Hypertension in the (mRen-2)27 Rat Is Not Explained by Enhanced Kinetics of Transgenic Ren-2 Renin

Pei Rong, Duncan J. Campbell, Sandford L. Skinner

Abstract—Enhanced efficiency of the reaction between transgenic Ren-2 mouse renin and endogenous rat angiotensinogen has been suggested as 1 mechanism that contributes to the accelerated hypertension and increased tissue angiotensin of the (mRen-2)27 transgenic rat. This was tested in a study conducted at pH 7.4 in vitro that compared the kinetic constants of purified mouse Ren-2 and rat renin (each at 100, 75, 50, and 25 pmol/L) reacting with physiologic concentrations of rat angiotensinogen (0 to 4 μmol/L). Under these conditions, the kinetic constants for Ren-2 (K<sub>m</sub> 1.8 μmol/L; K<sub>cat</sub> 0.07/s; and K<sub>cat</sub>/K<sub>m</sub> 0.04 L · μmol<sup>-1</sup> · s<sup>-1</sup>) were not different from rat renin. However, Ren-2 renin acting on its homologous mouse angiotensinogen was confirmed as being much slower. We conclude that hypertension in the Ren-2 rat is not related to renin kinetics. Other mechanisms are considered, with reference to human essential hypertension. (Hypertension. 2003;42:523-527.)

Key Words: renin-angiotensin system ■ angiotensinogen ■ blood pressure ■ hypertension, essential

The hypertensive (mRen-2)27 transgenic rat (Ren-2 rat) developed by Mullins et al<sup>1</sup> displays strong expression of the mouse Ren-2 transgene in several extrarenal tissues,<sup>2</sup> suppressed endogenous renal renin, and predominantly mouse inactive prorenin in the circulation secreted from extrarenal sources.<sup>3–5</sup> Most evidence indicates that it is the active component of mouse Ren-2 renin in tissues and plasma, reacting with the endogenous rat angiotensinogen (aogen), that induces and sustains the hypertension through conventional angiotensin II (Ang II)–dependent mechanisms, in particular because the hypertension is readily controlled by inhibition of the renin-angiotensin system (RAS).<sup>6</sup> However, this concept is at odds with the initial published evidence of suppressed or normal active plasma renin and angiotensin peptides in the adult Ren-2 rat,<sup>7,8</sup> reports that encouraged a search for additional hypertensive mechanisms. This transgenic rat strain has now been used widely to study various conditions relating to activation of the tissue RAS, including angiogenesis, cytokine activation, and profibrotic and inflammatory pathologic states, making it important to understand fully the fundamental processes inducing the severe hypertension.

That plasma Ang II is not suppressed is now apparent, with resting levels increased up to 4-fold in both young<sup>9</sup> and adult<sup>10</sup> Ren-2 rats. One would expect a similar elevation of plasma active renin, but this depends on the pH at which the activity is estimated,<sup>11,12</sup> because mouse and rat renins have different pH optima when acting on rat aogen (pH 8.5 and 6.5, respectively).<sup>5,11,12</sup> Measured at pH 7.4 the Ren-2 rat plasma renin reaction is a mixed activity, mainly owing to the mouse transgenic renin,<sup>11</sup> and increases in renin activity have been found under these physiologic conditions. The lower level of rat renin in plasma is consistent with its low but not absent level in the kidneys,<sup>11,12</sup> By comparison with other high-renin models that cause malignant hypertension, the ≈4-fold increase in plasma renin and Ang II would not seem a sufficient explanation for the degree of hypertension that develops rapidly in the mature animal. However, tissue levels of Ang II are also increased, by as much as 20-fold in the brain and 3-fold in the kidney,<sup>9</sup> and would be expected to have accelerating effects on the hypertensive process.

