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Research Article

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Gene Expression of the Renin-Angiotensin System in Human Tissues

Quantitative Analysis by the Polymerase Chain Reaction

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Abstract

Activation of tissue-specific gene expression of the components of the renin-angiotensin system (RAS) in humans may play an important role in cardiovascular regulation and pathophysiology. Studies of human tissue RAS expression, however, have been limited by the lack of availability of sufficient amounts of fresh human tissues and a sensitive method for detecting specific mRNAs. To demonstrate the presence of components of local RASs in humans we used the polymerase chain reaction (PCR) after reverse transcription to detect renin-, angiotensinogen-, and angiotensin-converting enzyme-mRNA in small quantities of human tissues. Results indicated that all components of the RAS were widely expressed in human organ samples. In order to study changes of gene expression in small tissue samples (e.g., renal biopsies) obtained from patients, we established a competitive PCR assay for quantification of renin, using a 155-basepair deletion mutant of the human renin cDNA as an internal standard. Renin-mRNA concentration was quantitated in the kidney (1.74±0.2 pg renin/μg total RNA), adrenal gland (1.15±0.15 pg renin/μg total RNA), placenta (0.7±0.1 pg renin/μg total RNA), and saphenous vein (0.02±0.01 pg renin/μg total RNA). The method described here may serve as a highly sensitive tool to quantify alterations in gene expression in man under various pathophysiologic conditions. This study should provide the methodological basis for future studies of tissue RAS in human physiology and disease. (*J. Clin. Invest.* 1993. 91:2058–2064.) Key words: gene expression • polymerase chain reaction • renin-angiotensin system • RNA

Introduction

The renin-angiotensin system (RAS)[1] is an important regulator of cardiovascular homeostasis. Originally defined as an endocrine system, recent animal data suggest that it also functions on the autocrine-paracrine level (1–3). The components of the RAS are present in many tissues and there is evidence for local angiotensin II (ANG II) biosynthesis. The physiological functions of such locally acting tissue RASs have been postulated (3, 4) and are still subject to further investigation. It should be pointed out that the definition of such tissue-based systems does not necessarily imply that all components of the RAS are present in the same cell. It is conceivable that they are produced by different cells and interact extracellularly or that some components are taken up from the circulation to interact locally. These tissue-based systems can be regulated independently of the endocrine RAS, and it has been suggested that the plasma RAS is predominantly important for acute regulatory mechanisms whereas the tissue RASs may be more involved in chronic aspects of cardiovascular regulation (3–6).

The genes of all RAS components have been cloned (see reference 5 for review) and numerous studies have investigated the gene expression and regulation of renin, angiotensinogen, and converting enzyme in physiological and pathophysiological processes. However, most of these studies have been carried out in animals and little is known about the expression and regulation of these genes in humans. This can in part be explained by the difficulties in obtaining sufficient quantities of viable tissue samples for RNA extraction and the low abundance of specific mRNAs in the tissues. These factors have made it very difficult in the past to investigate the mRNA expression of the components of the RAS using established molecular biological methods such as Northern and dot blotting. Consequently, the existence of tissue RASs in humans remains to be an important but contentious area of cardiovascular research.

The development of the polymerase chain reaction (PCR) as a new and extremely sensitive method to investigate small amounts of DNA and mRNA has made it possible to study the expression of genes in very small tissue samples such as human biopsies (7). Several methods for quantitative mRNA analysis using PCR have been published (7–9), and we have used a modification of one of these methods (competitive PCR) for the analysis and quantification of the mRNAs of the RAS in man. That allowed the investigation of the gene expression of renin, angiotensinogen, and angiotensin-converting enzyme (ACE) in a number of human tissue samples and the quantification of one of the RAS components (renin) in these tissues using the PCR method.

Methods

Materials. The experiments were approved by the institutional ethics committee. All tissues were obtained at the operating room and inspected by a pathologist, who removed a small sample for RNA extraction, which was immediately dropped into liquid nitrogen. In general, the sample size was 10–15 mg of tissue. With the exception of placenta (n = 3) and umbilical vein (n = 3), which were obtained from the obstetrics department, some tissue samples were taken from macroscopically healthy parts of organs which had to be removed because of

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1. *Abbreviations used in this paper:* ACE, angiotensin-converting enzyme; ANG II, angiotensin II; RAS, renin-angiotensin system.

