Extra-renal transcription of the renin genes in multiple tissues of mice and rats

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Communicated by François Jacob, April 17, 1989 (received for review February 9, 1989)

ABSTRACT Expression of the mouse renin genes (Ren-1 and Ren-2) and of the unique rat renin gene was determined in several extra-renal tissues of mice and rats by primer-directed enzymatic amplification of cDNAs. In addition to the adrenal glands, testis, and ovaries, renin transcripts are detected in the liver, whole brain, and hypothalamus and, at lower levels, in spleen, thymus, lung, and prostate. Expression of the rat renin gene correlates with that of the mouse Ren-1 gene with the notable exception of the submaxillary gland where renin transcripts are found only in mice. The levels of renin transcripts in the liver of females from both species are higher than in males. In mice, the relative levels of Ren-1 and Ren-2 transcripts vary widely in different tissues. These results support the hypothesis of a local renin-angiotensin system in multiple extra-renal sites and imply the existence of complex mechanisms of regulation of the renin gene, previously thought to be expressed in a tissue-specific manner.

Renin plays an important role in the control of blood pressure by cleaving angiotensinogen into angiotensin I. Circulating renin, in its active and inactive pro-renin forms, is produced by the juxtaglomerular cells of the kidney. In mice, the submaxillary gland (SMG) constitutes another major source of renin. The observation of renin activity in several other extra-renal tissues (for review, see ref. 1) has led to the suggestion of extra-renal renin-angiotensin systems. However, activity measurements are sometimes ambiguous due to nonspecific renin-like activities of other proteases and the possible contribution of blood-borne renin in tissue samples (1). The synthesis of renin in extra-renal sites has been supported by the detection of renin mRNA in tissues including the adrenal glands, testis, ovaries, brain, and heart (2–6). However, in the latter studies, renin mRNA levels are often close to the limit of detection by RNA blot analysis. Furthermore, the methods of detection used in studies of extra-renal renin gene expression have not allowed discrimination between transcripts of the two mouse renin genes, Ren-1 and Ren-2.

Strains of laboratory mice can be divided in two categories on the basis of SMG renin expression: Strains, such as BALB/c or C3H, that produce small amounts of SMG renin possess a single renin gene, designated Ren-1, which encodes for the circulating enzyme. Strains, such as DBA/2 or Swiss, which have an additional copy of the renin gene, designated Ren-2, exhibit 100-fold higher SMG renin (7–10) resulting from the higher SMG transcription of Ren-2. Renin activity is higher in the SMG of males and transcriptional regulation of Ren-1 and Ren-2 by androgens has been demonstrated (11). In contrast to the SMG, Ren-1 and Ren-2 are transcribed at the same level in kidney juxtaglomerular cells (12).

To elucidate the mechanisms underlying tissue-specific expression of the mouse and rat renin genes and the differential expression of the two mouse genes, several tissues have been examined for the presence of renin transcripts using a specific and much more sensitive method based upon amplification of renin mRNA sequences by the polymerase chain reaction (PCR) (13, 14).

MATERIALS AND METHODS

Amplification of Renin cDNA. Total RNA was prepared from tissue as described (15). Five micrograms of total RNA was used for cDNA first-strand synthesis (15) in a total volume of 15 μl using 0.1 μg of (dT)12–18 as primer and Moloney murine leukemia virus reverse transcriptase. After an incubation period of 1 hr at 37°C, the PCR with Thermus aquaticus DNA polymerase (14) was carried out directly on the entire cDNA reaction mixture or on a 1.5-μl aliquot in a final reaction volume of 100 μl. The concentration of the two PCR primers was in excess over the oligo(dT) and the mean length of the latter limited annealing at the temperature used for amplification. Therefore, only the amplified product primed by the two specific primers was obtained. Three dry blocks were used for the enzymatic amplification. Each cycle consisted of heating for 2 min at 95°C, followed by annealing at 52°C for 2.5 min, and elongation at 70°C for 2 min. This was repeated for 30 cycles. The last cycle was followed by an additional incubation period of 8 min at 70°C. After gradual cooling to room temperature, the DNA was precipitated with ethanol and dissolved in 100 μl of water.

