Hypertension in the Transgenic Rat
TGR(mRen-2)27 May Be Due to Enhanced Kinetics of the Reaction Between Mouse Renin and Rat Angiotensinogen

Yasuo Tokita, Roberto Franco-Saenz, Erwin M. Reimann, Patrick J. Mulrow

Abstract  The transgenic rat TGR(mRen-2)27, in which the Ren-2 mouse renin gene is transfected into the genome of the rat, develops severe hypertension with high adrenal renin and low kidney renin. These animals express both mouse and rat renin. To investigate the cause of hypertension in the TGR rat, we compared the kinetics of mouse renin acting on mouse and rat angiotensinogens. The optimum pH of the renin reaction in the Sprague-Dawley rat was 6.5, whereas the optimum pH of the reaction in the TGR rat was approximately 8.5. The optimum pH of the renin reaction in the DBA mouse was 6.0. Purified mouse Ren-2 renin acting on rat angiotensinogen showed a pH profile similar to that for the renin reaction in the TGR rat. The angiotensinogen concentration in pooled plasma from eight DBA mice was 104.5 ng angiotensin I/mL and was clearly lower than that in Sprague-Dawley rats (772.4±37.3 ng angiotensin I/mL; n=4). The reaction of purified mouse Ren-2 renin with rat angiotensinogen was 10 times faster than with mouse angiotensinogen. Plasma renin activity in DBA mice increased dramatically on addition of rat angiotensinogen (from 253.4±66.7 to 225 000±48 000 ng angiotensin I/mL per hour). Intravenous injection of 2 or 10 μL of DBA mouse plasma into the nephrectomized Sprague-Dawley rat increased the mean arterial pressure of the rat by 27.7±4.7 and 61.8±2.7 mm Hg, respectively, whereas injection of 200 μL of Sprague-Dawley rat plasma did not change the mean arterial pressure of the rat. From these measurements and from the kinetic parameters measured by Poulsen and Jacobsen (J Hypertens. 1986;4:175-180), we conclude that the molar concentration of mouse renin in the TGR rat is low compared with that of rat renin (1.5 versus 5.0 pmol/L) but that this low level of circulating mouse renin contributes significantly to plasma renin activity in the TGR rat. The elevation of renin activity and resulting hypertension in the TGR rat therefore can be attributed to the enhanced kinetics of mouse renin acting on rat angiotensinogen. (Hypertension. 1994;23:422-427.)

Key Words  •  renin-angiotensin system  •  animals, transgenic  •  renin  •  hypertension, renin-dependent  •  angiotensinogen

The renin-angiotensin system plays an important role in the development of hypertension. Recently, various transgenic animal models of hypertension have been developed to elucidate the mechanisms of hypertension. The transgenic rat TGR-(mRen-2)27, in which the mouse Ren-2 renin gene that codes for mouse salivary gland and kidney renin is transfected into the genome of the Sprague-Dawley rat, was the first genetic hypertensive rat model. In this model an overexpression of the transgene is found in the adrenal glands, and these rats show severe hypertension with remarkably high adrenal renin levels; however, the pathogenesis of the hypertension is not clear. We have reported that adrenal renin and circulating renin in TGR rats are probably derived primarily from the transfected mouse renin gene.2 On the other hand, angiotensinogen in the TGR rat is solely rat angiotensinogen. It is known that mouse renin can generate angiotensin I (Ang I) by reacting with rat angiotensinogen.7 If the circulating renin in the TGR rat is mouse renin, then the kinetics of the reaction between mouse renin and rat angiotensinogen may play a role in the pathogenesis of the hypertension. Poulsen and Jacobsen8 have reported that the reaction of mouse renin with rat angiotensinogen is significantly faster than the reaction of mouse renin with mouse angiotensinogen, and it was also 10 times faster than the reaction of rat renin with rat angiotensinogen.9 In an attempt to determine the species of circulating renin and to elucidate the pathogenesis of the hypertension in the TGR rat, in this study we investigated the optimum pH of the renin reaction and the rates of mouse renin acting on mouse and rat angiotensinogen.