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cancerous growth (kidney, $n = 4$; and adrenal, $n = 3$). Other samples such as heart (atrial appendages, $n = 3$), saphenous vein ($n = 3$), and aorta ($n = 2$) were obtained after cardiac bypass surgery.

RNA isolation. Total RNA was isolated from human tissues by a modification of the lithium chloride (10) or the guanidinium isothiocyanate methods (11). After isolation, total RNA samples were checked by gel electrophoresis in an 1% agarose gel stained with ethidium bromide after denaturation with 6 M glyoxal, 0.25 M DMSO, and 0.1 M NaH_2PO_4 (pH 7.0) at 50°C for 60 min. The concentrations of total RNA were calculated by spectrophotometric measurements at 260 nm wavelength.

Amplification method. For use in the polymerase chain reaction, total RNA was reversely transcribed to cDNA according to Wang et al (8). 1 μg of total RNA was dissolved in 20 μl of a reaction mixture containing 1 mM of dATP, dCTP, dTTP, and dGTP, 1 U of RNAsin (Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany) 100 pmol random hexamers (Boehringer Mannheim GmbH), 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM $MgCl_2$, 10 μg/μl nuclease-free bovine serum albumin, and 200 U of murine leukemia virus reverse transcriptase (MULV-RT; Gibco BRL, Eggenstein, FRG). After incubation for 45 min at 42°C, temperature was raised to 95°C and then quick chilled on ice. For amplification of the resulting cDNA, the sample volume was increased to 100 μl by a solution containing 50 mM KCl, 20 mM Tris (pH 8.4), and 2.5 mM $MgCl_2$ and 25 pmol of up- and downstream primers as well as 3 U of Taq polymerase (Perkin-Elmer/Cetus, Norwalk, CT). The thermal profile used on a Perkin Elmer/Cetus thermal cycler consisted of denaturation at 95°C for 1 min, annealing at 60°C for renin and 55°C for angiotensinogen and converting enzyme, respectively, for 1 min and of an extension temperature of 72°C for 1 min for 26 cycles. After PCR, 10 μl of loading buffer (50% glycerol, 10 mM Tris-HCl (pH 8.0) and 0.25% bromphenol blue and xylene cyanol) were added to each sample and the amplification products were checked for the predicted sizes by agarose gel electrophoresis and then submitted to Southern blot analysis.

Competitive PCR for quantification of human tissue renin-mRNA. Quantification of renin-mRNA was performed in presence of a defined concentration of a human renin-cDNA mutant as an internal standard. It contained a deletion between the primer binding sites of the oligonucleotides used for amplification of the endogenous renin gene product. Primer binding sites were not affected. From a HindIII/EcoRI fragment of human renin cDNA subcloned into a pGEM3x-vector, a 155-bp fragment was removed by ApaI, which cuts at bases 1023 and 1178 of the human renin cDNA, and subsequently religated. This resulted in an amplification product of 221 bp in length. The deletion mutant-cDNA was added to the PCR mixture after reverse transcription to compete with the endogenous renin-cDNA. 250–500 ng reverse transcribed RNA was mixed with the appropriate amount of mutant renin-cDNA, ranging from 0.11 to 0.66 pg, where neither the endogenous nor the mutant renin would completely suppress its counterpart. To create a standard curve for the estimation of endogenous renin, this mixture was then serially 1:2 diluted for five times. The standard curve was then used for quantification and not the individual PCR-samples, whereby minimizing sample-to-sample variations. Each dilution mixture sample was subsequently amplified as described above.

The amplification products were electrophoretically separated on 1% agarose gels and then vacuum-blotted on nylon membranes under defined conditions. The dilution curve of the endogenous and mutant renin-PCR products was reflected by the decreasing intensity of the autoradiographic signals. Analysis of radioactive signals was performed using a laser densitometer (LKB Produkter, Bromma, Sweden) and a computer based imaging system (Fuji). Optical densities were determined and regression analysis of the OD values in dependence of the corresponding amounts of internal standard or of total RNA, respectively, was performed using the statistical software package, CRUNCH. Measurements were omitted when the correlation coefficient was below 0.85. Renin-mRNA concentration was derived from the regression equations directly. Using the regression equation for the renin mutant, the OD value at a fixed number of renin mutant molecules was calculated. This OD value was then inserted into the regression equation of the standard curve for the endogenous renin gene to calculate the amount of total RNA, which was required to yield the same signal intensity for the endogenous renin gene. Using "micrograms of total RNA" as the reference basis, the picograms of renin-mRNA per microgram total RNA was obtained.

