Rapid Communication

Distinct Renin Isoforms Generated by Tissue-Specific Transcription Initiation and Alternative Splicing

Min Ae Lee-Kirsch, Francois Gaudet, M. Cristina Cardoso, Klaus Lindpaintner

Abstract—The aspartyl protease renin catalyzes the initial and rate-limiting step in the formation of the biologically active peptide angiotensin II. It is mainly synthesized in the kidney as a preprohormone and secreted via constitutive and regulated pathways. We identified a novel transcript of the rat renin gene, renin b, characterized by the presence of an alternative first exon (exon 1b) that is spliced to exon 2 of the known transcript, termed renin a. We demonstrated that renin b is exclusively expressed in the brain. In contrast, renin a was not expressed in the brain. Using primer extension assays, we mapped the transcriptional start site of this novel mRNA within intron 1 of the rat genomic sequence, suggesting the presence of a brain-specific promoter within intron 1. The presence of a brain-specific renin isoform is evolutionarily conserved, as demonstrated by the finding of renin b isoforms in mice and humans. The predicted protein renin b lacks the prefragment as well as a significant portion of the profragment and is therefore predicted not to be a secreted protein, unlike the classically described isoform renin a. As shown by in vitro translation of full-length renin b mRNA in the presence of microsomal membranes, renin b was not targeted into the endoplasmatic reticulum and remained intracellularly in transiently transfected AtT-20 cells. These findings provide evidence for a novel pathway of intracellular angiotensin generation that occurs exclusively in the brain. (*Circ Res*. 1999;84:240-246.)

Key Words: renin ■ renin-angiotensin system ■ alternative splicing ■ gene expression ■ brain

The renin gene, which encodes the enzyme that catalyzes the rate-limiting step in the formation of the biologically active peptide angiotensin II (Ang II), consists of 9 exons. They are arranged as 2 homologous clusters of 4 exons each, with the first exon being separated from the remainder of the gene by a 5-kb intron.[1] Renin is synthesized as a preprotein of 45 kDa. The first exon encodes the signal peptide that targets the nascent protein into the endoplasmic reticulum, where it undergoes glycosylation.[2] Renin is secreted via constitutive and regulated pathways as both inactive prorenin and active renin.[2]

Renin is primarily synthesized in the juxtaglomerular cells of the kidney and is released into the circulation after specific stimuli such as volume depletion. Among the classic functions of the humoral renin-angiotensin system (RAS) are vasoconstriction and stimulation of aldosterone release by the adrenal gland. Components of the RAS have also been demonstrated in the brain, both outside and inside the blood-brain barrier.[3–10] Recognized actions of the brain RAS include modulation of central regulation of blood pressure and sympathetic outflow, the release of hypothalamic and pituitary hormones, and central control of renal sodium handling.[3] These effects have been attributed to the action of Ang II via the AT_1 receptor subtype.[11,12] More recent studies

suggest additional functional roles of Ang II within the central nervous system, mediated primarily through the AT_2 receptor subtype, in processes such as neuronal development,[9,10] apoptosis,[13] and complex behavior.[14,15] Altered states of activation of the brain RAS have also been suggested as possibly contributing to or causing certain forms of hypertension.[16]

In the present study, we provide evidence for the presence of a novel molecular variant of renin, expressed exclusively in the brain, that arises from the use of an alternative promoter within intron 1 and from alternative splicing, resulting in a nonsecreted renin isoform.

Materials and Methods

Rapid Amplification of cDNA Ends (RACE)

Total RNA was extracted from frozen tissues by the guanidium thiocyanate–cesium chloride gradient method. First-strand cDNA was synthesized from 2 μg total RNA from rat brain and kidney tissues (Sprague-Dawley) with primer R1 (5′-ccctgatccatagtg-gatggtgaa-3′) located at position 7311 of the published rat renin sequence[1] using Superscript II RNase H⁻ Reverse Transcriptase (Life Technologies, Inc). The cDNA was column-purified, tailed with terminal transferase (Life Technologies) in the presence of dCTP, and then amplified in 2 sequential rounds of nested polymerase chain reaction (PCR) in a 25-μL reaction solution, containing 200 nmol/L of each deoxynucleotide triphosphate, 2 mmol/L mag-