Another factor examined by Tokita et al<sup>13</sup> was the possibility that an enhanced reaction between the heterologous reactants, mouse Ren-2 renin and rat aogen, might explain why in some reports the adult animal displays low/normal plasma active renin but that the hypertension is nevertheless Ang II dependent. These investigators used high concentrations of mouse salivary gland Ren-2 renin acting on mouse and rat aogens and found that the heterologous reaction was 10 times faster than the mouse homologous reaction. This confirmed the earlier conclusion by Poulsen and Jacobsen<sup>14</sup> of unusual renin kinetics in the normal mouse, wherein the turnover number was one quarter that of the homologous rat reaction. Tokita et al<sup>13</sup> then cited the kinetic data of Gukowska et al<sup>15</sup> for the homologous rat reaction and of Poulsen and Jacobsen<sup>14</sup> for the heterologous mouse renin on rat aogen reaction and proposed that the hypertension could be Ang II dependent, despite low molar concentrations of active mouse renin.
purified renin in plasma due to enhanced kinetics. However, such extrapolation from kinetic data obtained by different authors who used various conditions and reactants could lead to erroneous conclusions. Neither Tokita et al.13 nor other authors have directly compared, under plasma physiologic conditions at pH 7.4, the mouse Ren-2 renin on rat aogen reaction with the rat renin on rat aogen reaction, which is the appropriate experiment to test this notion and which is reported in this article.

Methods

Purified Renins
Male Swiss mouse submaxillary (SMG) Ren-2 renin and rat kidney renin were prepared as previously reported.16,17 Protein purity was >95%, as assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie blue staining. Specific activities by kinetic assay (see later sections) were 330 Goldblatt units (GU)/mg protein for Ren-2 and 410 GU/mg for rat renin compared with standard hog renin (65/119, National Institute of Biological Standards and Control, UK). Quantitative comparison with the renin used by various authors is difficult because a variety of substrates, reference standards, and reaction conditions have been used.16,17 Nevertheless, the activities of our preparations appear equivalent to other reports. For Ren-2 renin reacting with rat aogen at pH 7.4, it has been calculated that 1 nmol of the pure active enzyme is equivalent 16 GU of hog standard renin.20,21 This calculation included a molecular weight (MW) of 40 000 Da, which, though corroborated, is now identified as closer to 36 000 Da for nonglycosylated SMG Ren-2 renin.18,23 Rat renin MW, which is glycosylated, has been reported to be 36 000 to 42 000 Da.19 At MW 36 000 Da, our Ren-2 renin exhibited 11.9 GU/mmol, and rat renin, 14.8 GU/mmol. At MW 40 000 Da, the values are closer to 16 GU/mmol (13.2 and 16.4 GU/mmol, respectively). We accepted the established equivalence factor of 16 GU/mmol because the activities of our preparations were similar, within the context of the methods. Our pure rat renin generated 35.5 μg Ang I·h⁻¹·μg⁻¹ protein reacting with 0.975 μmol/L rat aogen at pH 7.4 (see following sections). This is 30% of the rate reported by Figueiredo et al.19 at a pH maximum of 6.5 for the rat reaction. Because equivalent purity is apparent, it is assumed that the difference relates to the pH, the concentration of aogen, the standard reference renin, and the presence of some denatured renin.

Angiotensinogen Preparations
Sprague-Dawley rats and BALB/c mice were exsanguinated by cardiac puncture after hepatic injection (200 and 20 U IV, respectively) under pentobarbitone anesthesia (60 mg/kg body weight) 24 hours after binephrectomy (BNx), and the plasma was separated at 4°C. Rats received dexamethasone 1 mg/kg SC, and mice, 0.3 mg dexamethasone each 8 hours after BNx to stimulate aogen synthesis. Residual renin in plasma was removed by antibody affinity chromatography.24 Rat aogen content was 7.8 μg/L renin sample, 30 μL renin sample, 30 μL rat aogen. The final 100-μL incubation for standard assay conditions comprised 20 μL renin sample, 30 μL protease inhibitors, 37.5 μL diluent, and 12.5 μL rat aogen. The incubated aogen concentration was 0.975 μmol/L. Ang I generated was estimated by radioimmunoassay with an in-house antibody and a 125I-Ang I tracer.24,25 In this assay, 1 GU of standard hog renin generated 87 μg Ang I/h, a value similar to that found by Devaux et al.26 (68 μg Ang I/h), although incubated at pH 6.5. For kinetic studies, renin and aogen were serially diluted with PBS with bovine serum albumin (see Results) and keeping incubate volume and protein concentration constant. Standard Ang I displacement curves were established for each set of circumstances.