Oligonucleotides used for PCR. Primers were selected with the computer program developed by Lowe et al. (12) which was licensed to M. Paul. Human renin cDNA was amplified by 21-mer oligonucleotides with the following sequences: AAATGAAGGGGGTGTCTGTGG as sense primer (bases 851–872) and (bases 1206–1227) AAGCCAATGCGGTTGTTACGC as antisense primer. This yielded an amplification product of 376 bp in length spanning the second and third exons of renin cDNA. The sense primer for the detection of ACE cDNA spanned oligonucleotide bases 492–512 (GCCTCCCCAACAAGACTGCCA) on exon 3, and the antisense primer spanned bases 860–880 (CCACATGTCTCCAGCCAGATG) which represent the junction of exons 5 and 6 of the human ACE-cDNA. Human angiotensinogen primers were situated over the fourth and fifth exon with the sense primer (bases 1209–1231) CTGCAAGGATCTTATGACCTGC and the antisense primer (bases 1404–1426) TACACAGCAAACAGGAATGGGC.

Generation of probes. Renin-cDNA was hybridized to a 1.3-kb-long rat renin cDNA-fragment yielding the complete rat renin cDNA after digestion with BamHI/HindIII. A plasmid vector (Bluescript KS, pB 35-19) containing 3,334 bp of human ACE cDNA was cut with EcoRI and BglII to yield a 1.7-kb fragment extending from position 690 to position 2326 of the ACE cDNA. A StuI/NcoI fragment of a pGEM5 vector producing human angiotensinogen cDNA sequences extending from position 234 to 1389 allowed detection of angiotensinogen-cDNA. All restriction fragments were separated from the vector on an agarose gel and isolated before radioactive labeling.

Hybridization methods. Southern blotting was performed as described (13). This method provided more reproducible results than direct labeling of amplified sequences. The amplified cDNA sequences were transferred from 1.3% agarose gels to nylon membrane (Pall, Dreieich, FRG) in a vacuum blot chamber (LKB Produkter) under defined and reproducible conditions using 0,25 N HCl for precipitation for 30 min and subsequent neutralization on 0.5 N NaOH and 1.5 M NaCl). Blotting was terminated after 2 h on 20× SSC (1× SSC: 0.15 M NaCl, 0,015 M sodium citrate). cDNA was cross-linked to the nylon membrane in a UV-linker (No. 1800, Stratagene Inc., La Jolla, CA). Membranes were prehybridized with 50% deionized formamide, 5× Denhard's solution, and 25 μg/ml herring sperm DNA for 4 h. Hybridization was done in the same buffer overnight at 60°C adding the corresponding probes, which were randomly labeled using [^{32}P]dCTP and purified on a Sephadex G-50 column. Nylon membranes were washed after hybridization at room temperature in 0.2× SSC and 0.1% SDS for 30 min and the three times at 56°C for 30 min. Blots were exposed for 6–36 h at −80°C to XAR x-ray films (Eastman Kodak Co., Rochester, NY) or to imaging plates for analysis using a radioactivity imaging system (Fuji, Bas 2000, Düsseldorf, FRG).

TCA precipitation of reversely transcribed RNA samples. To test the yield and the efficiency of the reverse transcriptase reaction as described (14), 1 μg of total RNA was subjected to reverse transcription as above, but 5 μM of radioactively labeled [^{32}P]dCTP was added to the reaction. The total volume of the RT rection was increased to 30 μl. Before the addition of enzyme, 1 μl of the reaction was removed for the determination of total counts and 1 μl was removed for the determination of TCA-precipitable counts (background). After 1 h of incubation at 42°C, 1 μl of the reverse transcription reaction was taken out for measurement of incorporated labeled dCTP. Samples were precipitated in cold 5% TCA and filtered on glass fiber filters (No 934 AH, Whatman Inc., Clifton, NJ) under a slight vacuum. Filters were dried and placed in scintillation vials. After addition of scintillation fluid samples were counted in a β counter (Packard Instruments, Inc., Downers Grove, IL). The amount of DNA synthesized was calculated by multiplying the fraction of total dCTP incorporated into TCA-precipitated counts per minute with the number of nanomoles of each dNTP in the reaction and the average weight of four dNTPs.