Circulation Research is available at http://www.circresaha.org

nesium chloride, 0.1 U Taq polymerase (Promega), 75 nmol/L of a poly-G anchor primer, and 200 nmol/L of the gene-specific primers R2 (5′-agaggttggctgaacccgtgtc-3′) and R3 (5′-tttgaaggtctgggatg-gggtacc-3′), respectively. In each reaction, an initial denaturation step for 2 minutes at 94°C was followed by 35 cycles of 94°C for 30 seconds, 56°C for 1 minute, and 72°C for 2 minutes and by a final extension at 72°C for 10 minutes. Amplification products were fractionated on a 2% agarose gel, purified, and cloned into the PCRII vector using the TA Cloning Kit (Invitrogen). Clones were sequenced with primer R3 on an ABI373A sequencer (Applied Biosystems). Cloning of the murine (C56 black) and human homologs from brain RNA (Clontech) was similarly carried out by RACE using primers M1 through M3 (M1: 5′-tgatccgtagtggatggt-gaagtc-3′; M2: 5′-cagaggactcatagaggttgtgaa-3′; and M3: 5′-agcaag-gtagaggcggctgcactt-3′) and H1 through H3 (H1: 5′-gacctctccaaac-atctgtgtcac-3′; H2: 5′-ccactgactgtccctgttgaatag-3′; and H3: 5′-gagggtgagttctgttccattgtg-3′), respectively. All newly determined sequences have been deposited with the GenBank database (accession numbers: AF117820, AF117821, AF117822).

Primer Extension Assay

Primer R4 (5′-tgatcctggtcatgtctactccccgctcctccaggatttc-3′) complementary to a sequence between position 5840 and 5879 in exon 2 of the rat renin DNA[1] was radiolabeled with $[\gamma\text{-}^{32}P]$-ATP (DuPont NEN) using T4 polynucleotide kinase (New England Biolabs) and was column-purified. After overnight hybridization at 45°C of 100 000 cpm of labeled primer against total RNA from rat kidney (40 μg) and brain (100 μg) in a buffer containing 80% formamide, 40 mmol/L PIPES, 400 mmol/L NaCl, and 1 mmol/L EDTA, and subsequent ethanol precipitation, reverse transcription was carried out at 42°C in the presence of 500 μmol/L of each deoxynucleotide triphosphate using Superscript II RNase H⁻ Reverse Transcriptase (Life Technologies). Extension products were purified by extraction with phenol/chloroform/isoamylalcohol (25:24:1) ethanol-precipitated and size-fractionated on a 5.5% denaturing polyacrylamide sequencing gel alongside a sequencing ladder obtained in a standard sequencing reaction using labeled M13$_{-40}$ primer on a pGEM vector (Promega) template.

RNase Protection Assay

Complementary RNAs were transcribed from 1 μg linearized plasmid pRen412[17] or pT3RR3K encompassing exons 1a, 2, and 3 and exons 1b, 2, and 3 of the rat renin gene, respectively, in the presence of $[\alpha\text{-}^{32}P]$-UTP (DuPont NEN) using the MAXIscript kit (Ambion). A rat β-actin cRNA was transcribed from the plasmid pSKrβact[17] and used as an internal control. Labeled probes were purified over Chromaspin-100 columns (Clontech). RNase protection assays were carried out using the RPAII kit (Ambion). Briefly, 100 000 cpm of labeled renin probe and 10 000 cpm of β-actin probe was hybridized overnight at 45°C against 40 to 100 μg total RNA or tRNA. Samples were digested with RNase A and T1, precipitated, and run on a 5.5% denaturing polyacrylamide sequencing gel.

In Vitro Translation

Full-length cDNAs for kidney and brain isoforms of the rat renin transcript were generated by reverse transcriptase–polymerase chain reaction (RT-PCR), cloned into the pCDNA3 vector (Invitrogen), and sequenced. mRNA was transcribed from 1 μg plasmid linearized at the 3′-end of the inserts (MEGAscript kit, Ambion). In vitro translation was carried out using 2 μg of synthetic mRNA in the presence of rabbit reticulocyte lysate (Promega), ^{35}S-methionine (DuPont NEN), and different amounts of canine microsomal membranes (Promega). After incubation at 30°C for 2 hours, the reaction products were fractionated over a 10% SDS polyacrylamide gel, dried, and exposed to x-ray film.

Transient Transfection of AtT-20 Cells and Measurement of Renin Activity

AtT-20 cells (American Type Culture Collection) were plated in F10 medium supplemented with 10% FCS (Life Technologies) at a density of 5×10^5 cells per 6-cm dish. After 16 hours, the medium was changed to growth medium (F10 supplemented with 15% horse serum and 2.5% FCS), and cells were transfected with 10 μg plasmid using the calcium phosphate precipitation method. Transfection efficiencies were monitored by cotransfection of the β-galactosidase gene. Each transfection experiment also included a control transfection with only the β-galactosidase gene. Cell extracts of this control yielded no measurable renin activity. Statistical analysis was carried out with Student t test, and $P<0.5$ was considered to be significant. Forty-eight hours later, cells were washed once with prewarmed medium, incubated in fresh medium, and collected 3 hours later. The medium was briefly centrifuged to remove debris, and the supernatant was transferred into fresh tubes containing protease inhibitor cocktail (1 mmol/L EDTA, 100 μg/mL PMSF, 2 μg/mL leupeptin, and 1 μg/mL pepstatin). Cells were washed twice with 1× PBS, scraped, pelleted, and lysed in 100 μL radioimmunoprecipitation assay buffer (150 mmol/L NaCl, 50 mmol/L Tris-HCl [pH 8], 0.1% SDS, 1% NP-40, and 0.5% deoxycholate) containing the protease inhibitor cocktail. Trypsin activation of prorenin and renin incubation in the presence of rat angiotensinogen were carried out as previously described.[17] Ang I was measured using the RIANEN radioimmunoassay kit (Amersham). Prorenin was calculated as the difference between renin with and without trypsin activation.

