Nonproportional Changes in Plasma Renin Concentration, Renal Renin Content, and Rat Renin Messenger RNA

NORIFUMI NAKAMURA, FLORENT SOUBRIER, JOEL MENARD, JEAN-JACQUES PANTHIER, FRANÇOIS ROUGEON, AND PIERRE CORVOL

SUMMARY The expression of the renin gene in rat kidneys was studied using mouse submaxillary gland renin complementary DNA. The length of rat renin messenger RNA (mRNA) was approximately 1600 nucleotides, similar to that of mouse submaxillary gland and kidney renin mRNA. Rat renin mRNA was quantified by a radiodensitometric complementary DNA hybridization assay. The effects of intense long-term stimulation and short-term inhibition of renin secretion on plasma renin concentration, renal renin concentration, and renin mRNA content were compared with those of controls. After 15 days of sodium depletion and captopril treatment, plasma renin concentration increased 46-fold, renal renin concentration only 1.5-fold, and renin mRNA content increased about threefold. Following a 1-hour infusion of angiotensin II in sodium-depleted and captopril-treated rats, plasma renin concentration decreased by 84% whereas no significant changes in either renal renin concentration or renin mRNA content were observed. These results show that sodium depletion and captopril treatment increase the level of renin gene transcription and renin biosynthesis. However, there are nonproportional changes in plasma renin levels, renal renin content, and its mRNA. These results suggest that newly synthesized renin is not stored in the kidney but is rapidly secreted into the blood. Short-term inhibition of plasma renin concentration by angiotensin II is most likely mediated by posttranslational mechanisms. (Hypertension 7: 855–859, 1985)

KEY WORDS • rat kidney renin messenger RNA • mouse submaxillary gland renin • complementary DNA • Northern blot hybridization • renin messenger RNA quantification

RENIN secretion from the kidney is regulated by several factors such as intrarenal baroreceptors, the renal sympathetic nerves, and various humoral substances. Studies of the regulation of renin secretion in rats have traditionally employed intact animals,1 isolated perfused kidneys,2–4 or semipurified juxtaglomerular cells.5–6 In such preparations, the alterations in renin secretion have been determined by measuring levels of plasma renin activity or plasma renin concentration in peripheral or in renal vein plasma, renal renin concentration, or by estimating the granulation of the juxtaglomerular cells. Using these techniques it has been possible to acquire considerable insight into the physiological mechanisms controlling renin secretion.1

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Such methods do not provide insight into the molecular mechanisms of renin production. In response to stimulation renin could be liberated from storage granules1 and (or) synthesized de novo as a preprorenin molecule, processed, and then secreted without storage. Studies of biosynthesis using labeled amino acids incorporated into the renin precursor have been limited because of the extremely small amount of renin in tissue. However, it is now possible to study the regulation of the renin gene at the transcriptional level by detecting the messenger RNA (mRNA) encoding for preprorenin in the rat kidney by DNA/RNA hybridization. Using a renin complementary DNA (cDNA) containing the entire coding sequence of mouse renin mRNA, we have characterized the rat renin mRNA that has a similar nucleotide length. A radiodensitometric hybridization assay was developed to quantify the rat renin mRNA in vivo experiments in which renin secretion was stimulated by sodium depletion and converting enzyme blockade and inhibited acutely by infusion of angiotensin II. The rat kidney renin mRNA content was compared with the renal renin content and the circulating renin levels. The results
show that sodium depletion induces a nonproportional increase in plasma renin levels, renin mRNA, and renal renin content.

**Materials and Methods**

Male Wistar rats (IFFA CREDO, L'Arbresle, France) (8 weeks old, 300 to 350 g) were used. For at least 1 week before the study, the rats were fed a standard laboratory diet (170 mEq of Na+/kg of food; UAR-France, Villemoisson-sur-Orge, France) and provided with tap water. The animals were then divided into three groups.

Group 1 (control group, n = 10) rats were given the standard laboratory diet and tap water. Beginning on the eighth day, they received distilled water by gavage twice a day for the following 7 days.

Group 2 (sodium-depleted and captopril-treated rats, n = 9) rats were maintained on a sodium-free diet (UAR-France) and distilled water for 7 days. In addition, they were injected subcutaneously with furosemide (20 mg/kg/day; Laboratoires Hoechst, Puteaux, France) for the 2 days preceding death. From the eighth to the fifteenth day they were given captopril (10 mg/kg in 1 ml of distilled water; Squibb Laboratories, Princeton, NJ, USA) twice a day by gavage.