Methods
Homozogous male transgenic TGR(mRen-2)27 rats (240 to 310 g) were obtained from Dr D. Ganten (Max Delbruck Center for Molecular Medicine). Male DBA mice (6 to 8 weeks old) and male Sprague-Dawley rats (225 to 250 g) were obtained from Harlan Sprague Dawley. All animals were housed under a 12-hour light/dark cycle and provided with water and a diet containing normal sodium (Wayne Lab Blox). Animals were killed by decapitation, and blood was collected from the neck vessels into chilled tubes containing Na2EDTA (4 mmol/L final concentration) for measurement of plasma renin activity (PRA), plasma renin concentration (PRC), and plasma angiotensinogen. Mouse Ren-2 renin purified to near homogeneity from mouse salivary gland was kindly provided from Drs K. Murakami and A. Fukamizu from the University of Tsukuba (Japan).
Measurement of Plasma Renin Activity and Plasma Renin Concentration

PRC was measured by radioimmunoassay of Ang I generated by plasma after the addition of rat angiotensinogen as we previously described. Briefly, 50 μL of plasma was incubated with 75 μL of rat angiotensinogen, 5 μL of 8-hydroxyquinoline, 5 μL of dimercaprol, 25 μL of Na2EDTA (4%), and 90 μL of Tris acetate buffer (0.1 mol/L, pH 7.4) for 1 hour at 37°C, and the generated 1 Ang I was measured with the RIANEN Angiotensin I kit (New England Nuclear Research Products). PRA was measured by the same method with additional Tris acetate buffer instead of rat angiotensinogen. Ang I generation was linear for 1 hour in Sprague-Dawley and TGR rats.

For measurement of PRA and PRC in mice, the assay incubation time was reduced to 5 minutes to prevent depletion of angiotensinogen. This was based on preliminary experiments in which we found that under our assay conditions Ang I generation is linear during the initial 5 minutes. Results were converted to nanograms Ang I per milliliter per hour. Therefore, the term PRA is used when endogenous angiotensinogen is the sole substrate; the term PRC is used when exogenous substrate is used.

Plasma Renin Activity and Plasma Renin Concentration in Mice

To study the effect of the addition of rat angiotensinogen to mouse plasma, PRA and PRC were measured in DBA mice. Eight male DBA mice (6 to 8 weeks old) were killed by decapitation, and blood was collected into chilled tubes containing Na2EDTA (4 mmol/L).

Injection of Mouse Plasma Into the Rat

To study the effect of mouse renin on rat angiotensinogen in vivo, mouse plasma was injected into nephrectomized rats. Male Sprague-Dawley rats (225 to 250 g) were nephrectomized bilaterally under ether anesthesia. Twenty-four hours after nephrectomy, the rats were cannulated with PE-50 catheters into the carotid artery and the jugular vein under ether anesthesia. Arterial pressure was monitored with a data-recording system (model 79, Polygraph, Grass Instrument Co.). After blood pressure stabilized, 200 μL of 0.9% saline or 200 μL of normal Sprague-Dawley rat plasma (PRA, 2.58 ng Ang I/mL per hour) was injected through the jugular vein catheter, and blood pressure was recorded. Rats were then injected with 2 or 10 μL DBA mouse plasma (PRA, 242 ng Ang I/mL per hour) diluted in 200 μL saline and 200 μL of Na2EDTA (4%) intravenously into the nephrectomized rats 5 minutes after the injection of 2 μL DBA mouse plasma and observed the change in arterial pressure.

Plasma Angiotensinogen in Mice and Rats

For comparison of the plasma angiotensinogen concentration in mice and rats, pooled mouse plasma (from eight DBA mice) and rat plasma (from four Sprague-Dawley or five TGR rats) were serially incubated for 30 minutes to 5 hours after addition of mouse or rat kidney renin. The Ang I generated was measured by radioimmunoassay, and the peak of Ang I production was taken as the angiotensinogen concentration in each plasma. Angiotensinogen concentration is expressed as nanograms Ang I equivalents per milliliter.

Reaction of Purified Mouse Submandibular Renin (Ren-2) With Mouse and Rat Angiotensinogen

The source of mouse angiotensinogen was nephrectomized (NEPEX) plasma obtained from 10 male DBA mice (20 to 30 g) and 13 to 16 hours after bilateral nephrectomy. The source of rat angiotensinogen was NEPEX plasma obtained from 10 male Sprague-Dawley rats (250 to 300 g) 48 hours after nephrectomy.