Results

Identification of a New Alternatively Spliced Renin Isoform

Using a plasmid encompassing the first 3 exons of the previously published rat renin cDNA as a probe for RNase protection assays, we noted that the protected fragment obtained from brain RNA was shorter than expected. To determine the nature of this difference, the PCR-based cloning strategy RACE was used. Sequencing of cDNAs obtained by RACE from kidney and brain led to the identification of 2 distinct renin mRNA species designated renin a and renin b, respectively. The distinct 5′-terminal sequences represent different first exons termed exons 1a and 1b, respectively, that are spliced to a common second exon. Although exon 1a represents the first published exon of the renin gene,[1] exon 1b has not been described previously. Databank searches showed that exon 1b, which is 46 bp in length, maps within the first intron of the published genomic sequence of the renin gene, from position 5083 to 5129[1] (Figure 1). The presence of a C at position 5112 in the published sequence that would give rise to an open reading frame was absent in our RACE clones and was shown to be a mistake in the published sequence by sequencing genomic DNA containing exon 1b from various rat strains. Exon 1b does not contain an open reading frame, and the nearest downstream methionine is located at position 5825 of the published sequence. Thus, the predicted renin b protein lacks the prefragment as well as a part of the profragment, which are encoded by exon 1a. RT-PCR with isoform-specific sense and antisense primers targeting various positions of the 2 transcripts and sequencing of PCR products yielded no evidence for further variations within the coding sequence of the renin gene (data not shown).

Renin b Is Also Present in Mouse and Human

To determine whether the finding of a brain-specific renin isoform is a species-specific phenomenon restricted to the rat or whether it represents an evolutionarily conserved biological phenomenon, we used RACE on RNA derived from mouse

A
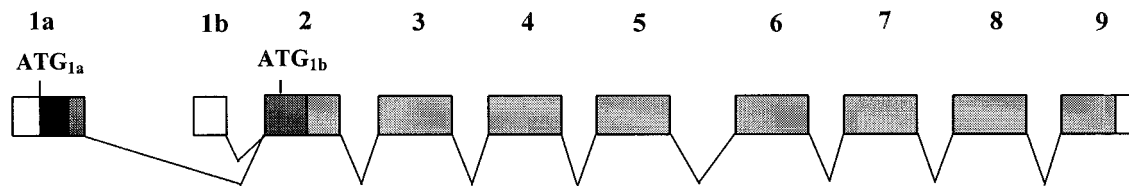
B

```
rat                                                                    CTTGAA TTTCCCCAGT
mouse                                       TCCGGC TGCTTTGAAG ATTTGATGAG AGGATACGCA
human                                     T TTTCCAGAGG ATTATGTAAC CCAAATCCAA GCACACACCA
```

```
                                                    exon 1b | exon 2
rat    CAGTGATGAT TGGAGGACAA CTGATTTGAG AATCTTGCTC AAGAAAATGC CCTCGGTCCG
mouse  TAGCACTTCC GATGCCAGGC GGGGAACGGT AATCCCGCTC AAGAAAATGC CTTCTGTCCG
human  CTCACCACAC AACAGCAAGT AAGCTGAGAG GATCTTCCTC AAGAGAATGC CCTCAATCCG
```

```
                                                  ↓
rat    GGAAATCCTG GAGGAGCGGG GAGTAGACAT GACCAGGATC AGTGCTGAAT GGGGTGAATT
mouse  GGAAATCCTG GAGGAGCGGG GAGTGGACAT GACCAGGCTC AGTGCTGAAT GGGGCGTATT
human  AGAAAGCCTG AAGGAACGAG GTGTGGACAT GGCCAGGCTT GGTCCCGAGT GGAGCCAACC
```

```
rat    CATCAAGAAG TCTTCCTTTA CCAATGTTAC CTCCCCCGTG GTCCTCACCA ACTACTTGGA
mouse  CACAAAGAGG CCTTCCTTGA CCAATCTTAC CTCCCCCGTG GTCCTCACCA ACTACCTGAA
human  CATGAAGAGG CTGACACTTG GCAACACCAC CTCCTCCGTG ATCCTCACCA ACTACATGGA
```

Figure 1. Alternative transcription initiation and splicing of the rat renin gene. A, Schematic presentation of the genomic organization of the rat renin gene. ATG_{1a} and ATG_{1b} denote the translation initiation sites for renin a and renin b, respectively. Coding regions for the signal peptide appear in black, the profragment in dark gray, and the active enzyme in light gray. Drawings are not to scale. B, Alignment of the exon 1b and exon 2 sequences of the rat, mouse, and human renin genes. Homologies are shaded gray. In-frame stop codons and start codons are underlined. The arrow indicates the carboxy terminus of the propeptide.

and human brain using analogous experimental conditions as for the rat. As shown in Figure 1, sequencing of RACE clones revealed the presence of brain-specific renin variants in both the mouse and human renin genes. As in the rat, an alternative first exon is spliced to a common exon 2. Comparison of the murine alternative exon 1b, which is 66 bp in length, with the genomic sequence demonstrated it to be located within the first intron of the mouse *Ren-1* gene.[18] The human exon 1b, which is 71 bp in length, also maps within the first intron of the human renin gene,[19] as could be shown by long-range PCR amplification of the first intron, followed by Southern blot analysis using the exon 1b sequence as a probe (data not shown).