Group 3 (angiotensin II-treated rats, n = 10) rats were treated in the same manner as those of Group 2. One day before being killed, the rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and a polyethylene catheter (PE-50) was placed into the jugular vein and brought to the surface at the back of the neck for angiotensin administration. (Ile5)-angiotensin II (Sigma Chemical Co., St. Louis, MO, USA) for the quantification of renin mRNA.

The samples were electrophoresed on 1.1% agarose gel in a 10 mM sodium phosphate buffer, pH 7.0, transferred to nitrocellulose paper (BA 85, Dassel, West Germany), prehybridized, and hybridized according to the procedure of Thompson. The RNA blots were washed with four changes of 2 x standard sodium citrate (1 x standard sodium citrate: 0.15 M NaCl, 15 mM sodium citrate, pH 7.0)/0.1% sodium dodecyl sulfate at room temperature and then washed twice with 1 x standard sodium citrate/0.1% sodium dodecyl sulfate at 50°C. Autoradiography was conducted with Kodak X-Omat AR film (Eastman Kodak, Rochester, NY, USA) at —70°C using an intensifying screen, and the autoradiogram was scanned with a Brucom densitometer (Brucom Industries Inc., Huntington Beach, CA, USA) for the quantification of renin mRNA.

Quantification of rat kidney renin mRNA was adapted from the method of Heinrich et al. as developed for assay for parathyroid hormone mRNA. The absorbances of the hybrid images were plotted against the various amounts of mouse submaxillary gland and rat kidney total RNAs applied on the gel. The relative changes in renin mRNA content between control and the other two groups were compared from the slope of each linear regression line.

**Results**

As shown in Table 1, a low sodium diet and converting enzyme (captopril) treatment dramatically increased plasma renin concentration (46-fold). At the same time renal renin concentration was slightly but significantly increased 1.5-fold (p < 0.01). One hour of intravenous angiotensin II infusion at a dose of 100 ng/min/kg decreased plasma renin concentration by 84% (p < 0.001) but had no significant effect on renal renin concentration.
Table 1. Renal and Plasma Renin Concentration in Treated and Untreated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>RRC (nmol ANG I/hr/mg protein)</th>
<th>PRC (pM ANG I/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>5.86 ± 1.14</td>
<td>26.08 ± 9.57</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>9.07 ± 2.61*</td>
<td>1193.18 ± 465.52*</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>8.32 ± 3.30† §</td>
<td>187.85 ± 54.76†</td>
</tr>
</tbody>
</table>

Values are means ± SD.

RRC = renal renin concentration; PRC = plasma renin concentration; ANG I = angiotensin I; Group 1 = control; Group 2 = sodium-depleted and captopril-treated rats; Group 3 = same as Group 2 plus angiotensin II infusion.

*p < 0.01, tp < 0.001, tp < 0.05 when compared with controls; §not significant, ‡p < 0.001 when compared with Group 2.

To identify and determine the size of renin mRNA obtained from the rat kidney, extracted total and poly(A) + -containing RNA from control rats were cross-hybridized with the mouse submaxillary gland renin cDNA probe (Figure 1). A mRNA population hybridized specifically with the mouse submaxillary gland probe. The 1.6-kilobase length of this mRNA is similar to that of the mouse kidney and submaxillary gland renin mRNA.

Renin mRNA was quantified by measuring the absorbance of the hybrid images at various dilutions of total RNA. Figure 2 (upper panel) shows that a regression line can be constructed using mouse submaxillary gland total RNA. When the linearity of the plot was analyzed, a high correlation coefficient (r = 0.99) was observed using points below 0.4 absorbance unit. Rat

\[ y = 0.015x - 0.03, r = 0.99; \]

\[ y = 0.039x - 0.02, r = 0.99; \]

\[ y = 0.031x + 0.01, r = 0.99. \]
kidney total RNA isolated from pooled kidneys of controls and renin-stimulated and angiotensin II-treated rats were electrophoresed, blotted, and hybridized together. Various RNA dilutions were used and repeated twice for each group. Separate regression lines were similarly constructed with three different concentrations of rat renin mRNA from each group, and the slope of each regression line was calculated. The ratio between each of these slopes was used for estimating the relative renin mRNA amount of the different rat groups. Figure 2 (lower panel) shows the differences between the slopes of the controls and those of the treated rats. The slope of the renin-stimulated and the angiotensin II-treated rats were respectively 3.0 and 2.8 times higher than that of the control group. This finding suggests that the renin mRNA content is increased about three times during renin stimulation when compared with that of controls. There was no significant difference between the slopes of renin-stimulated and angiotensin II-treated rats, which indicates that a similar renin mRNA content exists under these conditions.