Southern Blot Analysis. A 10-μl aliquot of the amplified cDNA was digested with Alu I (mouse samples only) before loading on a 2.5% agarose gel. The DNA was transferred by capillary action to a Zeta-bond nylon membrane. The internal probe was end-labeled in the presence of radiolabeled ATP by T4 polynucleotide kinase. The probe hybridization was carried out at 55°C in 6× SSC/10× Denhardt’s solution/0.7% SDS/25 mM sodium phosphate, pH 7.0/2 mM EDTA/sonicated salmon sperm DNA (100 μg/ml). The blot was washed in 3× SSC/0.5% SDS at 55°C. (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; 1× Denhardt’s solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin.)

RESULTS

For amplification of renin mRNA sequences, one cDNA strand was synthesized from total RNA in a small volume. The PCR was then directly performed on the cDNA reaction mixture. As shown in Fig. 1, the amplification primers corresponded to sequences located in a region of the gene

Abbreviations: SMG, submaxillary gland; PCR, polymerase chain reaction.

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that is common to the two mouse genes and the unique rat renin gene. Each primer was specific for sequences found in two exons of the genes to avoid confusion of the signal corresponding to a renin transcript with that resulting from a possible contamination of the RNA sample with DNA. For the mouse genes, the primers corresponded to sequences that are identical for Ren-1 and Ren-2 and amplified fragments of equal sizes (161 base pairs) were obtained. However, an Alu I restriction site unique to Ren-2 contained within the amplified fragment (Fig. 1) allowed discrimination between the transcripts of the two genes on the basis of size. Finally, the amplified cDNAs were hybridized to an internal probe to increase both the specificity and the sensitivity of the method.

When kidney or adrenal RNA samples from mice of the two-gene DBA/2 strain were subjected to 30 cycles of amplification, digestion with Alu I, and separation by agarose gel electrophoresis, fragments of the size expected for the mouse Ren-1 and Ren-2 transcripts were observed after ethidium bromide staining of the gel (data not shown). The fragments hybridized to the internal probe after Southern transfer (Fig. 2A, lanes 4 and 7). As expected, kidney or SMG RNA samples from the one-gene BALB/c strain analyzed with the same procedure produced only the fragment corresponding to Ren-1 (Fig. 2A, lane 5, and data not shown). The fragment corresponding to Ren-1 transcripts in the testis of DBA/2 mice was observed after staining of the gel but Southern transfer to a nylon membrane and hybridization with the internal probe was necessary to clearly show the presence of Ren-2 transcripts in the testis (Fig. 2A, lane 3). Similarly, Ren-1 transcripts were also found in the liver and brain of male and female mice, as well as in ovaries (Fig. 2).

In brain and ovaries, Ren-2 transcripts were found at levels similar to those of Ren-1, a situation similar to that found in the kidney or adrenal glands, whereas liver Ren-2 expression

![Fig. 1. Schematic illustration of the procedure for the enzymatic amplification of mouse renin cDNA sequences. (A) Structure of the mouse Ren-1 and Ren-2 genes with arrows indicating the position of oligonucleotide primers. The primers used for amplification of rat renin cDNA occupy equivalent positions in the unique rat renin gene whose structure is similar to that of Ren-1 and Ren-2. (B) Procedure for enzymatic amplification of cDNA sequences. The hatched box represents the internal probe used for Southern analysis of amplified products for both mouse and rat. Sequences of the oligonucleotides used in this work are as follows: mouse exon-7 primer, TCATGCAGGCCCTGGAAGC; mouse exon-9 primer, CTGGTCTCTCCTGTGGGT; rat exon-7 primer, TCATGCAAGCCCTGGGAGTC; rat exon-9 primer, GTCATCTCCTCTGAAGGAT; and internal probe, CGTAGCCGTAACGCAGGTGTGTAGGCCTCCTCCC. kb, Kilobase(s); bp, base pair(s).]

![Fig. 2. Detection of renin transcripts in mouse tissues by enzymatic amplification of cDNAs. (A) Southern analysis of amplification products from tissues of male mice. All samples originate from mice of the DBA/2 strain unless otherwise indicated. Lanes: 1, brain; 2, liver; 3, testis; 4, kidney; 5, kidney [BALB/c mice; 1 ng of poly(A)+ RNA]; 6, heart; 7, adrenal glands; 8, spleen. The number of cycles was 30. Exposure time was 1 hr (lanes 1–5) or 24 hr (lanes 6–8). (B) Southern analysis of amplification products from tissues of female mice (DBA/2 strain). Lanes: 1, adrenal glands; 2, liver; 3, brain; 4, ovaries; 5, spleen; 6, kidney; 7, heart; 8, lung; 9, thymus. The number of cycles was 30. Exposure time was 2 hr (lanes 1–9) or 24 hr (lanes 5–9).]
was lower than that of Ren-1. In addition to these tissues, weaker signals were obtained in the spleen and lung where only Ren-1 can be detected and in the thymus where both genes are expressed at similar levels. Surprisingly, we could not detect renin transcripts in the heart although renin expression has been reported for this tissue (4).