Mapping of the Transcriptional Start Sites of Renin a and Renin b

Primer extension analysis using a primer located at position 5840 within exon 2 of the rat renin sequence resulted in 2 unique extension products of 194 bp and 117 bp in size in kidney and brain, respectively, that are in agreement with the predicted length of renin a and renin b mRNA on the basis of the published start site for renin a and on the cDNA clones obtained by RACE for both renin a and renin b[1] (Figure 2).

Expression of Renin a and Renin b in Rat Tissues

Using a renin a–specific probe for RNase protection analysis, we found a 295-bp fragment corresponding to exons 1a to 3 that was protected in the kidney as well as in extrarenal tissues such as intestine, testes, adrenal, and whole embryo, whereas a shorter protected fragment of 183 bp, corresponding to exons 2 to 3, was found in the brain (Figure 3). In contrast, using a renin b–specific probe, we found a 229-bp fragment corresponding to exons 1b to 3 that was protected in the brain, whereas only the 183-bp fragment was apparent in all other tissues examined (Figure 3). RNase protection assays for renin, normalized for β-actin, on subregions of the brain revealed that the highest expression of renin b mRNA within the central nervous system is present in the midbrain, followed by medulla oblongata, hypothalamus/thalamus, and cerebellum, whereas renin a mRNA was undetectable. Neither renin a nor renin b expression was detectable in the cerebral cortex (Figure 3).

Unlike Renin a, Renin b Is Not Processed in the Presence of Microsomal Membranes

Renin b lacks the prefragment necessary for targeting the nascent polypeptide into the endoplasmatic reticulum and is
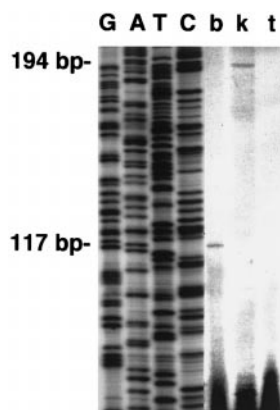
Figure 2. Mapping of the transcriptional start site of renin a and renin b by primer extension assay. Extension products of primer R4 located in exon 2 of the rat renin gene using 100 μg and 40 μg of RNA from brain (b) and kidney (k), respectively. A sequencing ladder primed by M13$_{-40}$ on pGEM vector was used as a size marker (G, A, T, C). t indicates tRNA control.

therefore expected not to be processed into the secretory pathway. For verification, full-length in vitro transcribed mRNAs for both isoforms were translated in the absence or presence of canine microsomal membranes. In the presence of microsomal membranes, translation of renin a mRNA led to a shift of the reaction products from 45 kDa toward a higher molecular mass, consistent with translocation and glycosylation of the nascent protein; conversely, a 42-kDa protein that did not undergo processing was seen when renin b mRNA was used as a template in the same experiment (Figure 4).

Unlike Renin a, Renin b Is Not Secreted by Transiently Transfected AtT-20 Cells

To further confirm that renin b is not a secreted protein, full-length cDNA clones of renin a and renin b were transiently transfected into AtT-20 cells, which are capable of correctly targeting and processing secretory proteins.[20] Renin activity in media and cell lysates with and without prior trypsin activation of prorenin to renin was determined after incubation in the presence of excess rat angiotensinogen, followed by measurement of generated Ang I by radioimmunoassay. As shown in Figure 5, expression of renin a in AtT-20 cells gave rise to prorenin as well as renin, both of which were also secreted, as shown by measurements of renin activity in the medium. In contrast, transfection of renin b into AtT-20 cells resulted in measurable levels of active renin only in cell lysates but not in the medium, consistent with the concept that the alternative transcript generates only active renin that is not secreted and remains intracellularly.

