Regulation of Renin Gene Expression in Hypertensive Rats

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SUMMARY A carboxy terminal renin complementary DNA (cDNA) clone from rat kidney was isolated, characterized, and used as a probe for renin messenger RNA (mRNA) quantification in normotensive and hypertensive rats. RNA blotting analysis detected renin mRNA in control kidney and brain. Deoxycorticosterone acetate (DOCA) and high salt (1%) treatment of experimental animals resulted in a greater than 95% decrease in the content of renin mRNA in the kidney, as compared with values in control rats receiving 0.4% NaCl in their diet. In contrast, high salt (1%) treatment alone caused only a twofold decrease in kidney renin mRNA content, as compared with values in controls. DOCA and low salt (0.04%) or low salt (0.04%) treatment alone caused a 1.5-fold increase in the kidney renin mRNA content, as compared with values in control rats. These results indicate that DOCA and salt have a synergistic effect in depressing renin mRNA levels in kidney. Clipping of the left renal artery caused a threefold increase in the steady state level of renin mRNA in the ischemic kidney and a 0.5-fold decrease in the hypertrophied kidney. The data are consistent with the hypothesis that blood pressure and other stimuli regulate the expression of the renin gene in vivo. (Hypertension 12: 405-410, 1988)

KEY WORDS • rat renin gene regulation • Goldblatt hypertension • renin • messenger RNA

RENIN is an aspartyl proteinase with an important function in the regulation of blood pressure and electrolyte balance. Physiological and pharmacological studies of the renin-angiotensin system over the last 10 to 20 years have demonstrated the participation of renin in the development of hypertension. Progress in the field of molecular biology has provided new insights into renin gene expression. Earlier studies have shown the presence of renin-like activity in a variety of mammalian tissues. Recently, the availability of renin cDNA probes has provided further information about the specificity of tissues in the expression of the renin gene. These studies have demonstrated the presence of renin messenger RNA (mRNA) in several tissues in humans, rats, and mice. In all species studied, the juxtaglomerular cells of the afferent arterioles of the kidney are a major site of renin gene expression. Renin mRNA has also been detected in human chorion; rat testis, adrenal, pituitary, ovary, uterus, and brain; and mouse adrenal, sublingual, and parotid salivary glands, testis, brain, and submandibular gland.

In the present article, we report the cloning of kidney renin complementary DNA (cDNA) corresponding to the carboxy terminal region of rat renin and its utilization in blotting analysis of RNA isolated from normotensive and hypertensive rats. We observed marked changes in renin gene expression under conditions of experimentally induced hypertension.

Materials and Methods

Materials were obtained from the following sources: a cDNA synthesis system and multiprime DNA labeling system from Amersham (Arlington Heights, IL, USA); Agt11 vector and packaging extracts from Stratagene (San Diego, CA, USA); EcoRI synthetic linkers, restriction and modification enzymes from New England Biolabs (Beverly, MA, USA); high concentration EcoRI from Boehringer-Mannheim (Indianapolis, IN, USA); deoxynucleoside triphosphates and Sepharose CL4B from Sigma (St. Louis, MO, USA); nitrocellulose membranes, 0.45 µm, from Schleicher and Schuell (Keene, NH, USA); ultra-pure CsCl, TEMED, and bacterial alkaline phosphatase and plasmid pUC19.
from Bethesda Research Laboratories (Gaithersburg, MD, USA); acrylamide, bisacrylamide, ammonium persulfate, and agarose from Bio-Rad (Rockville Centre, NY, USA); ultrapure urea from ICN (Cleveland, OH, USA); oligo-(dT)-cellulose Type 7 and Sephadex G-50 from Pharmacia (Piscataway, NJ, USA); formic acid and dimethyl sulfate from Aldrich (Milwaukee, WI, USA); piperidine from Fisher (Medford, MA, USA); hydrazine from Kodak (Rochester, NY, USA); [α-32P]deoxyctydine 5'-triphosphate (dCTP) and [γ-32P]adenosine 5'-triphosphate (ATP) from New England Nuclear (Boston, MA, USA). All other chemicals were of the highest purity commercially available.