The angiotensinogen concentration was 4827 ng Ang I/mL in the rat NEPEX plasma and 1294 ng Ang I/mL in the mouse NEPEX plasma. The rat NEPEX plasma was diluted with Tris acetate buffer (0.1 mol/L, pH 7.4) containing 5% bovine serum albumin to make the angiotensinogen concentration the same as in the mouse NEPEX plasma. PRA was 86.6 ng Ang I/mL per hour in the mouse NEPEX plasma and undetectable in the rat NEPEX plasma. Diluted purified Ren-2 mouse submandibular renin was added to both plasmas, and changes in renin activity in both plasmas were investigated. The concentration of the Ren-2 renin enzyme present in the highly purified mouse submandibular renin preparation was measured as follows: Three samples of the Ren-2 enzyme preparation and bovine serum albumin were analyzed by gel electrophoresis in the presence of sodium dodecyl sulfate. After fixing and staining with Coomassie blue, a protein band with a molecular weight of approximately 36,000, corresponding to the molecular weight of the A chain of mouse Ren-2 renin, was seen in each sample. The gels were scanned on an Ambis Gel Scanner, and the amount of protein in each band was measured in order to calculate the amount of Ren-2 renin, based on the known amount of serum albumin. The calculated concentration of the renin enzyme was 0.078±0.01 mg/mL.

Results

Nature of Plasma Renin in the TGR(mRen-2)27 Rat Based on pH Optimum

To investigate the nature of circulating renin in the TGR rat, we measured the optimum pH of the renin activity in the TGR rat and compared it with the optimum pH of the renin activity in the Sprague-Dawley rat. Renin activity in each plasma sample was measured at different pH values with the use of four different buffer solutions at the optimum pH range of each. When measured at a pH of 6.0 to 7.0, PRA was increased by a factor of approximately 1.5 in the TGR rat (Fig 1B), consistent with previous results. This increase was much more pronounced at pH values around 8.5, at which the increase was approximately 40-fold because of the markedly different pH profiles. The optimum pH of the renin reaction was 6.5 in the Sprague-Dawley rat (Fig 1A) and 8.5 in the TGR rat (Fig 1B). For determination of the basis for the different pH profiles, endogenous mouse renin was tested with mouse angiotensinogen (Fig 1C), and mouse Ren-2 renin was tested with rat angiotensinogen (Fig 1D). It is clear that the high pH optimum observed in the TGR rat is characteristic of mouse renin acting on rat angiotensinogen (Fig 1D), confirming the presence of circulating mouse renin in the TGR rat. The shoulder of increased activity at pH 6.5 in the pH profile of the TGR rat plasma probably represents the activity caused by rat renin.

Properties of Mouse Submandibular Renin (Ren-2 Renin)

To investigate the kinetic differences between mouse and rat renin further, we incubated purified Ren-2 renin with NEPEX plasma obtained from DBA mice and Sprague-Dawley rats. The rat plasma was diluted so that the angiotensinogen concentrations were the same in each plasma sample. Fig 2 shows that a given concentration of Ren-2 renin has a much higher activity toward rat angiotensinogen, confirming that rat angiotensinogen is a much better substrate for this enzyme than mouse angiotensinogen. The enhanced activity of mouse renin toward rat angiotensinogen demonstrates...
that low concentrations of mouse renin could induce hypertension in the TGR rat.

**Estimations of the Molarity of Circulating Renin**

The PRA of DBA mouse plasma was increased dramatically (from 253.4 ± 66.7 to 225,000 ± 48,000 ng Ang I/mL per hour, n=8) when rat angiotensinogen was added (Fig 3). PRA reflects the in vivo reaction between circulating renin and angiotensinogen. In the normal Sprague-Dawley rat, PRC was 6.8 ± 0.9 ng Ang I/mL per hour.
hour, and in the TGR rat it was higher, at 19.9±0.1 ng Ang I/mL per hour (Table). From kinetic constants reported previously it is possible to estimate the amount of circulating renin. These calculations are presented in the Table and indicate that the concentration of renin in mouse plasma is approximately 10 000-fold greater than the concentration of renin in rat plasma (50 versus 0.005 nmol/L). If one makes the simple assumption that the difference in renin activity between normal Sprague-Dawley rats and TGR rats is due to expression of the mouse renin gene, it can be calculated that the concentration of mouse renin in the TGR rat is 0.0015 nmol/L. Thus, the concentration of mouse renin (0.0015 nmol/L) in the TGR rat is lower than the concentration of rat renin (0.005 nmol/L) in the Sprague-Dawley rat. The increased renin activity and hypertensive effect of mouse renin in the TGR rat is therefore most likely due to the increased reactivity of mouse renin toward rat angiotensinogen. The high concentration of renin in the DBA mouse does not lead to hypertension, in part because the plasma concentration of angiotensinogen is approximately sevenfold lower than in the Sprague-Dawley rat (Fig 4, Table) (DBA mice, 104.5 versus Sprague-Dawley rat, 772.4±37.3 ng Ang I/mL), but this is probably not the only factor because PRA is still much higher (253 versus 6.8 [ng Ang I/mL]/h) despite the lower concentration of substrate (Table). Thus, the mouse appears to be resistant to renin-induced hypertension.