Discussion

Our results demonstrate that renin mRNA occurs in 2 isoforms: (1) termed renin a, is identical to the previously described renin transcript and (2) termed renin b, represents a new isoform. These isoforms are characterized by the presence of different first exons, exon 1a and exon 1b, respectively, and by highly specific and mutually exclusive expression patterns outside and within the central nervous system.
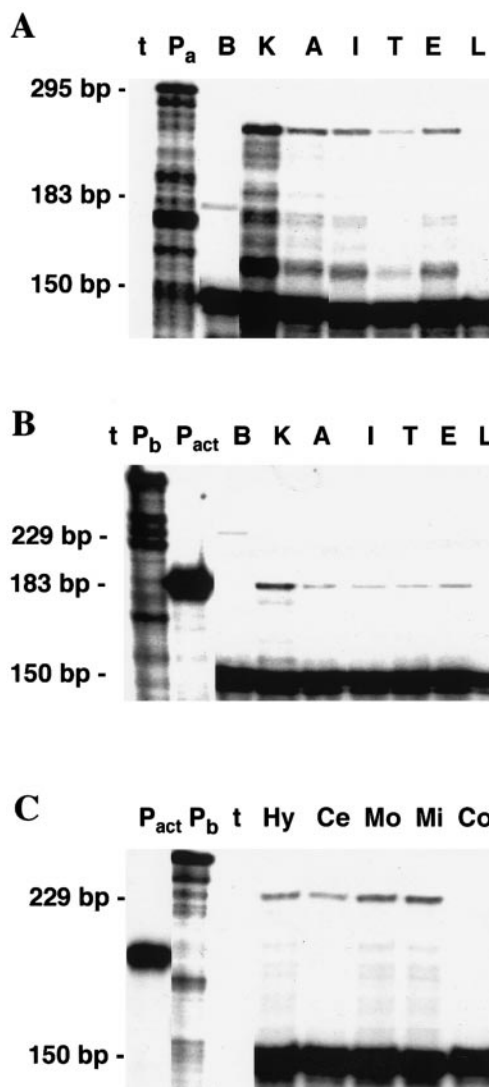

Figure 3. Expression of renin a and renin b in rat tissues. RNase protection analysis using 40 μg total RNA from kidney (K), 100 μg from whole brain (B), adrenal (A), intestine (I), testis (T), liver (L), and whole embryo (E) with probe pRen412/*Hind*III encompassing exons 1a to 3 (P$_a$, panel A) or pT3RR3K/*Pst*I encompassing exons 1b to 3 (P$_b$, panel B). All samples were cohybridized with a β-actin cRNA (P$_{act}$) probe as an internal control. In vitro transcribed probes P$_a$, P$_b$, and P$_{act}$ were 326 bp, 266 bp, and 178 bp, respectively, and included a vector-encoded sequence. The sizes of the protected fragments for P$_a$, P$_b$, and P$_{act}$ were 295 bp, 229 bp, and 150 bp, respectively. A shorter fragment of 183 bp corresponds to exon 2 and exon 3 sequences. Expression of renin b in subregions of the brain, midbrain (Mi), medulla oblongata (Mo), hypothalamus/thalamus (Hy), cerebellum (Ce), and cortex (Co) is shown in panel C. t indicates tRNA control.

Moreover, renin a mRNA gives rise to a secreted isoenzyme, whereas renin b translation results in an isoform that remains intracellularly. The newly identified exon 1b is located within the first intron of the renin gene and is spliced to exon 2. The intron-exon junctions of both renin a and renin b are flanked by typical consensus splice donor and acceptor sequences.[21] End analysis (5′-) by RACE and primer extension assays show the presence of 2 distinct 5′-leader sequences and transcriptional start sites, demonstrating the existence of 2
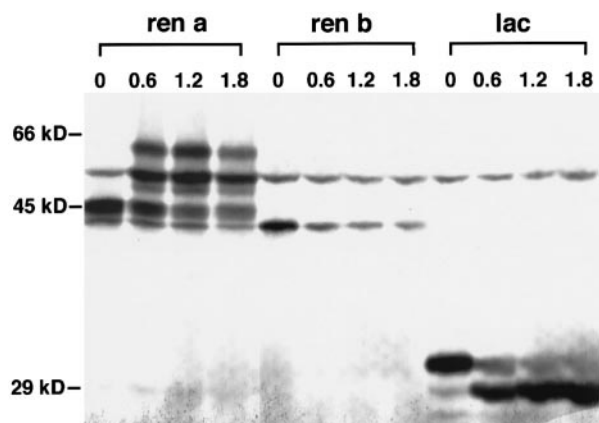
Figure 4. In vitro translation of renin a and renin b. Translation products of in vitro–transcribed mRNAs derived from full-length cDNA clones of rat renin a and renin b synthesized in the presence of 0, 0.6, 1.2, and 1.8 μL of canine microsomal membranes (0, 0.6, 1.2, and 1.8). Processing of renin a (ren a) from preprorenin to glycosylated prorenin leads to a shift in molecular mass of 45 kDa to 50 kDa. In vitro translation of renin b (ren b) yields a protein of 42 kDa, which is not processed in the presence of microsomal membranes. lac indicates *Escherichia coli* β-lactamase control for signal peptide processing.

unique primary transcripts that are presumably under the control of alternative promoters. As demonstrated by RNase protection assays, expression of the newly described isoform is restricted exclusively to the central nervous system; in contrast, we found no evidence for renin a expression in the brain, suggesting a high degree of tissue specificity of the alternative promoter. A pattern search for putative promoter elements in the sequence flanking exon 1b revealed no TATA box but revealed the presence of a number of consensus sequences such as activator protein-1 (AP-1) and AP-2 sites, glucocorticoid response elements (GREs), and Pit elements.