Construction of a cDNA Library

Double-stranded cDNA was synthesized from 5 μg of poly(A)+ RNA by the method of Gubler and Hoffman10 using a cDNA synthesis kit (Amersham). The cDNA was blunt-ended by sequential digestion with T4 DNA polymerase and Klenow enzyme. Following ligation of EcoRI linkers and digestion with EcoRI, the excess linkers were removed from the cDNA by gel filtration in Sepharose CL4B (Sigma). The cDNA was ligated to the EcoRI site of agt11 vector (Stratagene) and packaged in vitro using a packaging extract (Gigapack Gold, Stratagene). The titer of the amplified library was 9 × 10^10 plaque forming units (pfu)/ml.

Probe Preparation

The rat cDNA library was screened with a mouse renin cDNA, a gift from Dr. John Chirgwin. The probe was prepared by the multiprime DNA labeling method11 using a commercially available kit (Amersham). Briefly, 2 μg of plasmid pmRr49 harboring an 800-base pair mouse renin cDNA12 was digested with EcoRI, and the insert was excised from low gel temperature agarose and placed in a preweighted Eppendorf tube. Water was added at 3 ml/g agarose, and the mixture was boiled for 7 minutes, followed by incubation at 37 °C for 10 minutes. An aliquot of the solution containing 25 ng of DNA was mixed with random sequence hexanucleotides and the Klenow fragment of DNA polymerase I in the presence of 50 μCi [α-32P]dCTP (3000 Ci/mmol) in a total volume of 50 μl, as specified by the manufacturer. The reaction mixture was incubated at room temperature overnight. After the addition of 50 μl of 10 mM Tris HCl, 1 mM EDTA, pH 8, the probe was separated from unincorporated nucleotides and agarose fragments by a 20-second centrifugation through a "spin-column" containing Sephadex G-50 Fine.13 The specific activity of the labeled probe under these conditions was approximately 1 × 10^9 cpm/μg DNA. Before use the probe was boiled for 3 minutes and added to the hybridization mixture.

Screening of a cDNA Library

Plaque DNA was purified, and the longest insert was subcloned into pUC19 vector.

DNA Sequencing

DNA fragments were labeled at their 5’ ends by the polynucleotide kinase exchange reaction and sequenced by the chemical degradation method.15

Treatment of Animals

Male Wistar rats (Charles River Laboratories, Kingston, NY, USA) weighing 200 to 250 g were assigned to three groups. Group 1 (n = 3) was composed of intact rats, receiving a standard salt diet (0.4% NaCl) and tap water ad libitum for 4 weeks. Rats in Group 2 (n = 3) were subjected to unilateral nephrectomy followed by deoxycorticosterone acetate (DOCA; Percorten, Ciba), 15 mg/kg s.c. twice weekly for 3 weeks, and a 1% saline solution for drinking fluid. Group 3 (n = 3) consisted of rats in which a silver clip (internal diameter, 0.2 mm) was placed around the left renal artery (two-kidney, one clip hypertension, 2KIC). These animals were supplied with a standard salt diet and tap water for 4 weeks. Blood pressure was monitored every week by an automatic photoelectric tail-cuff method (IITC, Woodland Hills, CA, USA). Rats from Groups 2 and 3 had a systolic blood pressure over 200 mm Hg compared with 115 mm Hg in the controls (Group 1) by the end of the protocol. All animals were killed by decapitation upon completion of the protocol, and the kidneys, liver, heart, and brain (except for the cerebral cortex and cerebellum) were removed within 5 minutes, frozen in liquid nitrogen, and stored at -80 °C for further use.

RNA Isolation and Blotting Analysis

Total RNA was extracted by a modified method of Chirgwin et al.16 Frozen tissue was homogenized to a fine powder under liquid nitrogen, and 2-g
aliquoted were mixed with 12 ml of Buffer A (5 M guanidine thiocyanate, 50 mM Tris HCl, pH 7.5, and 20 mM EDTA, 0.7 M β-mercaptoethanol). After homogenization for 50 seconds using a polytron, sodium lauryl sarcosine (Sarcosyl) was added to a 0.5% final concentration and the homogenate was forced twice through a 22-gauge needle. The homogenate was mixed with 12 ml of 5.7 M CsCl, 50 mM Tris HCl, pH 7.5, and 20 mM EDTA, loaded into two quick-seal tubes (Model 342413, Beckman, Palo Alto, CA, USA) and centrifuged with slow acceleration in the Type 70.1 rotor at 65,000 rpm for 4 hours at 20 °C. The pellet was washed extensively with 70% ethanol and resuspended in sterile water. An aliquot of purified RNA was measured at 250, 260, 280, and 310 nm. The amount of RNA was calculated from the absorbance at 260 nm after subtraction of the 310-nm absorbance, assuming that 1 optical density 260 nm equals 40 µg of RNA. Poly(A)+ RNA was obtained through two cycles of fractionation in oligo-(dT)-cellulose.17

