Rats Transgenic for Human Renin and Human Angiotensinogen as a Model for Gestational Hypertension

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Abstract. Animal models of gestational hypertension are problematic. A novel mouse model was described earlier. The dams in that study were transgenic for human angiotensinogen and the sires for human renin; human renin was expressed in and produced by the placenta. This model was adapted to the rat, which has greater utility in terms of chronic instrumentation and physiologic measurements. Female rats transgenic for human angiotensinogen were mated with rats transgenic for human renin. Telemetry BP increased on day 5 of pregnancy from 110/80 mmHg to as high as 180/140 mmHg, while heart rate increased slightly. The renin transgene was expressed in the placenta, which resulted in increased human plasma renin concentration from 0 to 937 ± 800 ng angiotensin I ml/h; the values returned to 0 after delivery. Female rats transgenic for human renin that were mated with male rats transgenic for human angiotensinogen in contrast exhibited a decrease in BP. In these rats, human angiotensinogen in plasma remained undetectable. Double transgenic offspring of these transgenic rats developed hypertension and end-organ damage, regardless of the source of the transgenes. The conclusion is that transgenic rats that bear human renin and angiotensinogen genes make an attractive model for gestational hypertension. The rat model will have greater utility than the mouse model.

Chesley (1) followed families with preeclamptic women for more than three generations. He and his associates observed that daughters-in-law of preeclamptic women had a preeclampsia incidence of 6%, whereas 25% of sisters and 25% of daughters developed the condition. Segregation analysis favored a recessive gene hypothesis with a possible role for a fetal genotype. The renin-angiotensin system is expressed in normal placenta and has been implicated in preeclampsia (2). Genetic association studies have implicated the angiotensinogen gene M235T variant in preeclampsia (3,4). Further evidence comes from a report of a rare variant in a nulliparous 18-yr-old woman who developed severe preeclampsia early in the third trimester (5). The mutation consisted of a phenylalanine for leucine substitution at residue 10 of angiotensinogen (L10F). Kinetic studies with synthetic peptides spanning the renin-binding site showed that this substitution leads to a 10-fold decrease in the Michaelis-Menten constant (Km) of the renin reaction and a fivefold decrease in the catalytic constant (kcat). Thus, the catalytic efficiency (kcat/Km) was increased twofold. Takimoto et al. (6) described hypertension induced in pregnant mice by placental renin and maternal angiotensinogen. Mice were generated transgenic for the human renin (hREN) and human angiotensinogen (hAGT) genes. The homozygous model for the rat.

Materials and Methods

Sprague-Dawley rats harboring the complete genomic human AGT gene (TGR(hAGT)L1623), Sprague-Dawley rats bearing the entire genomic human REN gene [TGR(hREN)L10J], and nontransgenic Sprague-Dawley rats, weighing 230 to 350 g, were used for the experiments (7–10). Briefly, TGR(hAGT) show high hAGT gene expression in the liver, kidney, heart, aorta, brain, and adrenal glands. Their plasma hAGT concentrations exceed endogenous rAGT concentrations by 50- to 100-fold. The hREN gene is expressed predominantly in the kidneys. Active hREN is secreted into the circulation. The TGR were homozygous for their respective transgene and were normotensive because rat renin and angiotensinogen do not interact with the human components to produce Ang I. All rats were kept under standard conditions at 24 ± 2°C and were fed a commercial rat chow (No. C-1000, Altromin, Lage, Germany) containing 0.2% sodium by weight with free access to tap water. Mean BP, heart rate, and ambulatory activity were continuously recorded with a radiotelemetric system (Data Sciences International, La Jolla, CA) implanted into the infrarenal aorta and harbored in the abdominal cavity. Data were
averaged over periods of 60 min and then stored on a personal computer using the manufacturer’s DataQuest IV software. Six female hAGT TGR were mated with a hREN TGR male, after telemetry had been installed. Similarly, six female hREN TGR were mated with an hAGT male TGR. Sprague-Dawley females were mated with hAGT and hREN males as additional controls. Blood was obtained from the eye before pregnancy, late in gestation (approximately 8 d before giving birth), and 1 to 2 d after giving birth. Placenta and neonatal liver were obtained at sacrifice. Our techniques for measuring plasma renin activity (PRA), human and rat plasma renin concentrations (hPRC, rPRC), and human and rat AGT concentrations (hAGT, rAGT) are outlined in detail elsewhere (7). Briefly, we relied on enzymatic kinetic assays and a direct RIA for Ang I. The enzymatic assays are capable of distinguishing between the rat and the human renin-angiotensin systems. We used the human renin inhibitor remikiren, which blocks human but not rat renin. This approach allowed us to separate the rat and human systems by conducting assays with and without remikiren. Furthermore, concentrations for rat and human angiotensinogen were determined by using an excess of mouse submaxillary gland renin or human recombinant renin to produce equimolar amounts of Ang I after complete cleavage. Mouse submaxillary gland renin, in contrast to human renin, splits rat angiotensinogen very well. However, the mouse renin does not interact with the human angiotensinogen substrate. Our techniques for conducting RNase protection assays have been described (7,8). Values were calculated as mean ± SD. Statistical analysis was performed by ANOVA and t tests as appropriate with a significance level of P < 0.05.