Restriction enzyme analysis of PCR products. To test the specificity of amplified sequences the PCR products of renin, angiotensinogen, and ACE were digested with restriction enzymes and the specific restriction products were visualized on 1.5% agarose gels. The amplified renin sequence was digested with AccI, which cuts in position +913 of the cDNA, resulting in two fragments of 314 and 62 basepairs in length. The amplified ACE sequence was also digested with AccI, which cleaves at position 547 and should yield two fragments of 333 and 55 basepairs in length. The angiotensinogen sequence was digested with BstXI which cleaves in position 1365 and should result in two fragments of 217 and 61 basepairs in length.

Results

Analysis of renin-, angiotensinogen-, and ACE-mRNA expression in various human tissues. Specific mRNAs for all three components of the RAS could be detected in human tissues by PCR analysis (Figs. 1 and 2). All PCR products were found to be of the predicted size on agarose gels (Fig. 1). The specificity of PCR reaction was tested by restriction enzyme analysis of amplified products which resulted in fragments of correct size as well as by dideoxy sequencing (data not shown). Typically, PCR was performed using 1 μg of total RNA per sample, but clear signals could be obtained from concentrations as low as 16 ng with the same PCR protocol.

Tissue renin-, angiotensinogen-, and ACE-mRNA expression could be detected in the renal cortex and medulla, human adrenals, aorta, saphenous, and umbilical vein as well as placenta (Fig. 2). The same distribution was found for angiotensinogen- and ACE-mRNA, demonstrating that all these components of the RAS are coexpressed in the same tissues. Whereas angiotensinogen and ACE mRNAs were readily detectable in human heart tissue, renin mRNA was present in lower concentrations in cardiac samples, and the cycle number of the PCR reaction had to be increased to 35 to detect renin. No renin signal could be detected in human liver under the experimental conditions used.

Quantitative analysis of renin-mRNA expression in human tissues by competitive PCR. For quantification of renin-mRNA calculated as picograms renin per microgram of total RNA, we used a deletion mutant of human renin-cDNA as internal standard which was coamplified in the same vial (Fig. 3). Accordingly, renin mutant cDNA gave an amplification product of 221 bp in size compared to 376 bp for native renin cDNA. The use of the same primer binding sites results in identical amplification efficiency (8). When coamplified in the same reaction sample, both endogenous and mutant renin cDNA competed for primer bindings in a concentration dependent manner. Fig. 4 illustrates that raising amounts of mutant renin-cDNA from 0.1 to 10 pg increasingly inhibit amplification of renal endogenous renin-cDNA.

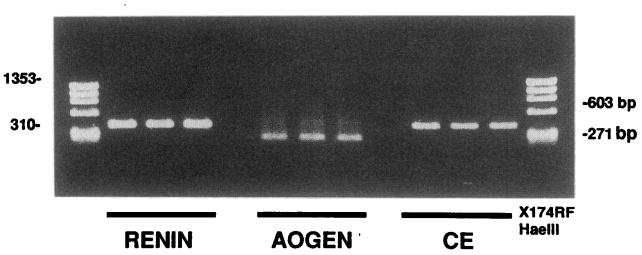


Figure 1. Detection of renin-, angiotensinogen-, and converting enzyme-mRNA expression by qualitative PCR. Amplification products of renin (376 bp), angiotensinogen (217 bp), and converting enzyme (388 bp) as obtained from renal total RNA (three samples each). Ethidium-bromide stained 1.2% agarose gel. PHIX174RF vector HaeIII-fragments are used as length markers.