Whereas exon 1a encodes the prefragment and part of the profragment of renin a, exon 1b does not contribute an in-frame start codon. Therefore, renin b encodes a truncated form of renin. As a result, the 2 proteins share most of their sequences except the N-terminus, given that a renin a internal methionine is predicted to serve as the start codon for the brain isoform. The first and second in-frame ATGs in exon 2 are not found within the context of a Kozak consensus sequence.[22] However, as suggested by size estimates on the
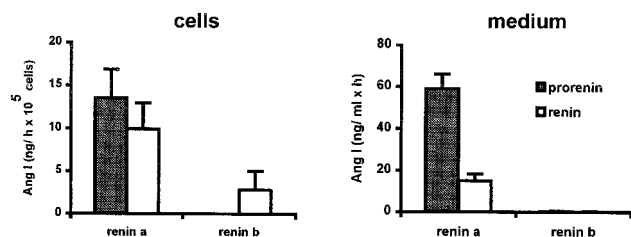

Figure 5. Renin activity in media and cell lysates of AtT-20 cells transfected with renin a and renin b cDNAs. Prorenin (solid bars) and renin (open bars) in cell lysates and media of AtT-20 cells transfected with full-length cDNAs for rat renin a and renin b. Values represent the mean±SEM of 3 independent transfections. Differences in renin activity between lysate and medium of cells transfected with renin b were statistically significant ($P<0.5$).

basis of our in vitro translation studies, initiation of translation begins at the first in-frame ATG within exon 2. Because no discernible targeting sequences are encoded downstream of this ATG, the resulting protein lacks the signal sequence that normally directs the kidney isoform, renin a, into the secretory pathway.[2] Furthermore, owing to the incomplete profragment, which sterically inactivates the active site within the cleft of the bilobar structure of the classic renin molecule,[2] we predicted that renin b would be synthesized as active renin only. Indeed, transfection experiments established that the polypeptide encoded by the renin b transcript is fully functional and capable of cleaving angiotensinogen to produce Ang I. Moreover, prior incubation of cell lysates with trypsin resulted in no additional renin activity, indicating that renin b, unlike renin a, is not synthesized as an inactive precursor. The observation that the renin b polypeptide yields active renin, despite the fact that it retains two thirds of the profragment, is consistent with earlier experiments demonstrating that N-terminal but not C-terminal fragments of the propeptide exhibit strong renin inhibitory properties.[23] Indeed, substitution of the first arginine residue of the human profragment with glutamine is known to yield active prorenin.[24] Thus, integrity of the N-terminal portion of the profragment, in particular the first arginine, which is highly conserved among rat, mouse, and human renins, is necessary for maintaining prorenin enzymatically inactive. The overall enzymatic activity of renin b appears to be lower, a feature that may be due to a shorter half-life of either the transcript or the protein, to differential translational efficiencies or enzyme kinetics, to different protein folding, or to different posttranslational processing.

Renin consists of 2 domains encoded by exons 2 to 5 and 6 to 9, respectively, which are thought to have arisen by duplication of an ancestral gene.[2,25] The finding of exon 1b in murine, rat, and human renin genes indicates a high degree of conservation throughout evolution. Given that compartmentalization of proteins is a feature characteristic of eukaryotic cells, one may speculate that the addition of the signal sequence encoded by exon 1a represents a later event in evolution, and that the existence of a nonsecreted renin isoform reflects the persistence of an archaic form of renin.

A number of genes have been shown to invoke controlled mechanisms of alternative RNA processing in the generation of protein diversity. The use of alternative promoters and/or of differential splicing allows the organism to tailor gene products to the functional demands of different cell types by tissue-specific regulation of expression. This mechanism is also used to generate proteins that are targeted to different subcellular localizations, thus achieving compartmentalization of function. For example, the angiotensin-converting enzyme gene is transcribed from an alternative promoter within intron 12 exclusively in the male gonad, resulting in a testis-specific isoenzyme; male mice lacking the angiotensin-converting enzyme gene demonstrate reduced fertility.[26,27] In the case of the yeast invertase gene, constitutive expression produces an mRNA encoding cytosolic invertase, whereas glucose repression regulates transcription from an alternative promoter, resulting in an mRNA encoding a signal sequence and leading to a secreted form of the enzyme.[28]