Results

Reaction Rates of Rat and Ren-2 Renins With Serial Dilutions of Rat Aogen
To establish ideal reaction conditions for kinetic studies (incubation period, amount of Ang I generated with respect to the Ang I displacement curve, and linearity of reaction velocity), enzyme-velocity plots were established for each renin concentration (100, 75, 50, and 25 pmol/L) under standard assay conditions (see Methods). From these plots, 75 pmol/L rat renin and 100 pmol/L mouse renin produced similar, convenient reaction velocities with the specific aogen concentration (0.975 μmol/L) used in the assay. This ratio of activities was close to that expected from the initial estimation of activities with respect to Goldblatt units (see Methods). A direct comparison was then made of substrate-velocity plots with these specific renin concentrations. Figure 1 illustrates that the substrate-velocity plots were virtually identical, as were the Lineweaver-Burk plots. The Table
Comparison of the Reactions of Ren-2 Renin on Mouse and Rat Aogens

Figure 2 shows the result of serially diluting rat aogen to equal the very low concentrations of mouse aogen obtained from the 24-hour BNx mice. The reaction was conducted with 500 pmol/L mouse Ren-2 renin, a concentration 5 times that used in Figure 1 to raise reaction velocity to a measurable level. The incubation period was prolonged to 12 hours for the homologous reaction. The rate of the heterologous reaction (Ren-2 renin on rat aogen) was considerably faster than the matched homologous mouse reaction, but the kinetic constants cannot be derived for mouse aogen, because maximum velocity was not approached. This experiment was conducted to test the hypothesis that the basis for the proposal of Tokita et al13 was reproducible under the present conditions.

Discussion

The proposition has been raised that enzymatic reactions in vivo of transgenic Ren-2 mouse renin versus normal rat renin are sufficiently different to account for the early reports of low-renin hypertension in the Ren-2 rat.13 For this to be the case, a higher affinity, maximum reaction rate, or catalytic rate might be expected for Ren-2 renin at pH 7.4 reacting with rat aogen. Our 2 renin preparations were of similar protein purity, and when matched for equal activity under standard assay conditions, virtually identical substrate-velocity and Lineweaver-Burk plots were obtained. The $K_m$ values are within a range (0.67 to 2.8 $\mu$mol/L) found by various authors14,15,27,28 for the homologous rat reaction and the heterologous Ren-2 renin on the rat aogen reaction14 between pH 6.5 and 7.5. The catalytic rate constant $K_{cat}$ depends on both the specific activity of the enzyme preparation and the $V_{max}$ achieved under the particular conditions of incubation. $K_{cat}$ for our rat renin acting on rat aogen at pH 7.4 was lower (0.09/s) than in 2 previous publications (0.87/s),14,15 although in 1 of those studies, the reaction was conducted at pH 6.5, at which the rate of Ang I formation is approximately double that at pH 7.5.13 The catalytic efficiencies of the reactions ($K_{cat}/K_m$), though identical, are also lower than previously published. On the other hand, the protein purity of our preparations as evidenced by SDS-PAGE was at least 95% and equivalent to previous purifications. However, activity rather than purity is more important in the present consideration. Our renin preparations, purified by similar methods and to similar extents, displayed specific activities and kinetic properties under the same conditions that are sufficiently close to conclude that no major kinetic difference exists in their reactions with rat aogen.

It has been shown for rat and human renin that $K_{cat}$ values are in the lower range for proteolytic enzymes reacting with their natural substrates, whereas for the mouse reaction (Ren-2 renin on mouse aogen), it is even lower.14 Our limited mouse data are consistent with this observation and illustrate the extreme differences in kinetics that Ren-2 renin can display for rat and mouse aogens. Substrate specificities and properties have been extensively investigated and reviewed14,15,29,30 and have included comparisons of whole plasma with purified aogen, without kinetic differences being apparent.

