Appropriate Regulation of Human Renin Gene Expression and Secretion in 45-kb Human Renin Transgenic Mice

Yan Yan, Lufei Hu, Rong Chen, Jean E. Sealey, John H. Laragh, Daniel F. Catanzaro

Abstract—To create physiological models of the human renin-angiotensin system in transgenic animals, the component genes should be expressed in the correct tissues and cells and respond appropriately to physiological stimuli. We recently showed that mice carrying a 45-kb human renin genomic fragment, containing approximately 25 kb 5'–flanking DNA and 6 kb 3'–flanking DNA, express the transgene in a highly cell- and tissue-specific pattern. More importantly, in contrast to previous models, human renin in the circulating plasma of these mice is derived exclusively from the kidneys. In the present study, we tested the responses of both human and mouse renal renin expression and secretion of the 45-kb hREN transgenic mice to a variety of physiological and pharmacological stimuli. A sodium-deficient diet, angiotensin-converting enzyme inhibition, and β, adrenergic stimulation each increased both human and mouse plasma renin concentration significantly, whereas elevated blood pressure and/or increased plasma angiotensin II levels suppressed them. Human and mouse renal renin mRNA levels changed similarly but to a lesser degree. These studies demonstrate that human renin synthesis and secretion respond appropriately in 45-kb hREN mice to physiological stimuli. This most likely results from appropriate cell-specific expression of the transgene conferred by the extended transgene flanking sequences. (Hypertension. 1998;32:205-214.)

Key Words: renin ■ kidney ■ mice, transgenic ■ regulation ■ gene expression ■ secretion

Renin in the circulation is derived only from the kidneys and disappears after bilateral nephrectomy.1-4 Because the cleavage of AGT by renin is the rate-limiting step that determines the activity of the system, the mechanisms that control renin secretion by the kidneys are important determinants of blood pressure. Human and animal studies and in vitro studies with kidney cell and tissue cultures have led to a fundamental understanding of the interrelationships between systemic, intrarenal, and cellular mechanisms that regulate renin synthesis and secretion, and the relationship of these processes to blood pressure. However, the dependency of renin-secreting juxtaglomerular cells on the intrarenal environment to maintain their prorenin-expressing phenotype and their ability to process and secrete renin have impeded further investigation of the cellular mechanisms involved in renin/prorenin regulation.

In the kidney, renin is synthesized only in the juxtaglomerular cells. These specialized epithelioid cells of the glomerular arterioles are transformed vascular smooth muscle cells. They represent <0.1% of cells in the kidney and are probably the only site of (pro)renin gene expression where the expressed prorenin is processed to renin. Prorenin processing occurs in specialized granules and is accompanied by changes in granule morphology that are unique to juxtaglomerular cells. Therefore, processing and secretion may require coding sequences that direct prorenin to the appropriate secretory pathway. When placed in culture, juxtaglomerular cells rapidly lose their ability to synthesize prorenin, to process prorenin to renin, and to secrete renin, suggesting that intrarenal factors may be important in maintaining their differentiated state. Moreover, the anatomic localization of juxtaglomerular cells in the kidney is critical in determining their ability to respond to changes in blood pressure, in the sodium chloride load sensed by the adjacent macula densa cells of the renal tubules, and changes in β-adrenergic stimulation. Taken together, these observations suggest that with our current state of knowledge, the physiological mechanisms of renin secretion can only be studied in the context of the intact kidney and require both regulatory and coding renin gene sequences.

Transgenic mice carrying human renin gene sequences provide a means to study human renin regulatory mechanisms in a native cellular environment. Insertion of human renin genomic fragments into mice also provides the opportunity to study the distribution of human renin gene expression in an animal model that permits the implementation of a greater variety of experimental protocols and the accessibility of tissues to determine protein and mRNA levels. The species specificity of the reaction between renin and its substrate AGT permits the plasma and tissue levels of mouse and human renin to be independently determined. Moreover, the species specificity of renin for...
its substrate should also prevent any effect of human renin unless human AGT is made available, either by infusion or by the production of doubly transgenic mice expressing both human renin and human AGT.\textsuperscript{5,6}

