Angiotensinogen Concentrations and Renin Clearance
Implications for Blood Pressure Regulation

Jürgen Bohlender, Joël Ménard, Detlev Ganten, Friedrich C. Luft

Abstract—Renin (REN) requires seconds to convert angiotensinogen (AGT) to angiotensin I. We tested the hypothesis that this long catalytic cycle might indicate an influence of AGT concentrations on REN clearance. We studied 2 transgenic rat (TGR) strains for human (h) AGT; one strain has hAGT values ∼7-fold higher than the other (68±18 versus 10±4 μg angiotensin I/mL). hREN (30 000 pg) was bolus-infused into both lines and into nontransgenic controls. The terminal half-life (T1/2β) was increased (130 versus 82 minutes) and the metabolic clearance rate (MCR) was decreased (0.83±0.29 versus 2.2±0.66 μL · min⁻¹ · g⁻¹) in the high hAGT strain compared with the low hAGT strain. The difference was not related to volume of distribution at steady state. Infused hREN blocked with remikiren resulted in T1/2β and MCR values that were not different from control values. Infused unblocked and blocked radiolabeled hREN was distributed similarly in the hAGT TGR strains. Infused mouse REN, which cannot convert hAGT, had similar T1/2β and MCR values in hAGT TGR. Measuring REN with direct radioimmunoassay or by enzyme kinetic assay gave similar results. We next crossed homozygous hAGT TGR from both strains with homozygous hREN TGR. Heterozygous offspring from the low hAGT TGR strain had plasma REN activity, hREN concentration, and rat AGT values that were no different from those of their parents. However, TGR offspring with high hAGT values had massively elevated plasma REN activity and hREN concentration as well as elevated blood pressure, even though both the hREN and rREN genes are downregulated. We conclude that increased AGT concentrations decrease REN MCR and increase REN T1/2β. The REN-AGT complex may stabilize plasma REN concentration and regulate plasma REN activity independent of renal REN secretion and angiotensin II–mediated feedback. These effects could augment angiotensin I generation and influence blood pressure. The notion that AGT is merely a passive substrate reservoir for REN should be revised. (Hypertension. 2000;35:780-786.)

Key Words: angiotensinogen ■ blood pressure ■ rats, transgenic ■ renin

The relation between renin (REN) and its substrate, angiotensinogen (AGT), has been investigated for decades. Ménard et al1 examined the REN-AGT reaction in estrogen-treated or nephrectomized rats and found that the increase in angiotensin I (Ang I) generation was not related to volume of distribution at steady state. Infused hREN blocked with remikiren resulted in T1/2β and MCR values that were not different from control values. Infused unblocked and blocked radiolabeled hREN was distributed similarly in the hAGT TGR strains. Infused mouse REN, which cannot convert hAGT, had similar T1/2β and MCR values in hAGT TGR. Measuring REN with direct radioimmunoassay or by enzyme kinetic assay gave similar results. We next crossed homozygous hAGT TGR from both strains with homozygous hREN TGR. Heterozygous offspring from the low hAGT TGR strain had plasma REN activity, hREN concentration, and rat AGT values that were no different from those of their parents. However, TGR offspring with high hAGT values had massively elevated plasma REN activity and hREN concentration as well as elevated blood pressure, even though both the hREN and rREN genes are downregulated. We conclude that increased AGT concentrations decrease REN MCR and increase REN T1/2β. The REN-AGT complex may stabilize plasma REN concentration and regulate plasma REN activity independent of renal REN secretion and angiotensin II–mediated feedback. These effects could augment angiotensin I generation and influence blood pressure. The notion that AGT is merely a passive substrate reservoir for REN should be revised. (Hypertension. 2000;35:780-786.)