In considering possible sources of error sufficient to invalidate our conclusion, there would have to be a major disparity in the enzyme specific-activity estimation or that the rat aogen preparation was not representative of in vivo conditions. As presented in Methods, the specific activities are similar to those reported in previous publications of rodent renin, but the question could be raised as to whether aogen prepared after BNx and/or dexamethasone stimulation represents its normal composition. However, kinetics are not affected by the changes in aogen glycoforms after BNx or hormone stimulation in the rat,29 and these procedures have been used routinely in many previous kinetic studies to augment the aogen level.

From these findings, we conclude that hypertension in the Ren-2 rat is not related to renin kinetics and logically rests with other mechanisms. In addition to the considerations mentioned in the Introduction, there are several hypertensive mechanisms not commonly appreciated that could result specifically from the increased tissue renin expression.

First, although renal juxtaglomerular cell renin and renal renin content are low in the Ren-2 rat, the renal content of Ang II is increased and probably mainly of plasma origin.9 However, renin and aogen are also expressed in the renal tubules of the normal mouse,31 and both renin expression32 and Ang II peptide33 become obvious in the proximal tubule of the Ren-2 rat with streptozotocin-induced diabetes. Renal Ang II type 1 receptors are also markedly increased in the Ren-2 rat,34 and tubular Ang II in various hypertensive models is considered to provide the necessary condition for the retention of sodium, despite high perfusion pressure.35

### Derived Kinetic Constants of Rat and Ren-2 Mouse Renins Reacting With Rat Aogen

<table>
<thead>
<tr>
<th>Renin</th>
<th>$V_{max}$ nmol/L per h</th>
<th>$K_m$ $\mu$mol/L</th>
<th>$V_{max}$ s$^{-1}$</th>
<th>$K_{cat}$ L/µmol per s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>25</td>
<td>1.9</td>
<td>0.09</td>
<td>0.05</td>
</tr>
<tr>
<td>Ren-2</td>
<td>25</td>
<td>1.8</td>
<td>0.07</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are from equations in Figure 1.
This would be expected to be at least a permissive hypertensive process in the Ren-2 rat.

Second, both blood pressure and peripheral vasoconstriction are reduced by bosentan (ET₄/ET₆ endothelin blockade) in Ren-2 rats, an effect seen also in normal animals infused with Ang II. Endothelin involvement in Ren-2 rat hypertension is controversial, but a specific action of Ang II to stimulate endothelin in a variety of systems is widely acknowledged.

Perspectives

Of the several other possible single causes of hypertension, such as increased adrenal secretions, increased sympathetic tone, increased sodium-proton exchange, or decreased nitric oxide production, none appear convincingly to the Ren-2 rat. An important feature of the hypertension of the Ren-2 rat is its sensitivity not only to RAS blockade but also to a variety of other antihypertensive agents or procedures that do not directly interfere with the RAS. This suggests that tissue renin synthesis, albeit manifest as high plasma prorenin, when resulting in at least some local Ang II production, might be viewed as a dispersed regulator of blood pressure that exploits many established hypertensive systems but is insufficient when acting alone as the single vasoconstrictor endocrine mediator. This concept is novel to the extent that it is the tissue RAS, including renal tubules, that is the proposed initiator, irrespective of plasma Ang II or renin activity level and largely independent of any direct Ang II vasoconstrictor action. Similar to the Ren-2 rat, prorenin exists in high concentrations in human plasma, but in humans, only half of the plasma prorenin originates from extrarenal sources.

Human plasma prorenin level is correlated inversely with blood pressure, and as for plasma active renin, is moderately decreased in essential hypertension. The inverse relation likely results from feedback suppression of juxtaglomerular cell renin synthesis and prorenin secretion due to the hypertensive and any extrarenal sources of plasma Ang II. This leaves open the possibility that tissue renin expression in humans, although causing considerable constitutive prorenin release, is also associated with local active renin and Ang II formation not accurately reflected in plasma levels. By analogy with the Ren-2 rat but in a much less fulminating manner, this could influence blood pressure incrementally through a variety of pathways over an extended time span and be an important contributing factor to essential hypertension in humans.

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References


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