Analysis of extra-renal renin transcripts was extended to the unique rat renin gene. The coding sequence of the rat gene resembles more closely that of the mouse Ren-1 than that of Ren-2 (16). As shown in Fig. 3, tissue distribution of the rat renin transcripts reflected that of Ren-1 with the notable exception of the SMG. Here, transcripts were undetectable in the rat, whereas they were easily detectable in mouse males and females of the low-producing BALB/c strain (ref. 17 and unpublished observations). Renin transcripts were also found in the rat prostate, the dissected hypothalamic region, and the pituitary. There appeared to be high levels of renin transcripts in the rat hypothalamus since the amplified renin fragment was observed after staining of the agarose gel (data not shown).

Sex-related differences in SMG renin expression are well-documented (11). Renin mRNA levels in the SMG of male mice are about 5 times higher than in females. Of all tissues that were analyzed in this study, the liver was the only other organ where a sex-related difference in levels of renin transcripts was apparent. In about a dozen liver samples from rat or different strains of mice (DBA/2 or Swiss), the intensity of the hybridization signal was always greater in females than in males (some of the data can be seen in Figs. 2 and 4). It is notable that the mRNA levels of angiotensinogen, the renin substrate, whose major site of production is the liver, are stimulated by estrogens in this organ (18).

To obtain an estimate of the relative levels of renin transcripts in different tissues, serial dilutions of the cDNA synthesis reaction mixtures from kidney, testis, liver, and spleen were subjected to the PCR. As can be seen in Fig. 5, for a given tissue, the intensity of signals resulting from serial dilutions does not decrease in the same ratio as the dilutions themselves. In this experiment, the number of amplification cycles was kept constant, and the efficiency of the procedure probably decreases as the amount of starting material increases. This was especially true for the kidney and testis samples, which show high levels of transcripts. Thus, to be able to estimate the relative levels of transcripts in various tissues, signals of similar intensities must be compared. The intensity of the hybridization signal obtained with a 1:100 dilution of the kidney sample was equivalent to that obtained with the undiluted testis sample. Thus, total renin transcripts in the kidney (Ren-1 plus Ren-2) may be more abundant than in the testis by about two orders of magnitude. Comparison of signals from serial dilutions of the testis, liver, and spleen samples indicates that testis have higher levels of renin transcripts than the liver by at least one order of magnitude and that the liver has more than the spleen by a comparable factor. These comparisons are very approximative and only reflect total tissue renin RNA levels regardless of the nature and number of cells in a given tissue that express the renin gene. For instance, the juxtaglomerular cells of the kidney represent only a small proportion of total kidney cells. Similarly, the low levels observed in tissues such as spleen, lung, and thymus might reflect either relatively higher amounts of transcripts in a cell type of low abundance in that tissue or low levels in the most abundant cell type. Therefore, on a cellular basis, relative renin mRNA levels may differ significantly from those deduced from data presented in Fig. 5.

**DISCUSSION**

Primer-directed amplification of cDNA sequences with the PCR has allowed us to detect renin mRNAs in multiple tissues of mice and rats. A procedure similar to that described in the present work has allowed determination of dystrophin transcripts in human muscle and non-muscle tissues (19). A considerable increase in sensitivity is the obvious advantage of the amplification method compared to RNA blot analysis, primer extension, S1 nuclease mapping, or in situ hybridization. The major drawback compared to in situ hybridization is the inability to identify which cells in a given tissue express the renin gene. Amplification of sequences from different exons, hybridization of the amplified fragment to the internal probe and generation of DNA fragments of the expected size after digestion with Alu I (Ren-2) confirm that the signal detected corresponds to renin transcripts. The inability to detect renin transcripts in the heart or the rat SMG make it appear unlikely that the small levels of transcripts detected in tissues such as the spleen, thymus, lung, and prostate are the result of a very low but existent “background” transcription of the genes or due to the presence of renin transcripts in a cell type common to all tissues. In this respect, renin-like activities in the arterial wall tissue of many species has been reported by several authors (1). Therefore, a very sensitive
method such as that used in this work should enable detection of renin transcripts in every tissue sample due to the presence of blood vessels in the sample. However, such was not the case and, therefore, the presence of renin or renin-like activity in blood vessel walls may not be a demonstration of the synthesis of the protein in this tissue.