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The relation between renin (REN) and its substrate, angiotensinogen (AGT), has been investigated for decades. Ménard et al1 examined the REN-AGT reaction in estrogen-treated or nephrectomized rats and found that the increase in angiotensin I (Ang I) generation was not related to altered mobility, molecular radii, size, or isoelectric point of AGT at different pH values. Instead, the increased velocity of the reaction was due to increased amounts of AGT. Increased plasma REN activity (PRA) in women ingesting oral contraceptives and during pregnancy has also been shown to be related to increased AGT production.2-3 In sheep and humans, the normal AGT concentration is less than the Keq and is therefore a determinant of Ang I production in vivo.4 Thus, AGT could be important in circulatory homeostasis by exercising control over Ang I and angiotensin II (Ang II) production. Cantanzaro et al5 recently attributed the absence of hypertension development in a transgenic mouse strain harboring human (h) REN and hAGT genes to an appropriate downregulation in that model. They suggest that increased AGT production would increase blood pressure only under the condition that REN production is inappropriately elevated and dysregulated. However, hAGT concentrations in their mice were modest. Tigerstedt et al6 observed that bilateral nephrectomy increased the pressor response to infused REN in an animal model, which could not be attributed to renal REN catabolism alone.7 Poulsen and Jacobsen4 described the surprising observation that the catalytic cycle between REN and AGT is rather slow and in the order of seconds. Therefore, we speculated that AGT might influence REN terminal half-life (T1/2β), thereby increasing Ang I production and blood pressure.

Methods

Animals

Male rats transgenic for the human AGT gene from 2 different lines, TGR 1623 and TGR 1663 (where TGR indicates transgenic rat[s]), were used. The transgene consisted of the entire genomic hAGT gene with a 1.6-kb 5′ flanking region and a 3.5-kb 3′ flanking region.9,10 Compared with the TGR 1663 strain, the TGR 1623 strain has ∼7- to 10-fold higher circulating hAGT concentrations. Het-
erozygous animals have approximately half the hAGT concentrations of homozygous animals, demonstrating a direct gene dosage effect. TGR are normotensive because of the species specificity of rat (r) REN and hREN, which cleave only their respective homologous substrates efficiently. Nontransgenic male Sprague-Dawley rats were used as controls. Rats weighed 350 to 450 g and were maintained under standard conditions. They received a standard diet (SSNIPP Spezialitäten GmbH) containing 0.25% NaCl and had free access to tap water. All experiments were performed according to American Physiological Society guidelines with permission.

Materials

Recombinant hREN and the hREN inhibitor remikiren (Ro42-5892) were gifts from Dr W. Fischli. Hoffmann-LaRoche AG, Basel, Switzerland. Recombinant hREN was obtained in vitro from cultured transfected Chinese hamster ovary cells after extraction and purification. Recombinant hREN was glycosylated and biochemically identical to purified renal hREN. Mouse submandibular gland REN was prepared as described. Purity of the preparation was determined with a gamma counter and corrected for sample weight. All other reagents were from Sigma Chemical Co. Radioactive hREN (125I) was custom-labeled by BIOTEZ Co by using the Bolton-Hunter method.

REN Disappearance Rates

Rats were anesthetized by intraperitoneal injection of ketamine with xylazine (50 and 10 mg/kg body wt, respectively). Both external jugular veins were exposed, and 2 polyethylene catheters (PE-10) were inserted into each vein and directed toward the heart. The catheters were fixed in position, and the veins were distally ligated. All tubing was rinsed with physiological saline containing 0.1 mg/mL BSA to prevent adhesion and subsequent REN loss. The solution was also used to dilute hREN or mouse (m) REN before infusion.

To determine plasma disappearance rates, recombinant hREN (30 ng) was infused as a bolus (200 μL) into one of the venous catheters; the other catheter was used to draw blood samples (200 μL) at regular time intervals by use of separate 1-mL syringes containing 10 μL of 6.25 × 10−6 mol/L Na2-EDTA. A control blood sample was drawn before the infusion (time 0); thereafter, samples were drawn at 1, 15, 30, 60, 90, 120, 150, and 180 minutes. To compensate for volume losses associated with blood sampling, an equal volume of physiological saline was reinfused after each sample. Plasma was immediately separated from blood by centrifugation at 4°C and shock-frozen in liquid nitrogen before storage at −70°C. Aliquots were thawed only once before determination of hREN, mREN, hAGT, and rAGT concentrations. Less than 2 mL blood was lost and replaced during the experiment, representing <10% of the total blood volume.

The protocol was used to test plasma disappearance rates of hREN in homozygous and heterozygous TGR 1623, TGR 1663, and non-TGR controls. In some experiments with homozygous TGR 1623, blood sampling was also performed at 10, 20, and 40 minutes (omitting the 15-minute sample). mREN was infused (12 μg) and similarly tested in homozygous TGR 1623 and non-TGR controls. We also tested recombinant hREN incubated with remikiren (1 × 10−7 mol/L) before infusion. Experiments were performed on different days, whereas groups of TGR and non-TGR were tested on the same occasion.