In previously established human renin transgenic models that used human renin genomic sequences with relatively short 5′-flanking DNA sequences (1 to 3 kb), human renin mRNA is expressed at a variety of extrarenal sites.\textsuperscript{7,8} Moreover, in 1 of these transgenic lines, human renin is secreted into the plasma from extrarenal sites, suggesting inappropriate processing and secretion.\textsuperscript{6} These mice also displayed a mixture of both appropriate and inappropriate responses of plasma renin and renal renin mRNA to physiological and pharmacological stimuli. When mated to human AGT–expressing mice, plasma levels of human renin were inappropriate high and the mice became hypertensive.\textsuperscript{5,6} Taken together, these observations suggest that the human renin genomic sequences used to generate these earlier models may lack some of the sequences required for appropriate cell- and tissue-specific expression.

Recently, we developed new transgenic mouse lines that contain 45-kb human renin genomic sequences\textsuperscript{4} with much longer 5′-flanking DNA sequences (≈20 kb) than previous models.\textsuperscript{7,8} In this model, human renin mRNA was expressed at fewer extrarenal sites, and more importantly, both mouse and human circulating renins were of renal origin exclusively.

In the present study, we investigated whether human renin secretion and human renal mRNA levels respond appropriately to a variety of physiological and pharmacological manipulations and whether these newly developed 45-kb hREN transgenic mice provide a suitable model for the study of human renin gene expression and secretion.

Methods

Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of Cornell University Medical College.

Transgenic mice were prepared using a 45-kb NotI-Sall fragment from a P1 human renin genomic clone.\textsuperscript{9} This fragment contains about 20 kb 5′- and 6 kb 3′-flanking DNA sequences plus all the coding and intervening sequences. The 45-kb hREN #2 line, which contains 4 to 5 copies of the transgene, was used for the present study. Founder mice were from C57BL/6J×CBA embryos and were backcrossed to C57BL/6J partners through 3 to 4 generations. Positive transgenic mice were identified by Southern blotting of genomic DNA purified from tail biopsy samples using a full-length hREN cDNA probe. All matings were to nontransgenic partners and generated the expected ratios of transgenic and nontransgenic offspring.

Spring. Mice were kept under standard conditions and fed a commercial mouse chow (No. 5008, Formulab) containing 0.28% sodium. They had free access to tap water unless otherwise indicated by the experimental protocol.

Blood samples (≈200 µL) were obtained by orbit sinus puncture of animals, which were lightly anesthetized with metapan and then perfused with saline without anesthesia, either after decapitation or by cutting the tail tip, showed that plasma renin levels were unaffected by anesthesia or by handling of the blood or plasma (not shown). Plasma was stored at −20°C. Mice were killed by spinal cord dislocation; tissues were removed quickly, frozen in liquid nitrogen, and stored at −70°C.

Experimental Protocols

All experiments used 6- to 8-week-old transgenic mice and nontransgenic littermates. Animals were subjected to one of the following experimental treatments: (1) Medium salt diet: normal mouse chow and free access to tap water for 16 days. (2) Low salt diet: sodium-deficient chow (0.014% sodium, Harlan Teklad, catalog no. 170950) for 16 days. (3) High salt diet: high salt chow (8% sodium, Harlan Teklad, catalog no. TD 92012) for 16 days. (4) Captopril treatment: captopril solution (0.5 mg/mL) as the drinking water and normal salt diet for 16 days. Blood samples were obtained at the end of the above treatments; kidney samples were obtained immediately after death. (5) Saline injection: 0.9% NaCl solution (Abbott Laboratories), 10 µL/g body wt SC 15 minutes before blood samples were taken. (6) Propranolol: (Sigma) 15 mg/kg SC 15 minutes before blood samples were taken. (7) Metaproterenol: (Sigma) 300 µg/kg SC 15 minutes before taking blood samples. (8) Propranolol/metaproterenol: 15 mg/kg propranolol SC 5 minutes before receiving 300 µg/kg isoproterenol SC. Blood samples were taken 15 minutes later. (9) AGT infusion: Mice were anesthetized with Inactin (100 mg/kg IP). Catheters were inserted with the aid of a dissecting microscope into the femoral artery for blood pressure measurement and blood sampling and into the jugular vein for AGT infusion. The arterial catheter was kept as short as possible to minimize blood loss during blood sampling. Catheters were flushed with a sterile diluted heparin solution (10 U/mL). The trachea was also cannulated to facilitate unobstructed spontaneous respiration. The mice were placed on a warming pad (39°C) throughout the surgical and experimental procedures. Purified AGT from human plasma (Scripps Laboratories, Catalog no. A1914) was used as the source of human AGT (2000 ng Ang I/mL); and pooled plasma from 24-hour bilaterally nephrectomized rats was used as a source of AGT (3000 ng Ang I/mL) for mouse renin.