Total RNA (25 µg) and poly(A)+ RNA (5 µg) from rat tissues were analyzed by Northern blot.18 Samples were electrophoresed on 1% agarose gel prepared in 20 mM 3-(N-morpholino)propanesulfonic acid, pH 7.0, 5 mM sodium acetate, 1 mM EDTA, 0.23 M 20 mM 3-(N-morpholino)propanesulfonic acid, pH 6.8, 5x Denhardt, 0.1% SDS, and 100 µg of salmon sperm DNA/ml and hybridized at 42 °C for 18 hours in the same buffer containing a 32P-labeled rat kidney cDNA probe.

The 480-base pair probe consisted of the insert of a rat kidney renin cDNA clone pUC-RRc4. This plasmid pRen-4419 was digested with HindIII and BamHI, and the excised insert was labeled as described. The specific activity of the labeled probes was approximately 1 × 109 cpm/µg DNA. The filters were washed three times at 30 minutes per wash at 65 °C in 0.1 × SSC, 0.1% SDS. The dried filters were exposed to x-ray film (Kodak XAR-5). Removal of the radioactive signal from Northern blots was accomplished by incubating the filters at 95 °C for 5 minutes in 0.1 × SSC, 0.1% SDS, and exposing the stripped filters to x-ray film overnight prior to hybridization with another probe. At least two different Northern blot analyses were performed for different experimental treatments. The integrated band densities on autoradiograms were determined by scanning (Shimadzu, Kyoto, Japan). The rat β-actin probe used for the control hybridizations of RNA blots was a gift from Dr. Stephen Farmer of Boston University.

**Results**

The nucleotide sequence of the insert of a rat kidney renin cDNA clone pUC-RRc4 is shown in Figure 1. The differences between this sequence and a recently published full-length renin cDNA from rat kidney18 are described in the legend to Figure 1. The reported distribution and relative abundance of renin mRNA in different tissues of humans, rats, and mice is shown in Table 1.

Northern blotting analysis of total RNA extracted from rat kidney and brain is shown in Figure 2A. DOCA–high salt (1%) treatment resulted in the...
disappearance of kidney renin mRNA. This finding was confirmed with poly(A)⁺ RNA (Figure 3, Lanes a and b). In 2KIC rats, the ischemic kidney showed a threefold increase in renin mRNA content, whereas the renin mRNA content in the hypertrophied kidney decreased 0.5-fold relative to the control values (see Figure 2A). Control experiments (see Figure 2B) showed that the tissue levels of actin mRNA were unchanged by the various treatments in the experimental animals.

Treatment of rats with either DOCA-low salt (0.04%) or low salt (0.04%) alone caused a 1.5-fold increase in the level of renin mRNA in the kidney compared with levels in the control animals receiving 0.4% NaCl in their diet (see Figure 3, Lanes e-g). In contrast, high salt (1%) caused a twofold decrease in renin mRNA content in the kidney (see Figure 3, Lanes c and d).

Discussion

Recent studies using renin and angiotensinogen cDNA probes showed that some rat tissues expressing the angiotensinogen gene do not express the renin gene (e.g., References 21, 25, 26). These findings imply that either angiotensinogen is exported from the tissue in question or renin is taken up from the circulation.

To further understand the role of renin in the regulation of blood pressure, we investigated the regulation of its synthesis in normotensive and hypertensive rat models. Since at the initiation of this study the rat renin cDNA probe was not available, we proceeded to isolate and characterize a rat kidney cDNA probe. This probe extends from the poly(A) tract to the codon specifying glycine 308 of the rat renin sequence.