In a separate protocol, we bolus-infused radioactively labeled (125I) recombinant hREN, with and without remikiren blockade, into heterozygous TGR 1623 and non-TGR controls (n = 3 per group). Rats were killed 180 minutes after the infusion, and tissue samples from liver, kidney, aorta, heart, adrenal gland, and skeletal muscle were obtained and weighed. Radioactivity in the samples was determined with a gamma counter and corrected for sample weight.

REN and AGT Concentrations

Human plasma REN concentration (hPRC) was determined by a commercial radioimmunoassay (RIA) kit (No. 79986, Sanofi Pasteur). The kit uses a pair of specific monoclonal antibodies to recognize active hREN. Remikiren and mREN do not interfere with the test. hPRC measurements were performed in duplicate. Pooled non-TGR plasma anticoagulated with 6.25 × 10−6 mol/L Na2-EDTA was used to dilute the plasma samples (1:10) before REN was assayed. The dilution ensured that the measurements were within the linear range of the assay. Linearity of hREN determinations in rat plasma was compared with hREN diluted in 2 mg/mL BSA. The 2 determinations were highly correlated (slope 1.05; r = 0.97, P < 0.001), thereby documenting the validity and reliability of the assay.

To confirm that hREN was enzymatically active, we also determined hPRA by an enzyme-kinetic assay (EKA) based on the release of Ang I from excess hAGT. In this assay, hAGT was constant and in excess. A pool of 48-hour bilaterally nephrectomized TGR plasma without any residual REN activity containing 297.5 nmol/mL hAGT and 3.6 nmol/L rAGT was used to provide constant substrate concentrations. This precaution made the determinations independent of the individual substrate concentrations in the plasma samples. In this model, hPRC and Ang II levels are correlated. Plasma hAGT and rAGT concentrations were determined by a similar EKA based on the complete cleavage of plasma AGT by either excess hREN or excess mREN to release equimolar amounts of Ang I. Ang I determination was performed by direct RIA. PRA at pH 7.4 and rREN and rAGT concentrations were also determined by EKA. Mouse plasma REN concentration was specifically determined by a direct RIA. We also controlled for confounding proteins in the serum of the differing rat strains. We subjected serum from a male heterozygous TGR 1623 and a male Sprague-Dawley control rat to conventional gel electrophoresis. Albumin fractions, as well as α, β, and γ subfractions, were compared after densitometry without notable differences.

Crossbreeding Experiments

Three homozygous male TGR 1623 and 3 homozygous male TGR 1663 were mated with 4 and 3 female hREN TGR, respectively. Human and rat PRC and AGT concentrations were determined in parents and offspring. Blood pressure was measured by the tail-cuff method during short ether anesthesia.

Statistics

The kinetics of the disappearance of hREN or mREN from plasma were analyzed by use of a pharmacological 2-compartment model. Linear regression coefficients (r values) were calculated. Disappearance curves were fitted into a 2-component exponential regression model. Half-time (T1/2) constants, distribution volume at steady state, and metabolic clearance rate (MCR) derived from the area under the concentration-time curve were determined. Mean ± SD values were calculated. Differences between groups were tested by ANOVA and by the Student t test as appropriate. StatView software and curve-fitting programs from PRISM software were used on a Macintosh personal computer.

Results

We first examined the effects of hREN bolus infusions on hPRC in the various rat models. hPRA at 1 minute after bolus infusion was 486 ± 97 pg/mL in 80 experiments, as determined by RIA. Mean hPRA did not vary significantly between experimental subgroups and strains. With 0.03 μg of human recombinant REN infused, the initial volume of distribution was calculated as 0.061 ± 0.014 L, which was equivalent to ≈0.14 mL/g body wt. We compared these data with values observed when mREN was infused (12 μg). mPRA was 374 ± 46 ng/mL in TGR 1623 and 379 ± 38 ng/mL in Sprague-Dawley controls (P = NS). The initial distribution volume was calculated as 0.032 ± 0.008 L, or ≈0.084 mL/g body wt. To compare REN disappearance kinetics without the bias of absolute plasma concentrations, the PRCs obtained
1623 had no significant further effect. Panel D shows plasma disappearance rates for mREN in heterozygous TGR 1623 and controls. No significant difference between the strains was observed. The TGR lines were L1623 and L1663.

during a single experiment were normalized for the individual starting concentration at the 1-minute time point (concentration at 1 minute equals 1.0, or 100%).