Although comparison of levels of amplified fragments only yields an approximation of the relative levels of transcripts in different tissues, one can clearly see that the major site of production of renin is the kidney (along with the submaxillary gland in mice). However, expression of the renin gene occurs in several other tissues. The testis and adrenal glands can be considered good sources of transcripts. Comparatively, the liver, ovaries, and brain show lower levels that are in turn distinctively higher than those observed in the spleen, lung, thymus, and prostate. As stated above, due to the inability of the method to tell in which cells of a given tissue the gene is transcribed, we cannot determine if the low levels observed in the spleen, lung, thymus, and prostate are the result of higher expression in a rare cell type of those tissues or of very low (perhaps less than one molecule of renin mRNA per cell) in a major cell type. Because the latter possibility cannot be entirely excluded at this time, the physiological significance of renin transcription in such organs still needs to be addressed with caution.

The presence of renin transcripts in several extra-renal sites argues for the existence of local renin–angiotensin systems. Physiological roles for a local renin–angiotensin system have been suggested in processes such as the inflammatory process, the regulation of the local vascular tone in endocrine organs to increase or decrease blood flow when hormone release needs to be modulated (20) or simply the production of angiotensin II for local physiological effect (21). A local renin–angiotensin system would require a lower synthesis of renin than that of the kidney, which must maintain circulating renin levels. This could explain the relatively lower levels of transcripts in extra-renal tissues. Another site of regulation of angiotensin II production might be found at the level of angiotensinogen gene expression. Angiotensinogen mRNA has been detected in tissues other than the liver, its major site of production, but it is not known if they are found in all tissues that produce renin transcripts. Measurement of angiotensinogen transcripts by the PCR should help to further clarify this issue.

The fate of the pro-renin synthesized in extra-renal tissues remains to be elucidated. Pathways for the proteolytic processing and secretion of renin in renal and extra-renal cells are not yet fully understood. Different pathways may be taken by the renin precursor according to the cell type especially in tissues such as the liver that lack the regulated pathway of secretion. Thus the possibility that most of the extra-renal renin exits the cells in the proenzyme form or is degraded cannot be excluded. For instance, it is known that Ren-2 is transcribed in the kidney to the same extent as Ren-1 but that the protein product of Ren-2 cannot be detected to a significant extent (11, 22).

The tissue distribution of the rat renin transcripts reflects that of the mouse Ren-1 gene with the major difference that renin transcripts are absent from the rat SMG. Previous attempts to detect renin transcripts in the SMG of rats by Northern blot or primer-extension analysis were unsuccessful (17). Analysis of the promoter regions of mouse, rat, and human renin genes carried out in this laboratory has yielded clues about the gene elements responsible for renin expression in the SMG (17).

Analysis of extra-renal expression of the renin genes suggests numerous sites of genetic regulation. In addition to the variable levels of renin expression found in various organs of mice and rats that might depend upon cis- and trans-acting elements, different balances between levels of Ren-1 and Ren-2 transcripts and different male/female ratios are observed in mouse tissues. With the notable exception of the SMG, the levels of Ren-2 transcripts are either similar to those of Ren-1 (e.g., in the kidney, adrenal glands, brain, and ovaries) or lower (e.g., in testis, liver, spleen, and lung). Transcriptional control of the two mouse renin genes by androgens in the SMG implies the presence of androgen response elements within the genes, and the higher renin expression in the liver of females from both mice and rats suggests the presence of other hormone response element(s).

Finally, the results of this study of renin gene expression support the hypothesis of local renin–angiotensin systems in multiple extra-renal tissues. This regulation is achieved by the interaction of multiple elements of a system whose complexity goes far beyond that of the simple example of tissue-specific expression that the renin genes were once thought to be.

We are grateful to Isabelle Chupin and Ruth Ladenheim for kindly providing the rat RNA samples and to Gordon Langsley, Georges Lutfalla, Roger Ollo, and Charles Roth for critical reading of the manuscript. This work was supported by grants from the Association pour la Recherche sur le Cancer (ARC), the Institut National de la Santé et la Recherche Médicale, and the Institut Pasteur de Paris. M.E. holds a Research Fellowship from the Medical Research Council of Canada.