After a 30-minute control period, AGT was infused (0.2 mL/h) for 30 minutes. Blood was drawn at 15 minutes before (as control), at the end of, and at 30 and 60 minutes after the infusion. The volume of the blood drawn was kept at a minimum (not to exceed 0.1 mL at each time point). In some experiments, the effect of losartan (3 mg/kg), an angiotensin type 1 receptor antagonist, was tested. Mice were killed with an overdose of pentobarbital (150 mg/kg IV) at the end of experiments.

Renin and AGT Assay

PRC was determined using a kinetic assay based on the ability of the renin to generate Ang I from its substrate.\textsuperscript{10} The Ang I generated from exogenous AGT added as the substrate was measured by radioimmunoassay. For most situations, 10 µL plasma (or a 1/5 dilution for mPRC assay) was incubated with 10 µL human substrate or 50 µL rat substrate (see below) at 37°C for 1 hour (for mPRC assay) or 3 hours (for hPRC assay) in the presence of 3 mmol/L EDTA and 0.04% PMSF at pH 7.5 (for mPRC assay) or pH 5.6 (for hPRC assay). An unincubated blank reaction was set up for each of the plasma samples to control for endogenous Ang I levels. TRC was measured after incubation with trypsin. The plasma concentration of prorenin (ProRC) was calculated as TRC minus PRC.
Mouse and human renin were distinguished by their substrate specificity. Mouse renin activity was assayed using rat AGT as the substrate, whereas human renin was assayed using human AGT as the substrate. Pooled plasma from 24-hour bilaterally nephrectomized rats was used as exogenous rat AGT (3000 ng Ang I/mL). Partially purified human plasma was used as exogenous human AGT (6000 ng Ang I/mL). Both samples were checked for the absence of residual renin. The cross-reactivity of renins with the opposite substrates, ie, human renin with rat substrate and mouse renin with human substrate, was <0.1%.

Human and mouse plasma AGT concentrations were estimated indirectly through Ang I generation from 10 μL plasma by adding excess Upjohn renin (for human AGT assay) or hog renin (for mouse AGT assay) in the presence of protease inhibitors.

**RNase Protection Assay**

Tissue samples were homogenized in RNA STAT-60 RNA/DNA/protein isolation reagent (LEEDO Medical Laboratories) with a Polytron homogenizer, and total cellular RNA was extracted following the manufacturer’s protocol. Total tissue RNA (10 μg) was hybridized to single-strand labeled antisense RNA probes generated by T7 polymerases using the Maxiscript kit (Ambion Inc). RNase protection assay was carried out using the RPAII kit (Ambion Inc). Protected products were separated by electrophoresis through 5% polyacrylamide/urea sequencing gels, visualized by autoradiography, and then quantified using a phosphorimager (Storm 860 and ImageQuant software, Molecular Dynamics).

The probes used were a partial hREN cDNA fragment (residues 550 to 730 in the coding sequence) cloned into pCRII (Invitrogen) and a partial mouse Ren-1 cDNA fragment (residues 184 to 418 in the coding sequence) cloned into pBSK(–) vector. A partial 18S cDNA from the Maxiscript kit (Ambion Inc) was used to normalize the amount of RNA in each assay.