Figure 1 shows time-dependent changes in hPRC from the initial infusion up to 180 minutes thereafter in homozygous and heterozygous TGR 1623 and TGR 1663 lines and in non-TGR controls. hPRC concentrations decreased exponentially over time in all models. Figure 1A shows the hPRC disappearance over time in heterozygous TGR 1623 (with the highest hAGT concentrations), TGR 1663 (with ~10-fold lower hAGT concentrations), and non-TGR (with no hAGT). hPRC is shown on a logarithmic ordinate. The dependence of hPRC clearance on hAGT concentrations is readily apparent. In non-TGR, hPRC decreased to 4% of its initial value by 180 minutes. In TGR 1663, the value at 180 minutes was twice the control value. In TGR 1623, hPRC was still at 29% to 34% of the initial value at 180 minutes. In homozygous TGR 1623 (with hAGT twice that of heterozygous rats), hPRC was 44% of the initial value at 180 minutes, and the effect appeared to reach a plateau. Figure 1B shows the effect of infusing hREN blocked by remikiren. Compared with unblocked REN, when hREN was blocked at its active site, hPRC was significantly lower and REN disappearance from plasma was significantly increased in all circumstances. At 180 minutes, all hPRC values were 3 to 4 times lower than the respective values in the parallel experiment shown in Figure 1A. We repeated these experiments with the EKA. Figure 1C shows the results for TGR 1623 homozygous and TGR 1623 heterozygous rats as well as non-TGR controls. These results substantiated the data obtained by the direct RIA shown in Figure 1A and 1B. Because the EKA cannot function when REN is blocked, the remikiren experiments were not repeated. We next infused mREN into these rat models as an additional control, as shown in Figure 1D. mREN does not significantly bind and cleave hAGT. When mREN was infused into homozygous TGR 1623 or non-TGR controls, there was no significant difference in the mPRC disappearance curves.

Table 1 summarizes the kinetic analysis. The exponential functions represent initial distribution (α) and terminal elimination (β) and are defined by the plasma disappearance curves in each model. Initial and terminal hREN T1/2 increased significantly with increasing hAGT concentrations (P<0.01). In contrast, remikiren-blocked hREN showed a significantly shorter model-independent T1/2 (P<0.001). The MCR decreased significantly (almost 10-fold) with increasing hAGT concentrations, whereas blocked hREN had a significantly higher MCR. In Figure 2, we show the influence of remikiren-blocked hREN on the hREN MCR in the various models. In non-TGR, remikiren increased the hREN MCR almost 2-fold. A similar effect was observed in TGR 1663 and TGR 1623. The lower hREN MCR of the hAGT TGR models is readily apparent. The volume of distribution at steady state was not significantly different in the various models. In any event, the volume of distribution at steady state was not increased when REN was blocked by remikiren. Thus, differences in hREN clearance cannot be attributed to an increased volume of distribution. We also infused radioactively labeled hREN, with and without remikiren blockade, into TGR and non-TGR as an additional control and sampled plasma and organs. There was no significant difference in the pattern of radioactivity accumulation for the various organs between the models. Radioactivity was highest in the liver, followed by the kidney, aorta, adrenal glands, and various other tissue samples (data not shown).

The results of our in vivo crossing experiments are shown in Table 2. PRA was not significantly different in the homozygous hREN TGR and TGR 1623 and TGR 1663 animal models. rAGT was significantly lower in female hREN TGR compared with the hAGT TGR lines. The animals were all normotensive. Heterozygous offspring from TGR 1663 had PRA, hPRC, and rAGT values no different