Principal central nervous system sites involved in cardiovascular control are the periventricular tissues surrounding the third ventricle, hypothalamic nuclei, the periaqueductal gray matter in the midbrain, and nuclei in the medulla oblongata.[3,4] Unlike the circumventricular organs, these sites are separated from the systemic circulation by the blood-brain barrier. The expression pattern of renin b mRNA, with the highest expression found in midbrain followed by hypothalamus/thalamus, medulla oblongata, and cerebellum, corresponds to these sites. Stimulation of central Ang II receptors by Ang II elicits systemic cardiovascular and behavioral responses and influences the secretion of hypothalamic and pituitary hormones. Thus, injection of Ang II into hypothalamic tissue or the lateral ventricle of rats elicits drinking behavior, sodium appetite, elevation of blood pressure, natriuresis, and the release of vasopressin.[3] Moreover, Ang II has been proposed to act as a neurotransmitter or neuromodulator, because neural pathways containing immunoreactive Ang II have been shown to connect areas responsible for cardiovascular and dipsogenic control.[4] More recent findings suggest additional roles of centrally generated Ang II in neuronal development,[9,10] apoptosis,[13] and complex behavior.[14,15] Angiotensinogen, the only known substrate for renin is expressed both in glial cells and neurons.[5,6] Along with the well-documented presence of angiotensin-converting enzyme[7] and angiotensin receptors[8–10] in the brain, our findings are consistent not only with the central generation of Ang II[16] but also provide a novel mechanism for intracellular generation of Ang II. The observation that only renin b but not renin a is expressed in the brain suggests that all centrally generated Ang II is attributable to renin b. This mechanism may explain earlier observations of intracellular renin activity in rat and mouse neuroblastoma cells.[29] In contrast to the classic humoral RAS, in which renin reaches its target via the circulation, this mechanism is of particular relevance for the central nervous system, a setting in which distribution of an inactive proenzyme via the circulation may appear redundant in view of the fact that cell of origin and target cell may be located next to each other or even be identical. Whether intracellular renin b interacts with the renin-binding protein, an intracellular protein of yet unknown function, remains to be investigated.[30]

The transcriptional start site of brain renin is located approximately 1 kb downstream of a polymorphic tandem repeat that has previously been used as a marker in linkage studies. Thus, the renin gene has been implicated as causally related to altered blood pressure regulation in rat models of hypertension such as the Dahl rat and the spontaneously hypertensive rat.[31–33] However, no mutations within the known coding regions have so far been identified.[34] It is noteworthy, in this context, that an increased activity of the brain RAS has been found in both these strains when compared with normotensive reference strains.[16,35,36] Alterations within regulatory sequences responsible for the expression of renin b, which may relate to this polymorphic site, may account for the observed differences.

In summary, we report the identification of a novel isoform of renin and demonstrate that this isoform is exclusively expressed in the brain and, more specifically, in regions known to be important centers for the integration of central control of the cardiovascular system; that the existence and expression pattern of this isoform are conserved in rats, mice, and humans; and that, as predicted by the absence of the prefragment and a portion of the profragment, this isoform is not secreted but remains as an enzymatically active protein in the intracellular space. Evidence for the physiopathological relevance of this brain-specific alternative pathway for Ang II generation must await more function-oriented studies.

References

1. Fukamizu A, Nishi K, Cho T, Saitoh M, Nakayama K, Ohkubo H, Nakanishi S, Murakami K. Structure of the rat renin gene. *J Mol Biol*. 1988;201:443–450.
2. Morris BJ. Molecular biology of renin I: gene and protein structure, synthesis and processing. *J Hypertens*. 1992;10:209–214.
3. Ganong WF. The brain renin angiotensin system. *Annu Rev Physiol*. 1984;46:17–32.
4. Lind RW, Swanson LW, Ganten D. Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. An immunohistochemical study. *Neuroendocrinology*. 1985;40:2–24.
5. Campbell DJ, Habener JF. Angiotensinogen gene is expressed and differentially regulated in multiple tissues of the rat. *J Clin Invest*. 1986;78:31–36.
6. Kumar A, Rassoli A, Raizada MK. Angiotensinogen gene expression in neuronal and glial cells in primary cultures of rat brain. *J Neurosci Res*. 1988;19:287–290.
7. Whiting P, Nava S, Mozley L, Eatham H, Poat J. Expression of angiotensin converting enzyme mRNA in rat brain. *Mol Brain Res*. 1991;11:93–98.
8. Mendelsohn FAO, Quirion R, Saavedra JM, Aguilera G, Catt KJ. Autoradiographic localization of angiotensin II receptors in rat brain. *Proc Natl Acad Sci U S A*. 1984;81:1575–1579.
9. Mukoyama M, Nakajima M, Horiuchi M, Sasamura H, Pratt RE, Dzau VJ. Expression cloning of type 2 angiotensin II receptor reveals unique class of seven-transmembrane receptors. *J Biol Chem*. 1993;268:24539–24542.
10. Kambayashi Y, Bardhan S, Takahashi K, Tsuzuki S, Inui H, Hamakubo T, Inagami T. Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition. *J Biol Chem*. 1993;268:24543–24546.
11. Murphy TJ, Alexander RW, Griedling KK, Runge MS, Bernstein KB. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature*. 1991;352:233–236.
12. Sasaki K, Yamano Y, Bardhan S, Iwai N, Murray JJ, Hasegawa M, Matsuda Y, Inagami T. Cloning an expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor. *Nature*. 1991;352:230–232.
13. Yamada T, Horiuchi M, Dzau VJ. Angiotensin II type 2 receptor mediates programmed cell death. *Proc Natl Acad Sci U S A*. 1996;93:156–160.
14. Hein L, Barsh GS, Pratt RE, Dzau VJ, Kobilka BK. Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice. *Nature*. 1995;377:744–747.
15. Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BLM, Inagami T. Effects on blood pressure and exploratory behaviour of mice lacking the angiotensin II type-2 receptor. *Nature*. 1995;377:748–750.
16. Ganten D, Hermann K, Bayer C, Unger T, Lang RE. Angiotensin synthesis in the brain and increased turnover in hypertensive rats. *Science*. 1983;221:869–871.