**Immunocytochemistry**

Tissues were removed from mice immediately after spinal cord dislocation and put in Bouin fixative (Poly Scientific). After fixation for 12 to 24 hours at room temperature, the specimens were dehydrated, embedded in paraffin, cut into 5-μm sections, and mounted on slides. The sections were then dewaxed in xylene, rehydrated, and washed before incubation with the primary antibody at 4°C overnight. Bound antibody was visualized by immunogold silver staining (IGSS) using the AuroProbe LM and IntenSE M kits (Amersham). The primary polyclonal anti-human renin antibodies BR1-512 and R15 (the kind gift of Dr P. Corvol, INSERM, Collège de France, Paris) were used at dilutions of 1:1000 and 1:500, respectively.

**Statistical Analysis**

All values are expressed as mean±SEM. Comparisons between various treatment groups were tested by ANOVA. The significance of multiple comparisons was assessed by the Scheffe’s test. A value of P<0.05 was considered statistically significant.

**Results**

**Effects of Dietary Sodium and Converting Enzyme Inhibition**

Mouse and human PRC and ProRC were measured after 16 days of the different salt diets (Figure 1A). Among animals on a medium (normal) salt diet, mouse PRC was about 30-fold higher than human PRC, but the percentage of renin was similar for both mouse and human, suggesting similar rates of prorenin processing to renin (Table). Human PRC in transgenic mice fed a low salt diet was significantly greater than in mice fed a medium salt diet (2.8-fold) or high salt diet (3.7-fold). Although human PRC in the high salt group was 24% lower than in the medium salt group, this difference was not significant. A similar pattern was observed in the mouse PRC of both transgenic and nontransgenic mice. Among transgenic mice, mouse PRC was significantly greater in mice fed a low salt diet than for either the medium salt (3.2-fold) or high salt (3.2-fold) diets. Among nontransgenic mice, only the difference in mPRC between low and medium salt diets was significant (2.5-fold); the 1.5-fold difference between low and medium salt groups fell slightly short of statistical significance (P=0.06). In the medium salt group, the mouse
PRC was 2-fold higher in nontransgenic compared with transgenic animals, but this difference also fell short of statistical significance (P=0.07). There were no differences between diet groups in either mouse or human prorenin levels. ANOVA revealed a significant difference in mouse prorenin levels between transgenic and nontransgenic mice across diets (P=0.03). However, within each treatment group these differences were not significant. Similar responses to dietary salt restriction have been observed in humans and rats.12,14,15

Treatment with captopril caused a dramatic increase in both mouse and human PRC compared with mice on a normal salt diet (Figure 1B). Captopril treatment stimulated human PRC 28-fold in transgenic mice and mouse PRC 26- and 20-fold in nontransgenic and transgenic mice, respectively. In captopril-treated mice, the 2-fold difference in the mouse PRC between control transgenic and nontransgenic mice was amplified, resulting in a highly significant 2.5-fold difference in the stimulated levels (P=0.0009). Captopril treatment stimulated human ProRC 3.8-fold in transgenic mice and mouse ProRC 8-fold and 13-fold in nontransgenic and transgenic mice, respectively. These observations suggest that mouse and human prorenin may be secreted from the kidneys when renin secretion is highly stimulated, but normally the mouse kidney secretes predominantly renin. For both dietary salt and captopril treatments, there were no significant effects of gender, transgenic parent, or date of experiment.

**Effects of Dietary Sodium and Captopril on Renal Renin mRNA Levels**

Mouse and human renal renin mRNA levels were determined by a ribonuclease protection assay using probes that distinguish between mouse and human renin mRNA (Figure 2A and 2B). Quantification of the data is shown in Figure 2C and 2D. Generally, changes in both mouse and human renin mRNA followed the changes in circulating renin levels, although the magnitude of the changes was not as great. Among animals fed diets containing different amounts of salt, human renin mRNA levels in the low salt treatment group were significantly higher than in the high and medium salt groups (Figure 2A and 2C). The lower human renin mRNA levels in the high compared with the medium salt group were not significant. Mouse renin mRNA levels also showed significant differences between diets. Differences between high and low salt diets and high and medium salt diets were highly significant, and the difference between the medium and low salt groups was at the borderline of significance (P=0.055). Although mouse renin mRNA levels for transgenic animals on each diet were slightly lower than nontransgenic animals, these differences were not significant.

Captopril treatment caused a significant increase in both mouse and human renin mRNA, and captopril-stimulated mouse renin mRNA levels were significantly lower in transgenic than in nontransgenic mice (Figure 2B and 2D).