Figure 1. Plasma disappearance curves for bolus-infused REN. The values were normalized for REN concentration at 1 minute (100%, or 1.0). Panel A shows disappearance curves for hREN in nontransgenic Sprague-Dawley rats (controls) and heterozygous TGR 1663 or TGR 1623. With increasing plasma hAGT concentrations, the MCR of hREN decreased. Panel B shows the same experiment for hREN blocked by remikiren before infusion. The disappearance rate was markedly increased in all models. Panel C shows the results of a control experiment in which unblocked hREN was infused into controls and heterozygous or homozygous TGR 1623; both strains had hAGT levels sufficiently high to achieve a plateau. The hPRC was measured by EKA, demonstrating that hREN was enzymatically active. In TGR, the plasma elimination of hREN was again significantly decreased, whereas doubling of the plasma hAGT concentration in homozygous TGR
TABLE 1. Plasma AGT Concentrations and Kinetic Decay Constants After Intravenous Injection of hREN and mREN Into TGR and Nontransgenic S-D Control Rats

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>RI Method</th>
<th>n</th>
<th>hAGT, ( \mu g ) Ang I ( \cdot mL^-1 )</th>
<th>rAGT, ( \mu g ) Ang I ( \cdot mL^-1 )</th>
<th>hPRC180min, ( % )</th>
<th>( \alpha ), s(^{-1}) ( \cdot 10^{-3} )</th>
<th>( \beta ), s(^{-1}) ( \cdot 10^{-3} )</th>
<th>MCR, ( \mu L \cdot min^{-1} \cdot g^{-1}BW )</th>
<th>Vss, ( \mu L \cdot g^{-1}BW )</th>
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<tr>
<td>L1623 Het</td>
<td>RI 8</td>
<td>8</td>
<td>85±32( ^* )</td>
<td>1.2±0.2</td>
<td>9.4±3.6†</td>
<td>27.4±16.1</td>
<td>8.5±3.4†</td>
<td>2.22±0.56†§</td>
<td>246±63</td>
</tr>
<tr>
<td>L1623 Het</td>
<td>RI 8</td>
<td>8</td>
<td>68±18( ^* )</td>
<td>1.1±0.1</td>
<td>29.2±5.0</td>
<td>23.1±11.1</td>
<td>5.2±2.5</td>
<td>0.83±0.29(</td>
<td></td>
</tr>
<tr>
<td>L1663 Het</td>
<td>RI 16</td>
<td>11</td>
<td>11±4</td>
<td>1.1±0.1</td>
<td>2.8±1.3†</td>
<td>56.4±19.8†</td>
<td>12.9±5.3†</td>
<td>4.72±1.56†</td>
<td>235±54</td>
</tr>
<tr>
<td>S-D</td>
<td>RI 8</td>
<td>8</td>
<td>...</td>
<td>1.0±0.1</td>
<td>4.0±0.4†</td>
<td>47.3±19.6</td>
<td>19.5±4.0†</td>
<td>5.49±0.82†</td>
<td>201±31</td>
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<tr>
<td>L1623 Hom</td>
<td>EKA 3</td>
<td>205±31*</td>
<td>1.2±0.2</td>
<td>44.3±4.1†</td>
<td>ND</td>
<td>3.9±1.2†</td>
<td>0.46±0.17†</td>
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<tr>
<td>L1623 Het</td>
<td>EKA 7</td>
<td>114±32</td>
<td>1.1±0.2</td>
<td>34.9±7.2†</td>
<td>ND</td>
<td>5.0±1.6†</td>
<td>0.82±0.20†</td>
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<tr>
<td>S-D</td>
<td>EKA 5</td>
<td>5</td>
<td>...</td>
<td>1.1±0.1</td>
<td>4.3±1.1</td>
<td>44.2±15.3</td>
<td>10.1±2.7</td>
<td>2.78±0.35</td>
<td>194±25</td>
</tr>
<tr>
<td>S-D</td>
<td>mREN RI 8</td>
<td>8</td>
<td>...</td>
<td>1.1±0.2</td>
<td>5.4±1.8</td>
<td>34.3±14.3</td>
<td>11.4±2.1</td>
<td>1.26±0.18</td>
<td>85±17</td>
</tr>
<tr>
<td>L1623 Hom</td>
<td>mREN RI 8</td>
<td>183±57</td>
<td>1.2±0.2</td>
<td>5.9±1.9</td>
<td>37.4±11.4</td>
<td>12.5±2.6</td>
<td>1.34±0.23</td>
<td>84±14</td>
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Values are mean±SD. RI indicates REN inhibitor; hPRC, hPRC 180 minutes after injection expression percentage of the value at 1 minute (100%); \( \alpha \) and \( \beta \), logarithmic decay constants for distribution (\( a \)) and elimination (\( b \)) phase; Het, heterozygous; Hom, homozygous; S-D, Sprague-Sawley; and ND, not determined, a 1-compartment model (\( b \)) fit best. Initial doses were as follows: hREN, 0.030 \( \mu g \); mREN, 12.0 \( \mu g \); mean plasma hREN or mREN concentration 1 min after bolus infusion 486±162 pg \( \cdot mL^{-1} \) and 376±97 ng \( \cdot mL^{-1} \), respectively.