### Injection of Mouse Plasma Into Rats

The effect of small amounts of mouse renin on rat blood pressure was demonstrated by injecting a small volume of mouse plasma into Sprague-Dawley rats (Fig 5). Injection of 200 μL saline did not change mean arterial pressure of the nephrectomized rats, nor did injection of 200 μL rat plasma (93.4±8.1 to 95.5±8.5 mm Hg, n=5, P=NS). However, injection of 2 μL DBA mouse plasma dissolved in 200 μL saline clearly increased the mean arterial pressure of rats (92.0±8.2 to 119.7±111.9 mm Hg, n=5, P<.005). Moreover, injection of 10 μL DBA mouse plasma increased mean arterial pressure from 96.2±5.3 to 158.0±7.1 mm Hg (n=5, P<.001), showing a dose-response relation. Injection of the Ang II antagonist DuP 753 (1 mg/kg dissolved in 100 μL saline) abolished the effect of mouse plasma and lowered mean arterial pressure to baseline levels (Fig 5). One would expect that 2 μL mouse plasma would result in a renin concentration of approximately 0.01 nmol/L in rat plasma, assuming a concentration of 50 nmol/L in mouse plasma (Table) and a plasma volume of 10 mL in the rat. From these data it appears that the temporary presence of 0.01 to 0.05 nmol/L mouse renin in the plasma of rats results in marked hypertension. In the TGR rat the estimated concentration of mouse renin is 0.002 nmol/L (Table). Because this is a chronic level of expression, it may be sufficient to produce the observed hypertension.

### Discussion

The studies described here were carried out to clarify the pathogenesis of hypertension in the TGR rat. In previous studies enhanced mineralocorticoid production was reported in TGR rats. However, treatment of these rats with the mineralocorticoid receptor antagonist spironolactone did not influence blood pressure. On the other hand, treatment with the angiotensin-converting enzyme inhibitor captopril (10 mg/kg per day) reduced the blood pressure of TGR rats by 40 to 60 mm Hg and treatment with the Ang II receptor antagonist DuP 753 (10 mg/kg per day) also lowered the blood pressure of these rats. These findings clearly indicated

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### Estimation of the Molar Concentration of Plasma Renin in DBA Mice, Sprague-Dawley Rats, and TGR(mRen-2)27 Rats

<table>
<thead>
<tr>
<th>Plasma Sample</th>
<th>Species</th>
<th>Renin Activity (ng Ang I/mL)/h</th>
<th>Renin, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBA mouse</td>
<td>Mouse</td>
<td>253</td>
<td>67</td>
</tr>
<tr>
<td>DBA mouse</td>
<td>Mouse</td>
<td>19.9</td>
<td>1</td>
</tr>
<tr>
<td>SD rat</td>
<td>Rat</td>
<td>19.9</td>
<td>0.0055</td>
</tr>
<tr>
<td>TGR rat</td>
<td>Mouse</td>
<td>6.8</td>
<td>0.0023</td>
</tr>
<tr>
<td>TGR rat</td>
<td>Mouse</td>
<td>13.1</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

**Notes:**
- Ang I indicates angiotensin I; SD, Sprague-Dawley; and TGR, TGR(mRen-2)27.
- *From Poulsen and Jacobsen* after conversion to new units.
- †Calculated from the Michaelis-Menten equation: \( v = \frac{K_{\text{m}} \cdot [\text{ENZ}] \cdot [S]}{K_{\text{m}} + [S]} \), where \( v \) is renin activity, [ENZ] is molar concentration of renin, and [S] is angiotensinogen concentration.
- ‡Assuming that all activity is due to mouse renin and that no rat renin is present in TGR rat plasma.
- §Assuming that the amount of mouse renin activity is represented by the difference between renin levels in TGR and SD rats.
that the hypertension of these animals is due to hyper-
function of the renin-angiotensin system. However, plasma renin and kidney renin levels were reported to be low in TGR rats compared with Sprague-Dawley rats. In previous experiments we confirmed that kidney renin in TGR rats was significantly lower than in Sprague-Dawley rats. In contrast to the previous findings, however, in our experiments PRC was significantly higher in the TGR rat than the Sprague-Dawley rat. This discrepancy existed even when the Hanover strain of Sprague-Dawley rats, the strain from which the TGR rat is derived, was used as control. The reason for this discrepancy in results is not clear but may stem from differences in diet, the renin substrate used for the reaction, or slight differences in the pH of the renin reaction.