Results

Figure 1 shows the mean BP values of 6 hAGT TGR dams mated with a male hREN TGR. The mean BP values were variable during day and night and therefore display a circadian variation. The same is true for heart rate. The rats developed hypertension abruptly 10 ± 1 d before delivery and had sustained hypertension (approximately 160 ± 10 mmHg, P < 0.01) until shortly before delivery, when BP decreased slightly. Within 3 d, the BP had returned to below normal. Heart rate increased (approximately 50 bpm, P < 0.05) after the BP increase by approximately 1 d, until delivery. Figure 1B shows the telemetry values from a single representative animal. Mean BP increased by approximately 60 mmHg. Six hREN TGR dams that were mated with a male hAGT TGR, conversely, showed no increase in either mean BP or heart rate. Instead, BP tended to decrease during pregnancy. Sprague-Dawley dams that were mated with either hREN or hAGT TGR showed BP and heart rate responses no different from hREN TGR dams that were mated with hAGT males (not shown).

Table 1 shows PRA, hPRC, rPRC, hAGT, and rAGT in maternal plasma. hAGT dams had no detectable hPRC before mating. However, at late gestation, their hPRC values had increased to 937 ± 800 ng Ang I ml/h. After gestation, the hPRC values again were not detectable. Their hAGT values were 133 ± 77 µg Ang I/ml before pregnancy, decreased to 43 ± 29 µg Ang I/ml at late gestation, and increased to 85.6 ± 19.9 µg Ang I/ml after delivery. hREN dams had hPRC values of 14.8 ± 6.1 Ang I/ml before conception, which increased to 552 ± 266 ng Ang I/ml at late gestation and decreased to 5.9 ± 2.6 ng Ang I/ml after delivery. However, their hAGT values remained undetectable before, throughout, and after pregnancy. Sprague-Dawley dams that were mated with hREN males had high detectable hPRC values (1640 ± 713 ng Ang I/ml/h) at late gestation with normal PRA values, whereas hPRC was negative before and after pregnancy. Sprague-Dawley dams that were mated with hAGT males had normal PRA values and undetectable plasma hAGT concentrations throughout pregnancy.

Figure 2 shows RNase protection assays from placenta and neonatal liver for hAGT (A) and hREN (B). hAGT mRNA expression was detected in placenta from hAGT TGR dams and from hREN TGR and Sprague-Dawley dams that were mated with hAGT TGR males. No hAGT was detected in Sprague-Dawley dams that were mated with hREN TGR males. hREN expression was present in placentas from hREN TGR dams that were mated with hAGT TGR males, from hAGT TGR dams that were mated with hREN TGR males, and from Sprague-Dawley dams that were mated with hREN TGR males. Double (d) TGR offspring from either hAGT TGR dams that were mated with hREN TGR males or hREN TGR dams that were mated with hAGT TGR males developed hypertension detected at approximately 30 d of age, which increased by day 50 (8).

Heart weights of hAGT dams that were crossed with hREN males were 0.294 ± 0.01 g/100 g body wt, compared with 0.235 ± 0.02 g/100 g body wt (P < 0.05) for hREN dams that were crossed with hAGT males. Pregnant Sprague-Dawley control dams had relative heart weights of 0.255 ± 0.13 g/100 g body wt, which was also less than the hypertensive cross (P < 0.05). The body weights of the groups were not significantly different. Furthermore, the mean number of offspring per dam was four per pregnancy in the hypertensive, compared with eight per pregnancy in the nonhypertensive crosses (P < 0.05).

Discussion

We showed that hAGT TGR dams developed marked hypertension in the last two trimesters of pregnancy along with an increase in heart rate when the animals were mated with hREN TGR males. In contrast, hREN TGR dams showed no change in BP or heart rate during pregnancy when mated with hAGT TGR males. hAGT TGR dams also showed during pregnancy a marked increase in hPRC, which disappeared after delivery. In contrast, hREN TGR dams showed no detectable hAGT during pregnancy, despite having been mated with hAGT males. Although both transgenes were expressed in placenta during pregnancy, only hREN was produced in sufficient quantities to be detectable in maternal plasma and to increase BP. The experiments show that placental tissue, of either maternal or fetal origin, is capable of expressing, producing, and secreting active human renin. These findings are in accord with the observations by Takimoto et al. (6) in transgenic mice. Their hAGT TG mice dams also showed hREN expression in the placenta when mated with hREN TG mice males. Furthermore, hREN concentration increased in the placental tissue accordingly.