\( ^* \)P<0.01 vs L1663; †P<0.05 vs no Ri (-); ‡P<0.03 vs S-D; with Ri; and ||P vs L1663 or S-D without Ri.

AGT has previously been solely considered as a substrate reservoir for REN around its \( K_m \) value.\(^7,14\) This situation would predict an increase in PRA with increasing AGT concentrations. Although AGT levels may physiologically vary by an order of magnitude, the demonstration of any significant AGT-related influences on PRA in vivo has been difficult. The subsequent increase in Ang II production would immediately decrease REN secretion from the kidneys. In experimental models with sodium depletion and maximal stimulation of REN secretion, AGT can support PRA and blood pressure.\(^19\) Furthermore, in some clinical situations (eg, hepatic cirrhosis), low plasma AGT concentrations may correlate with low PRA.\(^20\) A malignant tumor with excess production of AGT has been described in association with high blood pressure.\(^21\) Elevated plasma AGT concentrations can also be found during pregnancy or after pharmacological stimulation with estrogens or glucocorticoids.\(^3,22\) Any independent effect of AGT on PRA and blood pressure in these situations is nevertheless obscured by parallel changes occurring in other homeostatic systems.

The most likely explanation for the prolongation of hREN clearance in TGR is significant hREN binding by AGT, preventing hREN degradation. Compared with unbound hREN, relatively stable hREN-hAGT complexes are less likely to leave the circulation or to be taken up by cells. In vitro experiments indicate that hREN can bind rAGT with a \( K_m \) value even lower than that for hAGT.\(^23,24\) However, rAGT cleavage by hREN is negligible. hAGT cleavage by hREN is also a slow process that takes >1 to 2 seconds per cycle.\(^8\) This low turnover characterizes one of the slowest enzymatic reactions. In comparison, other enzymes, such as carbonic anhydrase, requires 1/1 000 000 less time to complete a cleavage cycle.\(^25\) Thus, rAGT acts as an hREN inhibitor rather than a substrate.\(^24\) In terms of Michaelis-Menten kinetics, about half of hREN would exist as a complex with its substrate at any given time at the accepted \( K_m \) values.

Figure 2. MCRs for heterozygous (het) TGR 1663, TGR 1623, and non-TGR (control) are shown. MCR of hREN significantly decreased with increasing hAGT concentrations by a factor of 3 to 4. When hREN was blocked by remikiren (RI), MCR increased in all tested models by a factor of 2 to 3. The TGR lines were L1663 and L1623.

Discussion

We showed that the kinetics of hREN are a function of plasma hAGT concentrations. The higher the hAGT concentrations are, the longer the hREN terminal T1/2, the lower the MCR, and the greater the propensity to increase blood pressure through the generation of Ang II. These results may explain the putative increase in blood pressure observed under the condition of increased AGT production in persons with the T variant of the M235T AGT substitution.\(^16\) These data aid in the understanding of the increased PRA found in their homozygous parents.