**Recruitment of Human Renin Expressing Cells in Glomerular Arterioles**

Previous studies suggest that the increase in renal renin mRNA caused by converting enzyme inhibition is not limited to an increase in the level of renin mRNA per juxtaglomerular cell but is also accompanied by an increase in the cellular population of renin-expressing cells. This increase is manifested by the recruitment of renin-expressing cells in afferent arterioles.16 To examine the effects of captopril treatment on the localization of human renin in the transgenic mice, we carried out immunocytochemistry on the kidneys of captopril-treated mice using a specific anti-human renin antibody (R15) and an antibody that recognizes both mouse and human renins (BR1-5) (Figure 3).

The percentage of juxtaglomerular apparatus stained with either antibody was higher in the captopril-treated mice than in the controls, and the staining in the captopril-treated mice was more intense. The distribution of renin staining along the afferent arteriole was also markedly different in captopril-treated mice. In kidneys from control animals, staining was confined to the juxtaglomerular region (Figure 3, Control). In contrast, in kidneys from captopril-treated animals, renin staining extended along the arterioles distal to the glomerulus (Figure 3, Captopril).

**β-Adrenergic Control of Renin Secretion**

Renal sympathetic nerve activity stimulates renin release.17 Isoproterenol administration increased both human and mouse PRC up to 10-fold compared with saline control (Figure 4). This effect was completely blocked by prior administration of propranolol, although acute propranolol treatment alone did not suppress human or mouse renin secretion significantly. Because the half-life of renin in the circulation is about 20 minutes,18,19 the PRC would be unlikely to decrease greatly during a 15-minute infusion of isoproterenol. As in the chronic treatments described above, mPRC was somewhat lower in transgenic compared with nontransgenic mice, although this difference was not significant.

**AGT Infusion**

The endogenous plasma renin concentration in mice is normally extraordinarily high (>200 ng Ang I/mL per hour when measured using homologous mouse substrate), but AGT levels are low and limit Ang I production by mouse renin. To assess regulation of renin secretion in response to elevated blood pressure and/or plasma Ang II levels, we increased mouse blood pressure by infusion of rat or human AGT. Figure 5A shows the blood pressure response to 2 doses of nephrectomized rat plasma in an anesthetized male mouse. Intravenous injection of 10 μL rat plasma with AGT concentration at 3000 ng Ang I/mL raised blood pressure by 14 mm Hg, while injection of 30 μL plasma raised blood pressure by 25 mm Hg. Losartan reduced base-
line blood pressure and blocked the effect of AGT administration, indicating that the rise of blood pressure was Ang II dependent.

Nephrectomized rat plasma or human AGT were also infused for 30 minutes at a rate calculated to elevate plasma AGT levels to approximately 200 ng Ang I/mL (Figure 5B). Plasma AGT levels produced by infusion of nephrectomized rat plasma declined more rapidly than those produced by infusion of human AGT, possibly because of the lower clearance and/or cleavage of human AGT from the mouse circulation. Infusion of saline alone had no effect on circulating AGT levels. However, during the course of the experiment, blood pressure of the saline-infused mice fell, presumably due to the effects of anesthesia (Figure 5C). Infusion of either nephrectomized rat plasma or human AGT transiently increased blood pressure 25% and 10%, respectively, while during the same period, infusion of saline decreased blood pressure 15%. These blood pressure changes were reflected in both the mouse and human PRCs (Figure 5D and 5E). As blood pressure fell, PRC rose. Conversely, the rise in blood pressure associated with infusion of either rat or human AGT dampened the rising mouse and human PRC. When the infusion was terminated, blood pressure fell and PRC rose. Human PRC in transgenic mice infused with nephrectomized rat plasma also decreased, although less than the mouse PRC (not shown). Although there were differences in the extent and the time course of the effects of nephrectomized rat plasma and pure human AGT, both caused an increase in blood pressure that was mirrored in reciprocal changes in both mouse and human PRC.