Discussion

Rat renin mRNA was easily characterized using the mouse renin cDNA by Northern blot analysis under stringent conditions of hybridization. This finding was an expected result because of the relatively recent time of divergence between the two species. Rat kidney renin mRNA has about the same length (1600 nucleotides) as mouse and human renin, which indicates that it probably encodes for a 45- to 50-kDa renin precursor. We have previously shown that mouse submaxillary gland renin is synthesized as a preprorenin precursor and is processed into an active renin molecule (38 kDa) after release of a 45 amino acid prosegment. The recent cloning of human renin has led us to postulate the same type of processing. The molecular weight of active renin in the rat kidney is around 35 to 37 \times 10^3, which again suggests a similar processing of the enzyme.

Rat kidney renin mRNA was quantitatively measured by a specific densitometric Northern blot hybridization assay. This assay and the expression of the results were performed essentially as recently described by Heinrich et al. for parathyroid hormone mRNA. The specificity of the assay was measured by the analysis of the hybrid images obtained at 1.6 kb. Such an assay is obviously more specific than the dot blot hybridization assay, which may give a high nonspecific background level. Indeed, as an example, rat liver total RNA gave a nonspecific signal using the dot blot assay whereas no specific hybrid image could be detected on Northern blot experiments under the same stringent conditions. The radiodensitometric hybridization assay was sufficiently sensitive to detect renin mRNA in normal rats as well as under conditions of renin stimulation or inhibition.

Various concentrations of renin mRNA produced proportional increases in absorbance as in the case of homologous hybrids (mouse renin mRNA/mouse renin cDNA) as well as in the case of the heterologous hybrids (rat renin mRNA/mouse renin cDNA). The results could not be expressed as rat renin mRNA concentration because of the lack of rat renin mRNA standard. Close dilutions of RNA allowed us to construct a representative regression line between the various RNA concentrations and the absorbances for each of the experimental groups. It was then possible to compare the slopes of the regression lines constructed from the various mRNA samples and, therefore, we were able to deduce the relative increase of renin mRNA under various conditions. The relative increase in renin mRNA could be due to an increase of the transcription rate of the renin gene and (or) the rat renin mRNA stability.

The combination of sodium depletion and converting enzyme blockade increased plasma renin concentration about 46-fold but increased the renal renin content only slightly (1.5 times), as already reported under similar conditions. At the same time, there was an increase in renin mRNA. This increase in renin biosynthesis could result from an increase in the transcription of the renin gene by previously functioning juxtaglomerular cells or by the recruitment of additional secretory cells. In fact, the major finding is that sodium deprivation in conjunction with converting enzyme blockade did not induce a parallel increase in plasma renin levels and renin biosynthesis.

It could be hypothesized that under such conditions prorenin is synthesized at a higher rate, processed rapidly, and secreted into the plasma without significant storage. In favor of this hypothesis is the fact that during adrenalectomy and sodium depletion the juxtaglomerular cells appear degranulated. In addition, De Senarclens et al. have shown that when renin secretion, previously stimulated by adrenalectomy associated with sodium depletion, is abruptly stopped by 48 hours of treatment with deoxycorticosterone acetate and salt, the secretory granules reappear together with an increase in the inactive form of renin within the kidney. This hypothesis would fit well with the model that we have proposed recently in which the renin granules act mainly as storage granules in the human juxtaglomerular cells.

One hour of angiotensin II infusion decreased plasma renin concentration by 84%. This decrease is likely due to a suppression of renin secretion by the juxtaglomerular cells. At the same time, renal renin concentration decreased slightly but not significantly and renin mRNA was not affected. The absence of changes in rat renin mRNA suggests that the decrease in plasma renin concentration was due to posttranslational mechanisms. It does not exclude the possibility that the renin gene transcription had been stopped by angiotensin II treatment and that the renin mRNA measured 1 hour after angiotensin II infusion reflected the stability of this mRNA.

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