be expected from either inhibition of ACE or AT₁ receptors. Removal of ANG II activity either by reducing its synthesis or preventing the ligand from binding to the AT₁ receptor stimulated increased ACE and ACE2 gene expression in both cardiac and renal cortical tissue. Because the increases in ACE2 gene expression were associated with parallel rises in cardiac and renal cortical ACE2 activity, these data provide newer evidence for a role of ACE2 in the regulation of blood pressure and the response to blockade of the RAS. Additionally, our experiments showed that both forms of interfering with the actions of ANG II resulted in increases in renal renin, angiotensinogen, and cardiac, but not renal cortical, AT₁ receptor mRNAs. Likewise, the study in Lew.Tg(mRen2) rats provides new information on the expression of the mas receptors, recently identified to mediate the vasodilator and antipro-
liferative actions of ANG-(1–7) (30, 32). Whereas lisinopril or losartan treatments did not alter mas receptor mRNA in the heart of Lew.Tg(mRen2) rats, both agents reduced the transcription of the mas receptor gene in the kidney.

As in experiments reported elsewhere (12, 16, 17, 35), changes in plasma angiotensins reflected the mechanism of action of the given drugs because lisinopril therapy is associated with a substantial reduction in plasma ANG II, whereas ANG II levels rise substantially in rats medicated with the AT1 receptor blocker. In contrast, both forms of therapy are associated with increases in plasma ANG-(1–7) levels that reached statistical significance in rats medicated with lisinopril. The larger increase in plasma ANG-(1–7) found in lisinopril-treated rats reflects the fact that ACE metabolizes ANG-(1–7) into ANG-(1–5) (6). In previous experiments, it has been ascertained that the increases in plasma ANG-(1–7) contribute to the antihypertensive effect of these treatments because inhibition of endogenous ANG-(1–7) activity with a selective antibody or an ANG-(1–7) receptor antagonist partially reversed the antihypertensive effects of these medications (16, 17).

The hemodynamic effect of RAS blockade in congenic rats was not associated with significant changes in either water intake or renal excretory capacity in animals given lisinopril, whereas rats medicated with losartan showed reduced water intake and increased urinary osmolality associated with natriuresis and kaliuresis. The increases in sodium and potassium excretion found in Lew.Tg(mRen2) rats medicated with losartan is not the product of increased food intake because no differences in this variable were found among animals given vehicle, lisinopril, or losartan. Mitchell and Mullins (21) first documented the dependence of tubuloglomerular balance on increased ANG II in (mRen2)27 transgenic rats. While our experiments suggest a direct effect of AT1 receptor blockade on tubuloglomerular function independent of blood pressure, the possibility that a part of these differences is related to the greater control of arterial pressure in animals medicated with the ACE inhibitor cannot be excluded. Proteinuria, present in congenic Lew.Tg(mRen2) rats, was equally reversed by both forms of treatment.

In congenic hypertensive rats, the fall in urinary excretion rates of ANG-(1–7) in response to the treatment regimens mimicked the fall in peptide excretion reported by us previously in (mRen2)27 transgenic hypertensive (35) rats and in aged Wistar rats (18). These findings contrast with the augmentation in urinary levels of ANG-(1–7) found in normotensive Sprague-Dawley (35) and Lewis rats (12). The effects on urinary ANG-(1–7) found in congenic rats suggest that intrarenal uptake of ANG-(1–7) may be augmented in congenic Lew.Tg(mRen2) rats, because these effects are associated with downregulation of mas receptor mRNA as well as high plasma levels of the peptide. A similar argument can be advanced regarding urinary levels of ANG II because RAS blockade was associated with lower urinary excretion rates of ANG II, somewhat paralleling the decreases in urinary excretion rates of ANG-(1–7). This interpretation is in keeping with our previous findings in Wistar rats medicated with either enalapril.
sensitive Na and diuretic agent in part through inhibition of the ouabain-function, sodium excretion, and maintenance of hypertension.

et al. (24) and Navar and Nishiyama (25) showed that intrarenal ANG II provides the basis for sustained actions on renal vasa recta and glomeruli of losartan-treated rats (18). Navar effect because cardiac AT1 receptor mRNA, but not renal AT1 receptors in vasa recta, glomeruli of losartan-treated rats (18). Navar et al. (24) and Navar and Nishiyama (25) showed that intrarenal ANG II provides the basis for sustained actions on renal vasa recta and glomeruli of losartan-treated rats (18). Navar et al. (24) and Navar and Nishiyama (25) showed that intrarenal ANG II provides the basis for sustained actions on renal vasa recta and glomeruli of losartan-treated rats (18). Navar et al. (24) and Navar and Nishiyama (25) showed that intrarenal ANG II provides the basis for sustained actions on renal vasa recta and glomeruli of losartan-treated rats (18).

Another important finding in this study concerns the effects of the therapies on the expression of the mas receptor. Santos et al. (30) identified the mas protein as a functional receptor for ANG-(1–7), whereas we showed that the mas receptor mediates the antifibrogenic effect of the peptide on isolated cardiac myocytes (32). In congenic Lew.Tg(mRen2) rats, we now show that reversal of hypertension is associated with decreased renal cortex, but not cardiac, mas receptor mRNA. The reduction in renal mas receptor gene expression suggests that down-regulation of the renal cortex mas receptor mRNA may be secondary to increased binding of ANG-(1–7) to tubular cells. Whereas further work will be required to ascertain the specific factors associated with these changes, our data do establish a baseline for further understanding of the mechanisms that result in the reversal of the hypertensive process and the correction of renal damage as assessed from the correction of the proteinuria.

In summary, the new congenic model of hypertension created by expression of the Ren-2 gene in Lewis rats maintains the trait of hypertension while avoiding the incidence of a rapidly evolving malignant hypertension observed in the (mRen2)27 transgenic rats (23, 35). When compared with previous data in (mRen2)27 transgenic rats, Lew.Tg(mRen2) congenic hypertensive rats have comparable levels of arterial pressure and heart rate, but they display less polydipsia and polyuria (35), a lesser reduction in renal concentrating ability, and urinary sodium and potassium excretion (35). Both transgenic and congenic rats do not respond to blockade of the RAS by augmenting the excretion of urinary ANG-(1–7), although the changes in plasma ANG II and ANG-(1–7) in response to the drugs are comparable (35).

ACKNOWLEDGMENTS

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REFERENCES