Figure 2. RNase protection assay of renal RNA for animals fed salt-modified diets (A) and treated with captopril (B). The positions of hREN-, mRen-1C-, and 18S rRNA–specific protection products are indicated. Numbers at the bottom of the figure indicate the amount of human or mouse renin mRNA in each sample (arbitrary unit, normalized by the amount of 18S rRNA). Exposure time was 16 hours. Quantification of renal renin RNA levels in animals fed different salt diets (C) and in captopril-treated mice (D). Data were quantified from RNase protection assays shown in panels A and B after normalization from measurements of samples run in both assays.

*P<0.0001 compared with control; †P<0.05 compared with low salt. Open bars indicate nontransgenic; hatched bars, transgenic.
The greater effect of the rat substrate and its more rapid clearance is most likely due to the faster rate of cleavage of rat substrate by mouse renin compared with the reactions of mouse and human substrates and their homologous renins. These observations indicate that mouse and human renin secretion responds appropriately to changes in blood pressure or angiotensin levels that are induced by the administration of rat or human AGT.

Discussion

To establish the relevance of the mouse as a host organism in which to construct models of the human RAS, it is important to demonstrate that human renin gene expression and secretion are regulated appropriately in the mouse. In strains of laboratory mice, PRCs are extraordinarily high, often exceeding 200 ng Ang I/mL per hour compared with normal human levels of 0 to 10 ng Ang I/mL per hour. Despite the high PRC, mouse plasma generates angiotensin in the same range as human plasma because plasma concentrations of AGT are very low, and the rate of cleavage of mouse AGT by mouse renin is lower than that of their human counterparts. In the few studies that have examined the physiological regulation of plasma renin levels in mice, renin secretion in mice was regulated similarly to that of humans and other species. However, the high plasma renin levels in mice may have required adaptive modifications to the mechanisms controlling mouse renin gene expression and secretion that may preclude the appropriate physiological regulation of human renin synthesis and secretion.

We recently generated several transgenic mouse lines containing a 45-kb hREN genomic fragment, much longer than previously established lines that contained hREN transgenes of 13 to 15 kb. In each of these lines, human renin gene expression was restricted to a much narrower range of tissues than earlier transgenic models containing shorter hREN transgenes. Importantly, circulating plasma human renin levels in 45-kb hREN mice were copy-number dependent, and when a single copy was present, were close to the normal human range, suggesting that the level of plasma renin is determined by information encoded in the renin gene rather than by the species in which it is expressed. Moreover, plasma human renin in these mice disappeared 24 hours after nephrectomy, demonstrating that it originates exclusively from the kidneys. The results of the present study show that the synthesis and secretion of human renin in 45-kb hREN transgenic mice respond appropriately to all physiological and pharmacological stimuli that were tested, and similarly to mouse renin in direction, although less so in absolute amount. This is illustrated in Figure 6, which shows the relationship between mouse and human PRC for each determination in every experiment reported in the present study. Irrespective of the nature of the stimulus or the experimental system (chronic, acute, conscious, or anesthetized), mouse and human PRCs were highly correlated in a linear fashion. Moreover, all the responses were similar to those previously reported for human renin secretion. This would suggest that

Figure 3. Serial sections of kidneys from a transgenic mouse fed a normal salt diet (control) and a transgenic mouse treated with captopril for 16 days. Panels A, B, E, and F were from the control mouse; and panels C, D, G, and H were from the captopril-treated mouse. Panels A, C, E, and G stained for both mouse and human renin (BR1-5); and panels B, F, D, and H stained for human renin (R15). Upper panels (A–D) are from lower power magnification (×6), and lower panels (E–H) are central portions of the upper panels at higher magnification (×40).

Figure 4. β-Adrenergic control of human and mouse renin secretion. S indicates saline; P, propranolol; I, isoproterenol; and P+I, propranolol followed by isoproterenol. *P<0.0001 compared with I. Open bars indicate nontransgenic; hatched bars, transgenic.
the intrarenal mechanisms that direct mouse renin synthesis and secretion can also regulate the intrarenal expression of the human gene, as well as the processing and secretion of human prorenin and renin. In addition, our studies revealed some nuances of the mouse RAS and the expression of human prorenin and its processing to renin in mice that may contribute to our understanding of the RAS. In particular, we observed that the proportion of human prorenin processed to renin more closely resembled the mouse proportion, suggesting that this characteristic of prorenin processing is a property of the host rather than the gene. In transgenic animals, mouse renin and renin mRNA were suppressed, suggesting an interaction between mouse and human renins manifested at a transcriptional level. We also observed much higher total renin (renin + prorenin) expression per unit of renin mRNA for the mouse than the human gene.