The Ang I-generating activity of the plasma in the TGR rats was completely inhibited by the renin inhibitor CP 71362, indicating that the Ang I-generating activity in the plasma of the TGR rats is true renin.

The kinetics of the renin reaction vary among different species. Poulsen and Jacobsen reported that the reaction of pure mouse renin with rat angiotensinogen is faster than the reaction with homologous angiotensinogen, and it was also 10 times faster than the reaction of rat renin with rat angiotensinogen reported by others. It is also known that the reaction of human renin with sheep angiotensinogen is 10 times faster than the reaction with human angiotensinogen. Thus, the renin reaction has interesting characteristics, not only in that the kinetics are different from species to species but also the rate of the reaction can change by using angiotensinogen from other species. In this study, the reaction of mouse renin with rat angiotensinogen was 10 times faster than the reaction with mouse angiotensinogen. On the other hand, it was also reported that mice have a relative deficiency of angiotensinogen. In the present study, we confirmed that the angiotensinogen concentration in mice was clearly lower than that in rats. Therefore, the dramatic increase in mouse renin activity after addition of rat angiotensinogen depends on both the increase in the substrate concentration in the assay and the increase in the speed of the renin reaction. Weaver et al. showed an elevation of the blood pressure of mice after injection of nephrectomized rat plasma, and Poulsen and Jacobsen reported that mouse renin reacts faster with rat angiotensinogen than with the homologous substrate. Recently, Kimura et al. developed a hypertensive transgenic mouse by transfection with the rat angiotensinogen gene. The blood pressure elevations in the mice therefore may depend not only on the increased amount of angiotensinogen but also on the enhancement of the kinetics of the renin reaction.

We reported that circulating renin in the TGR rat is probably derived from the transfected mouse renin gene. In the present study, the pH curve of renin activity in the TGR rat showed a pattern similar to that of the reaction between mouse renin and rat angiotensinogen. This finding provides additional evidence that mouse renin contributes to most of the circulating renin activity in the TGR rat.

We have shown that bilateral nephrectomy increases adrenal renin in the TGR rat and Sprague-Dawley rat. On the other hand, bilateral nephrectomy increased PRA in the TGR rat, whereas in the Sprague-Dawley rat PRA was undetectable 20 hours after nephrectomy. This finding clearly indicates an extrarenal source for plasma renin in the TGR rat.

In a transgenic rat in which the human renin gene was transfected, the transfected renin gene and the host renin gene were coexpressed. Therefore, in the transgenic TGR(mRen-2)27 rat the rat renin gene and the mouse renin gene probably are coexpressed. However, according to the results of the pH profile of the renin reaction and our previous data using an antibody that inhibited mainly rat renin, most of the circulating renin activity appears to result from mouse renin. The underexpression of the rat renin gene may result from inhibition of kidney renin by the hypertension or from negative feedback from Ang II generated from the reaction of mouse renin with rat angiotensinogen. The activity of plasma renin in the TGR rat was equal to 1.5 to 2.3 pmol/L of purified Ren-2 renin in the same assay conditions. Considering the fact that mouse renin reacts more quickly with rat angiotensinogen than with mouse angiotensinogen, the absolute amount of mouse renin...
enzyme protein in the TGR rat is less than in the normal Sprague-Dawley rat, which was calculated to be 5 pmol/L.

We conclude that the dramatic increase in mouse PRA by addition of rat angiotensinogen and the elevation of blood pressure in the nephrectomized rat after injection of DBA mouse plasma are primarily due to the increase in kinetics of the mouse renin reaction with rat angiotensinogen as the substrate. Therefore, the hyper-tension in the TGR(mRen-2)27 rat may be due to the enhanced kinetics of the reaction between mouse renin and rat angiotensinogen.

Acknowledgments

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References

Hypertension in the transgenic rat TGR(mRen-2)27 may be due to enhanced kinetics of the reaction between mouse renin and rat angiotensinogen.
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