Takimoto et al. (6) observed that their mice developed
proteinuria and histologic changes in the kidneys and heart when becoming hypertensive. The glomeruli were enlarged and the hearts were hypertrophied. The authors also reported convulsions in 15% of their hypertensive pregnant mice. We have not yet performed detailed histology of our hypertensive pregnant rats; however, we have considerable histologic information on the offspring. dTGR die of cardiac hypertrophy and vasculopathy at 7 to 8 wk of age. They exhibit increased oxidative stress at the level of the vessel wall and express surface adhesion molecules, monocyte chemoattractant protein-1, and tissue factor. The small vessels in the heart and kidney show fibrinoid necrosis, round cell infiltrates, and mi-
Values are mean ± SD.

Table 1. The maternal plasma renin-angiotensin system throughout pregnancy

<table>
<thead>
<tr>
<th>Stage</th>
<th>Dam</th>
<th>Sire</th>
<th>PRA (ng Ang I/ml⁻¹h⁻¹)</th>
<th>hPRC (ng Ang I/ml⁻¹h⁻¹)</th>
<th>rPRC (ng Ang I/ml⁻¹h⁻¹)</th>
<th>hAGT (µg Ang I/ml⁻³)</th>
<th>rAGT (µg Ang I/ml⁻³)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepregnancy</td>
<td>hAGT</td>
<td>hREN</td>
<td>0.66 ± 0.35</td>
<td>0</td>
<td>5.0 ± 4.6</td>
<td>133 ± 77</td>
<td>0.63 ± 0.12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>hREN</td>
<td>hAGT</td>
<td>1.73 ± 1.07</td>
<td>14.8 ± 6.1</td>
<td>13.5 ± 6.3</td>
<td>0</td>
<td>0.47 ± 0.09</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>S-D</td>
<td>hREN</td>
<td>1.17 ± 0.80</td>
<td>0</td>
<td>nd</td>
<td>0</td>
<td>0.62 ± 0.06</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>S-D</td>
<td>hAGT</td>
<td>1.90 ± 1.32</td>
<td>0</td>
<td>nd</td>
<td>0</td>
<td>0.68 ± 0.04</td>
<td>3</td>
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<tr>
<td></td>
<td>–</td>
<td>hREN</td>
<td>2.29 ± 1.95</td>
<td>4.5 ± 3.6</td>
<td>193 ± 89</td>
<td>1.15 ± 0.31</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>hAGT</td>
<td>2.34 ± 1.25</td>
<td>29.5 ± 10.8</td>
<td>5.1 ± 2.8</td>
<td>0</td>
<td>0.76 ± 0.17</td>
<td>6</td>
</tr>
<tr>
<td>Late gestation (~8 d before delivery)</td>
<td>hAGT</td>
<td>hREN</td>
<td>349 ± 280</td>
<td>937 ± 800</td>
<td>&lt;S</td>
<td>43 ± 29</td>
<td>1.3 ± 0.68</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>hREN</td>
<td>hAGT</td>
<td>5.4 ± 2.8</td>
<td>552 ± 266</td>
<td>&lt;S</td>
<td>0</td>
<td>0.51 ± 0.07</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>S-D</td>
<td>hAGT</td>
<td>9.3 ± 2.0</td>
<td>1640 ± 713</td>
<td>&lt;S</td>
<td>0</td>
<td>0.68 ± 0.03</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>S-D</td>
<td>hREN</td>
<td>13.2 ± 4.0</td>
<td>0</td>
<td>nd</td>
<td>0</td>
<td>0.91 ± 0.39</td>
<td>3</td>
</tr>
<tr>
<td>1 to 2 d after delivery</td>
<td>hAGT</td>
<td>hREN</td>
<td>3.6 ± 1.6</td>
<td>16.1 ± 5.6</td>
<td>85.6 ± 19.9</td>
<td>0.47 ± 0.09</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hREN</td>
<td>hAGT</td>
<td>4.5 ± 4.8</td>
<td>5.9 ± 2.6</td>
<td>14.5 ± 6.6</td>
<td>0</td>
<td>0.28 ± 0.04</td>
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<tr>
<td></td>
<td>S-D</td>
<td>hREN</td>
<td>4.1 ± 4.2</td>
<td>0</td>
<td>nd</td>
<td>0</td>
<td>0.43 ± 0.06</td>
<td>3</td>
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<tr>
<td></td>
<td>S-D</td>
<td>hAGT</td>
<td>0.9 ± 0.6</td>
<td>0</td>
<td>nd</td>
<td>0</td>
<td>0.39 ± 0.06</td>
<td>3</td>
</tr>
</tbody>
</table>

a The values are shown according to maternal and paternal transgene carrierness. The characteristics of the male TGR breeders are shown. All TGR were homozygous for their respective transgene. hAGT, human angiotensinogen TGR; hREN, human renin TGR; S-D, nontransgenic Sprague-Dawley rats; PRA, plasma renin activity; hPRC and rPRC, human and rat plasma renin concentration; hAGT and rAGT, human and rat plasma angiotensinogen concentration; nd, not determined; <S, below assay sensitivity (~1 ng Ang I/ml⁻¹h⁻¹).