In the present study, we found that the proportion of mouse and human total renins detected in the plasma as prorenin (~40%) was lower than in humans and rats (~90%). This is consistent with the findings of earlier studies that reported values of 30% to 50%. Although Poulsen and coworkers reported a value of 80% to 90% prorenin for male mice, they commented that the proportion of prorenin measured in the plasma of female mice was around 50%. In the present study, mouse prorenin levels were similarly higher in male than female mice on a normal salt diet, but these differences disappeared in the responses to dietary salt or captopril; none of the differences were significant, possibly due to the small number of males that were tested (9 of 36). Data presented in another study of human renin transgenic mice suggest that proportion of prorenin in C57BL/6J mice is about 9%. Therefore, the levels of prorenin detected in mouse plasma may depend on the strain and gender and may also be affected by the methodology, especially considering the errors that may occur when subtracting high PRC values to determine the prorenin concentration. However, it is important that the proportion of human prorenin was similar to that of mouse prorenin, suggesting that mouse and human prorenins are processed similarly by the kidneys.
Changes in both mouse and human renin mRNA levels were smaller than the changes in the PRC noted previously in rats. Despite this disproportionality, both mouse and human renin mRNA levels were highly correlated to their respective plasma levels of prorenin, renin, and total renin (Figure 7). Although some of this effect is due to the greater increase in renin relative to prorenin, suggesting increased prorenin processing in the kidney, the changes in total renins were much greater than the changes in renin mRNAs. It is also noteworthy that the ratio of total renin to renin mRNA for the mouse gene was much higher than for the human transgene. Mouse and human renal renin mRNA were very similar for each treatment. Across the various salt intake groups, mouse renin mRNA levels averaged 2.2 ± 0.2 times human renin mRNA levels (n = 13), whereas this pattern was reversed in captopril-treated animals in which human renin mRNA was 2.5 ± 0.5 times the mouse renin mRNA (n = 4). Because the human renin transgene in the line of 45-kb hREN mice that was tested is present in approximately 4 copies, and both human PRC and hREN mRNA levels appeared to be copy-number dependent, at relatively low expression levels each copy of the mouse renin gene might be more highly expressed than the human renin gene. However, at high levels of expression, under conditions in which the concentration of transcription factors might be limiting (see below), the relative level of transcription of mouse and human genes might be more closely related to the copy number. The greater mouse PRC and ProRC observed per unit of mRNA suggests the involvement of posttranscriptional mechanisms, possibly a higher translational efficiency for mouse renin mRNA, or a greater stability of the protein in the juxtaglomerular cells or the plasma. However, since the rate of angiotensin formation for mouse renin acting on rat substrate is about 5 times higher than for human renin acting on human substrate, part of this effect may be due to the kinetics of the system used to measure renin concentrations by enzymatic activity.