from those of their parents. Offspring from TGR 1623, however, had PRA and hPRC concentrations that were massively elevated, and some animals also had elevated rAGT concentrations. The blood pressures were elevated in the hAGT 1623×hREN 10J cross. Plasma hAGT concentrations in both offspring lines were less than half the concentrations in their homozygous parents.
Long-lived complexes may show a dissociation into distinct forms of complexed and uncomplexed enzyme with biologically distinct behavior. This situation is typical for proteinase inhibitors that stably bind to activated enzymes while the complex is rapidly cleared from the circulation.26 Our findings suggest that uncomplexed REN is cleared from plasma more rapidly than is the larger REN-AGT complex. AGT has an extremely slow elimination rate from plasma with a T1/2 of up to 10 hours,27.28 and complexed AGT may follow these kinetics unless released from AGT after its cleavage. In vitro Michaelis-Menten kinetics predict an increased complex formation for REN with increasing AGT concentrations. We demonstrated such a quantitative effect of increasing hAGT concentrations on increasing REN T1/2; however, we do not believe that this difference can explain a 5-fold difference in metabolic REN clearance. hAGT concentrations in homozygous TGR 1623 may have protected the enzyme against degradation. The formation, such as in homozygous TGR 1623, may have formed complexes with hREN, the presence of increasing AGT concentrations on increasing REN T1/2 and decreasing REN clearance. hAGT concentrations in heterozygous TGR was 100 times higher in TGR than in control rats; however, we do not believe that this finding indicates that TGR and Sprague-Dawley rats do not differ in their capacity to eliminate foreign proteins from the circulation. Finally, we found no differences in the organ degradation between mREN and hAGT at 24.7 μmol/L without any significant cleavage.30 The Km value is 3 times lower for mREN cleaving rAGT than for homologous rREN, whereas mREN cleaves this substrate 60 times more efficiently than does rREN.8 We infused a 400-fold higher dose of mREN, compared with hREN, into TGR and non-TGR without any significant difference in plasma mREN kinetics in the models. This finding also supports our hypothesis.

We cannot exclude different cellular hREN elimination in TGR compared with non-TGR or humans. However, several arguments speak against such a possibility. Aside from blood pressure and slight renal function differences, TGR and non-TGR are similar.10 Serum protein electrophoresis showed no significant plasma protein differences in the strains. REN is mainly cleared by liver cells (60%), followed by the kidney (20%) and other organs.31 Glomerular filtration rate and renal blood flow are 25% lower in double TGR than in control rats; however, we do not believe that this difference can explain a 5-fold difference in metabolic REN clearance. There is no known scavenger receptor specific for REN degradation. Instead, it is cleared by nonspecific receptors recognizing glycosylated protein moieties or by general protein metabolism.32,33,34 However, the serpin-enzyme complex receptor could be involved in the clearance of complexed REN.35 Sessler et al36 have demonstrated different plasma disappearance kinetics of REN with different degrees of glycosylation in rats. However, they did not investigate the influence of different AGT concentrations on this effect. Ex vivo studies with rat livers placed in an organ bath perfusion system showed a high first-pass clearance effect on hREN, amounting to 19%.37 This finding may explain why even very high amounts of mREN (12 μg) infused into TGR or non-TGR, compared with lower amounts of hREN (30 ng) infused into Sprague-Dawley rats, were cleared without any significant decrease in clearance rates. Our results on REN metabolism in non-TGR were in accord with those reported in the literature.38–40 Furthermore, this finding indicates that TGR and Sprague-Dawley rats do not differ in their capacity to eliminate foreign proteins from the circulation. Finally, we found no differences in the enzyme complex receptor could be involved in the clearance of complexed REN.35 Sessler et al36 have demonstrated different plasma disappearance kinetics of REN with different degrees of glycosylation in rats. However, they did not investigate the influence of different AGT concentrations on this effect. Ex vivo studies with rat livers placed in an organ bath perfusion system showed a high first-pass clearance effect on hREN, amounting to 19%.37 This finding may explain why even very high amounts of mREN (12 μg) infused into TGR or non-TGR, compared with lower amounts of hREN (30 ng) infused into Sprague-Dawley rats, were cleared without any significant decrease in clearance rates. Our results on REN metabolism in non-TGR were in accord with those reported in the literature.38–40 Furthermore, this finding indicates that TGR and Sprague-Dawley rats do not differ in their capacity to eliminate foreign proteins from the circulation. Finally, we found no differences in the organ pattern for hREN degradation between TGR and non-TGR when 125I-labeled blocked and unblocked hREN was used.