17. Lee MA, Bohm M, Kim S, Bachmann S, Bachmann J, Bader M, Ganten D. Differential gene expression of renin and angiotensinogen in the TGR(mREN-2) transgenic rat. *Hypertension*. 1995;25:570–580.

18. Burt DW, Mullins LJ, George H, Smith G, Brooks J, Pioli D, Brammar WJ. The nucleotide sequence of a mouse renin-encoding gene, Ren-1d, and its upstream region. *Gene*. 1989;84:91–104.

19. Morris BJ. New possibilities for intracellular renin and inactive renin now that the structure of the human renin gene has been elucidated. *Clin Sci*. 1986;71:345–355.

20. Moore H-PH, Walker MD, Lee F, Kelly RB. Expressing a human proinsulin-cDNA in a mouse ACTH-secreting cell. Intracellular storage, proteolytic processing, and secretion on stimulation. *Cell*. 1983;35:531–538.

21. Mount SM. A catalogue of splice junction sequences. *Nucleic Acids Res*. 1982;10:459–468.

22. Kozak M. An analysis of 5′-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res*. 1987;15:8125–8148.

23. Cumin F, Evin G, Fehrentz J-A, Seyer R, Castro B, Menard J, Corvol P. Inhibition of human renin by synthetic peptides derived from its pro-segment. *J Biol Chem*. 1985;260:9154–9157.

24. Yamauchi T, Nagahama M, Watanabe T, Ishizuka Y, Hori H, Murakami K. Site-directed mutagenesis of human prorenin. Substitution of three arginine residues in the propeptide with glutamine residues yields active prorenin. *J Biochem (Tokyo)*. 1990;107:27–31.

25. Holm I, Ollo R, Panthier J-J, Rougeon F. Evolution of aspartyl proteases by gene duplication: the mouse renin gene is organized in two homologous clusters of four exons. *EMBO J*. 1984;3:557–562.

26. Kumar RS, Kusari J, Roy SN, Soffer RL, Ganes C. Structure of testicular angiotensin converting enzyme. *J Biol Chem*. 1989;164:16754–16760.

27. Krege JH, John SWM, Langenbach LL, Hodgin JB, Hagaman JR, Bachman ES, Jennette JC, O'Brien DA, Smithies O. Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature*. 1995;375:146–148.

28. Carlson M, Botstein D. Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. *Cell*. 1982;28:145–154.

29. Okamura T, Clemens DL, Inagami T. Intracellular renin in neuroblastoma cells. *Proc Natl Acad Sci U S A*. 1981;78:6940–6943.

30. Takahashi S, Ohsawa T, Miura R, Miyake Y. Purification and characterization of renin binding protein (RnBP) from porcine kidney. *J Biochem (Tokyo)*. 1983;93:1583–1594.

31. Rapp JP, Wang S-M, Dene H. A genetic polymorphism in the renin gene of Dahl rats cosegregates with blood pressure. *Science*. 1989;243:542–544.

32. Kurtz TW, Simonet L, Kabra PM, Wolfe S, Chan L, Hjelle BL. Cosegregation of the renin allele of the spontaneously hypertensive rat with an increase in blood pressure. *J Clin Invest*. 1990;85:1328–1332.

33. Pravenec M, Simonet L, Kren V, Kunes J, Levan G, Szpirer J, Szpirer C, Kurtz T. The rat renin gene: assignment to chromosome 13 and linkage to the regulation of blood pressure. *Genomics*. 1991;9:466–472.

34. Alam KY, Wang Y, Dene H, Rapp JP. Renin gene nucleotide sequence of coding and regulatory regions in Dahl rats. *Clin Exp Hypertens*. 1993;15:599–614.

35. Samani NJ, Swales JD, Brammar WJ. A widespread abnormality of renin gene expression in the spontaneously hypertensive rat modulation in some tissues with the development of hypertension. *Clin Sci*. 1989;77:629–636.

36. Teruya H, Muratani H, Takishita S, Sesoko S, Matayoshi R, Fukiyama K. Brain angiotensin II contributes to the development of hypertension in Dahl-Iwai salt-sensitive rats. *J Hypertens*. 1995;13:883–890.