Mice carrying the human renin transgene had lower levels of mouse renin, prorenin, and renin mRNA than their non-transgenic littermates. Differences in mouse plasma renin and prorenin levels between transgenic and nontransgenic mice were accompanied by proportional differences in the mRNA levels (Figure 7). Thus, plasma renin and prorenin levels for transgenic and nontransgenic mice fell on the same line when plotted against renal renin mRNA levels. In short-term experiments, β-adrenergic stimulation increased both human and mouse plasma renin levels ≈ 10-fold, although mouse PRC tended to be higher in nontransgenic than transgenic mice. Taken together, the findings of long- and short-term studies suggest that the expression of human renin either depletes or prevents the accumulation of mouse renal renin to normal levels. There are at least 2 possible explanations for this phenomenon. The first is that human and mouse renin gene expression in juxtaglomerular cells might share the same trans-acting factor(s). The quantities of the factors could limit the amount of renin mRNA that can be produced, even under conditions of normal salt loading and in the absence of pharmacological interventions. This hypothesis is consistent with the observation that high demand for renin secretion results in recruitment of afferent arteriolar vascular smooth muscle cells, in addition to any increase in renin gene expression that may occur per cell, suggesting that there may be a limit to the amount of renin that can be made by any one juxtaglomerular cell. The other possibility is that human renin may feed back to the mouse renin system, either directly through some unidentified mechanism or by cleavage of mouse AGT to produce Ang I. It has been reported that overexpression of a nonprocessable form of mouse prorenin in transgenic mice is associated with a suppression of renin activity from the endogenous mouse gene, suggesting the existence of a direct mechanism. Although we have never been able to detect cleavage of rodent substrate by human renin in vitro, prolonged incubation in plasma in vivo might initiate the formation of sufficient Ang II to suppress renin secretion and synthesis as part of the normal homeostatic feedback control. Further studies will be required to determine the mechanism whereby human gene expression inhibits expression of the endogenous mouse gene.
Previous studies with mice transgenic for the human renin gene reported 2.4-fold increases in both mouse PRC and mouse renal renin mRNA after 2 weeks of sodium depletion. In another study of hREN transgenic mice, mouse PRA was unchanged after 10 days of diet containing either no salt (0.01% Na) or high salt, although human PRC was reduced approximately 2-fold by high salt feeding and increased by captopril treatment 25- to 40-fold. When these and similar hREN transgenic mice were crossed with mice expressing the human AGT (hAGT) gene to yield doubly transgenic mice expressing both hREN and hAGT, the plasma renin activity was elevated and blood pressure increased 30 to 40 mm Hg. If the RAS were normally regulated, an increase in blood pressure should decrease renal renin secretion and normal blood pressure should be restored. In 1 of these lines, human renal renin mRNA actually increased in the doubly transgenic hREN×hAGT animals. Increased human RAS activity and blood pressure were also observed in doubly transgenic hREN×hAGT rats. While the shorter hREN transgenes used in these studies may express human renin in the renal juxtaglomerular cells and respond appropriately to physiological stimuli, this may be obscured by inappropriately regulated secretion of human renin from extrarenal sites. In the present study, infusion of rat or human AGT into 45-kb hREN transgenic mice increased blood pressure and decreased plasma renin concentration.

In summary, both human PRC and mouse PRC responded similarly to salt treatments, ACE inhibition, acute β-antagonist and agonist treatment, and blood pressure and/or Ang II. Overall, these results indicate that renal renin synthesis and secretion of mouse and human renins in 45-kb hREN transgenic mice respond in parallel to various physiological and pharmacological manipulations similarly in humans and other species. The species specificity of renin for its substrate permits the replacement of the mouse RAS with its human counterpart by inserting the human genes encoding renin and AGT into mice lacking the endogenous mouse AGT gene. Such mouse models provide the opportunity to study the physiology of a normal human RAS and its disruption in hypertension. Transgenic models of the human RAS permit the implementation of a greater variety of experimental protocols and the accessibility of tissues to determine protein and mRNA levels. In addition, human RAS mouse models would be useful for the development and testing of antihypertensive therapies, especially those that involve renin inhibitors that tend to be species specific. However, for such models to be physiologically meaningful requires that the renin and angiotensin genes be expressed at appropriate sites and be regulated by relevant physiological mechanisms. This is particularly important because of the complex interplay between the parameters that control blood pressure and the activity of the RAS, and the reactive changes that occur when any one parameter is altered. By extending the human renin transgene sequences, we have accomplished a much tighter pattern of cell- and tissue-specific expression that is reflected in more appropriate physiological responses. These mice should provide valuable models to study the role(s) of the RAS in blood pressure homeostasis.

Acknowledgments
This work was supported by National Institutes of Health grant DK-45982 (to Dr Catanzano) and by the generous support of the Greenburg, Wallace, and Wolk Funds. Dr Catanzano is an Established Investigator of the American Heart Association.


Appropriate Regulation of Human Renin Gene Expression and Secretion in 45-kb Human Renin Transgenic Mice

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_Hypertension_. 1998;32:205-214
doi: 10.1161/01.HYP.32.2.205

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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