We tested whether increasing human AGT concentrations can increase hPRC in an in vivo model by crossbreeding rats and humans.22 Because rat and human AGT can 

<table>
<thead>
<tr>
<th>TGR Line</th>
<th>Gender</th>
<th>Type</th>
<th>n</th>
<th>PRA, ng Ang l·mL⁻¹·h⁻¹</th>
<th>hPRC, ng Ang l·mL⁻¹·h⁻¹</th>
<th>rAGT, ng Ang l·mL⁻¹·h⁻¹</th>
<th>hAGT, ng Ang l·mL⁻¹·h⁻¹</th>
<th>BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>hREN 10J</td>
<td>F</td>
<td>Hom</td>
<td>7</td>
<td>1.8±1.9</td>
<td>17.7±8.1</td>
<td>0.61±0.06†</td>
<td>...</td>
<td>110±8</td>
</tr>
<tr>
<td>hAGT 1663</td>
<td>M</td>
<td>Hom</td>
<td>3</td>
<td>3.7±3.6</td>
<td>...</td>
<td>1.13±0.28</td>
<td>17.5±1.3</td>
<td>116±5</td>
</tr>
<tr>
<td>hAGT 1623</td>
<td>M</td>
<td>Hom</td>
<td>3</td>
<td>2.6±0.8</td>
<td>...</td>
<td>1.22±0.21</td>
<td>272±43</td>
<td>114±6</td>
</tr>
<tr>
<td>hREN 10J×hAGT 1663</td>
<td>M+F</td>
<td>Het</td>
<td>23</td>
<td>3.0±1.8</td>
<td>14.4±9.4</td>
<td>0.92±0.26</td>
<td>5.5±3.2</td>
<td>119±15</td>
</tr>
<tr>
<td>hREN 10J×hAGT 1623</td>
<td>M+F</td>
<td>Het</td>
<td>18</td>
<td>228±187*</td>
<td>536±498*</td>
<td>1.62±1.23</td>
<td>77±34*</td>
<td>186±26*</td>
</tr>
</tbody>
</table>

(34–596) (60–1727) (0.5–4.1) (44–113)
homozygous for the hREN transgene with homozygous TGR 1663 and TGR 1623. PRA, hPRC, and hREN were not suppressed in heterozygous double TGR 1663 offspring. In contrast, PRA, hPRC, and hREN were massively elevated in heterozygous high hAGT TGR 1623 offspring, in line with our prediction. Earlier, we demonstrated Ang II–sensitive regulation of hPRC in hREN TGR and downregulation of hREN and rREN gene expression in the kidneys of double TGR.13,32 This downregulation in REN gene expression was similar to that reported by Catanzaro et al. In hREN TGR x hAGT TGR 1663, hPRC was higher than expected for heterozygous hREN TGR because hREN binding apparently compensated for the gene-dose effect and the downregulation of the REN genes by increasing Ang II production. PRA was not generally elevated, and the animals remained normotensive. In contrast, the effect of higher hAGT levels in hREN TGR x hAGT TGR 1663 on hREN clearance probably caused significant hREN accumulation in the circulation, with elevated PRA and hypertension. In transgenic mice heterozygous for the hREN and hAGT transgenes, the plasma hAGT levels were <10 μg Ang I/mL.4,1,42 hPRC was lower than in heterozygous monospecific hREN control mice, but PRA was unchanged. Possibly, the human AGT concentrations were not sufficiently high to increase hPRC, as was the case for our hREN TGR x hAGT TGR 1663 strain of rats.

Our data suggest that hREN-hAGT binding directly stabilizes hPRC at its physiological level for a given REN secretion rate also in humans. The plasma levels would be lower without REN binding by AGT and prevention of REN degradation. Higher AGT concentrations directly prolong REN survival in the circulation with a higher AGT to Ang I conversion rate per molecule until degradation occurs, thereby supporting blood pressure. This effect would create a more sensitive REN secretory phenotype, inasmuch as a lower renal REN secretion rate, or a smaller change of the REN secretion rate, would be sufficient to maintain the same PRA. AGT may similarly regulate local tissue REN metabolism or influence the exchange of REN between fluid or tissue compartments across concentration gradients. The mechanism may also contribute to the prolonged pressure effect of REN observed after bilateral nephrectomy when REN is degraded much more slowly, in parallel with increasing AGT concentrations.6,7,43 We conclude that AGT represents more than a simple substrate reservoir for REN but is an active participant in the normal PRA and REN concentration regulation, independent of renal REN secretion or Ang II–mediated feedback. Our findings have implications for the regulation of the REN-angiotensin system that have not been previously appreciated. Finally, these observations not only are applicable to circulating REN but also have relevance for the activity of REN at the tissue level.

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