Blotting analysis of total RNA showed that the relative abundance of renin mRNA in brain is approximately 20% of that in kidney (see Figure 2A). The most interesting aspect of this study is the absence of renin mRNA in the kidneys of rats treated with DOCA-high salt (1%; see Figures 2A and 3, Lanes a and b). This finding is consistent with previous work showing a 98% suppression of plasma renin activity in DOCA-salt-treated rats over the span of 6 to 9 weeks. This tissue-specific effect suggests either a suppression of renin gene transcription or a rapid degradation of renin mRNA (or both). Uptake of DOCA is mediated by the mineralocorticoid receptor, and its effect on steady state renin mRNA levels may reflect a direct effect on renin gene transcription, as has been documented for other hormone systems.

To decouple the effects of DOCA from salt on renin gene expression, rats received high salt (1%) or DOCA-low salt (0.04%) or low salt (0.04%) alone, in their diets. DOCA-low salt or low salt alone led to a small increase in mRNA levels in kidney compared with levels in control animals receiving 0.4% NaCl in their diets (see Figure 3, Lanes e-g). In contrast to the precipitous decrease in renin mRNA caused by DOCA-high salt (1%; see

Supplementary Table 1

Tissue Distribution and Relative Abundance of Renin mRNA in Humans, Rats, and Mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Human Probe</th>
<th>Reference</th>
<th>Rat Probe</th>
<th>Reference</th>
<th>Mouse Probe</th>
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<tr>
<td>Kidney</td>
<td>+ + + +</td>
<td>h 2, 20</td>
<td>+ + +</td>
<td>r</td>
<td>+ +</td>
<td>m 6, 7, 21, 22*</td>
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<tr>
<td>Heart</td>
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<td></td>
<td>m 21, 23</td>
<td>+</td>
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<tr>
<td>Liver</td>
<td>−</td>
<td>h 20</td>
<td>m 21, 23</td>
<td>−</td>
<td>m 6, 8, 21</td>
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<tr>
<td>Brain</td>
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<td></td>
<td>r</td>
<td>m 21, 23</td>
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<tr>
<td>Testis</td>
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<td>Uterus</td>
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<td>4</td>
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<td>m 21, 23</td>
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<td>Chorion</td>
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<td></td>
<td>+ + + +</td>
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<tr>
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<td></td>
<td>+ + +</td>
<td>m 16</td>
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<td>NA</td>
<td></td>
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<td>−</td>
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<tr>
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<td></td>
<td>+ + + m</td>
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</table>

Symbols indicate abundance of renin mRNA as follows: − = absence; + = traces (barely detectable on blotting analysis of total RNA); + + = small quantities, + + = moderate quantities; + + + = large quantities; NA = data not available.

The following designations are used for probes: h = human; r = rat; m = mouse.

In addition to these tissues, the use of mouse probes detected no renin mRNA in the following mouse tissues: intestine, pancreas, muscle, tongue, lacrimal, seminal vesicle, preputial, epididymis, coagulating, hardenan, placenta, mammary.

**Solution hybridization.

†In situ hybridization.
RAT RENIN mRNA/Makrides et al.

**Figure 2.** Blotting analysis of RNA isolated from rat tissues. The RNA was electrophoresed on 1% agarose gels, transferred to nitrocellulose filters, and hybridized with a rat kidney renin cDNA clone pUC-RRc4 (A) or with a rat B-actin probe (B). All lanes contained 25 μg of total RNA. Before hybridization with the actin probe, the renin band was erased as described in Materials and Methods. The DOCA-salt treatment contained 1% NaCl. The exposure time of the x-ray film was 6 days for Panel A and overnight for Panel B, using intensifying screens. Kidney+ = ischemic kidney; Kidney− = hypertrophied kidney.

**Figure 3.** Effect of DOCA and salt treatment on renin mRNA content in rat kidney. Lanes a and b, c and d, and e-g represent three different experiments. Lanes a and b contained 5 μg of poly(A)+ RNA; Lanes c and d contained 50 μg of total RNA; lanes e−g contained 25 μg of total RNA. Nitrocellulose filters were hybridized with rat kidney cDNA clones pUC-RRc4 or pRen-44. The exposure time of the x-ray film was 2 days for Panel A and overnight for Panel B, using intensifying screens. actin probe, Barbara Hertkorn for her skillful technical assistance, and Andrea Karis Bonias for the word processing.

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