P < 0.05 compared with the same previous value.

P < 0.01 compared with the same previous value.

P < 0.05 compared with the other experimental groups.

P < 0.01 compared with the other experimental groups.

crothrombosis. The transcription factors NFκB and AP-1 are activated and the endothelin system is recruited (8,11). All of these features have been described in preeclampsia (12). The PRA, hPRC, and hAGT values of hAGT TGR dams that were mated with hREN males are in the range at which we would expect vasculopathy, in addition to BP increases (11).

The uteroplacental unit may be important to the development and maintenance of preeclampsia. Evidence has been provided by studies showing defective cytotrophoblast invasion associated with a failure to express surface adhesion molecules associated with a vascular endothelial phenotype (13). A clinical report of a patient with an extraterine placenta and preeclampsia provides support for the notion that the condition is maintained by the placenta (14). The patient delivered her intra-abdominal pregnancy; however, the placenta could not be removed immediately. Hypertension and proteinuria persisted in this patient until the placenta was removed at a second operation. In our model, the placenta with ample hREN production is responsible for hypertension in the pregnant hAGT TGR. This state of affairs is analogous to the patient with the L10F mutation in the angiotensinogen gene (3). The altered kinetics engendered by the mutation results in an increased angiotensinogen conversion to Ang I and subsequently to Ang II by the Ang I converting enzyme. Renin acts relatively slowly in a matter of seconds to convert AGT to Ang I. We showed recently by adapting the dTGR model that renin half-life is a function of AGT concentrations (15). hAGT TGR produce AGT to excess. The subsequent hREN production by the placenta likely reacted locally and in the circulation with a longer hREN half-life than would be expected had the AGT concentrations been lower. We believe that the decrease in hAGT observed in late pregnancy in our model may be related to consumption of the substrate in the face of the massively high hREN concentrations. In an earlier report on human renin effects in hAGT TGR, we observed a similar effect that we were able to demonstrate quantitatively (16).

In our model, hREN production in the placenta was predominantly responsible for hypertension in the dams. The number of offspring dictates the number of placentas and thereby quantitatively influences hREN production. An additional component from the kidneys of hREN-positive fetuses may also play a role. We observed that rREN was not expressed in the placenta. However, our RNase protection assay was not as sensitive as other methods we might have applied, such as reverse transcription PCR. Nevertheless, species differ considerably in terms of renin production in the placenta. Rat, swine, cattle, and cats express relatively little or no detectable renin in the placenta, whereas humans and rabbits produce placental renin in generous amounts (17).

We believe that our model will have utility in the study of hypertension in pregnancy. Other animal models are available; however, each has certain limitations (18). Administration of NG-nitro-L-arginine methyl ester to pregnant rats results in hypertension (19); however, a defect in nitric oxide production (20).

Furthermore, prostaglandins maintain renal vasodilation and hyperfiltration during chronic nitric oxide synthase blockade in conscious pregnant rats (21). A single dose of adriamycin has
offspring outcome, an important issue in human preeclampsia (24). By the use of BP telemetry, continuous renal blood flow measurements, and on-line cardiac output determinations, techniques that all can be done far more readily in rats than in mice, we should be able to define better the hemodynamics of pregnancy as well as alterations that occur in gestational hypertension.

**Acknowledgments**

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**References**


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**Figure 2.** RNase protection assay with hAGT and rAGT expression (A) and hREN and rREN expression (B) in placenta and neonatal liver. One μg RNA per sample was tested. hAGT was expressed in placenta in all groups except for female Sprague-Dawley rats that were mated with hREN TGR. rAGT was present in all placentas with a weak expression level in hAGT transgenic females (A, lane B). hREN was expressed in the placenta in hREN TGR females that were mated with an hAGT TGR male, in hAGT females that were mated with an hREN TGR male, and in Sprague-Dawley female rats that were mated with an hREN TGR. rREN was not detectable. Neonatal liver (offspring) showed appropriate rAGT and hAGT expression but no renin expression. rAGT and hAGT, rat and human angiotensinogen; hREN and rREN, human and rat renin; GAPDH, glutaraldehyde-phosphate dehydrogenase; NC, negative control; P, assay probes.