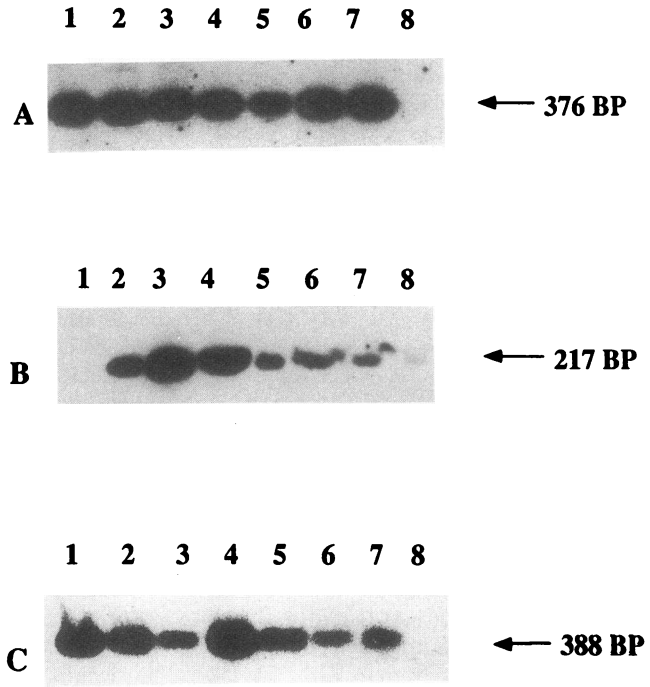


Figure 2. Tissue-specific expression of renin-, angiotensinogen- and converting enzyme-mRNA expression in man. PCR was carried out in 25 cycles with the exception of cardiac renin, which was amplified in 35 PCR cycles. Southern blotting of amplified sequences. (*A*) Renin: lane *1*, renal cortex; lane *2*, renal medulla; lane *3*, adrenal gland; lane *4*, aorta; lane *5*, heart; lane *6*, placenta; lane *7*, umbilical vein; lane *8*, control. (*B*) Angiotensinogen: lane *1*, control; lane *2*, renal cortex; lane *3*, renal medulla; lane *4*, adrenal gland; lane *5*, heart; lane *6*, aorta; lane *7*, placenta; lane *8*, saphenous vein. (*C*) ACE: lanes *1–3*, kidney; lane *4*, adrenal gland; lane *5*, aorta; lane *6*, heart; lane *7*, saphenous vein; lane *8*, control.

Before quantitative analysis, minor amounts of reversely transcribed RNA (100 ng) were subjected to PCR in presence of increasing amounts of renin mutant cDNA, to determine the amount of internal standard which would allow both native and mutant renin-cDNA to be amplified. After that, 250–500 ng of native reversely transcribed RNA were coincubated with the appropriate amount of mutant renin-cDNA ranging from 0.11 to 0.66 pg depending on the abundance of endogenous renin mRNA. To enhance accuracy, a standard curve for the quantification of the native renin was established by serial dilutions of the original mixture (Fig. 5). The decreasing intensities of the native and mutant renin-PCR products reflect the raising degree of dilution.

To evaluate the efficiency of the reverse transcriptase reaction, TCA precipitation of total RNA was verified. Results indicated that cDNA first strand synthesis under the conditions used had an efficiency of 52.6±7.4% (n = 8). This value was used as a correction factor for the calculation of renin mRNA concentrations in the tissues. Since the addition of a cDNA control for PCR quantitation does not allow direct evaluation

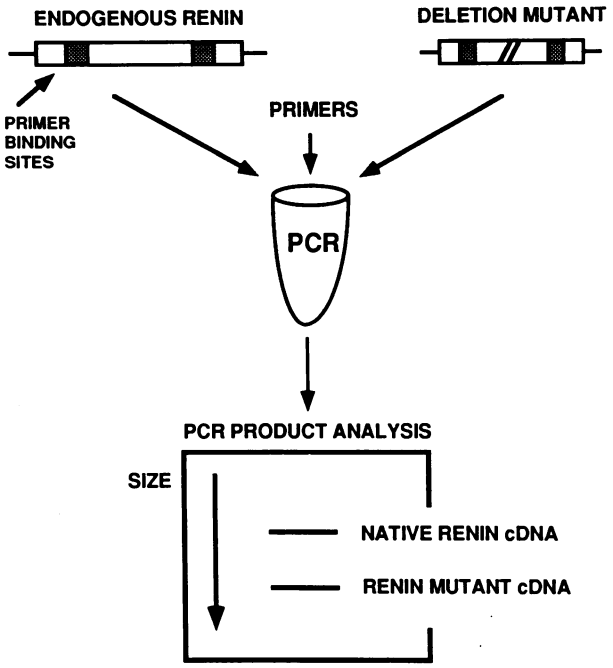


Figure 3. Quantitative PCR: a deletional mutant of the endogenous renin gene using the same primer binding sites is coamplified in the same reaction tube avoiding sample-to-sample variations. The short version of the renin-cDNA serves as an internal standard when added to the reaction mixture in known concentration. After PCR, the amplification products can easily be separated by gel electrophoresis and further processing by southern blotting.

of the efficiency of the reverse transcription reaction, several experiments were performed to verify the reproducibility of this reaction. Total RNA was reversely transcribed and amplified using the renin-specific primers in 10 experiments performed on different days and with different solutions. Results

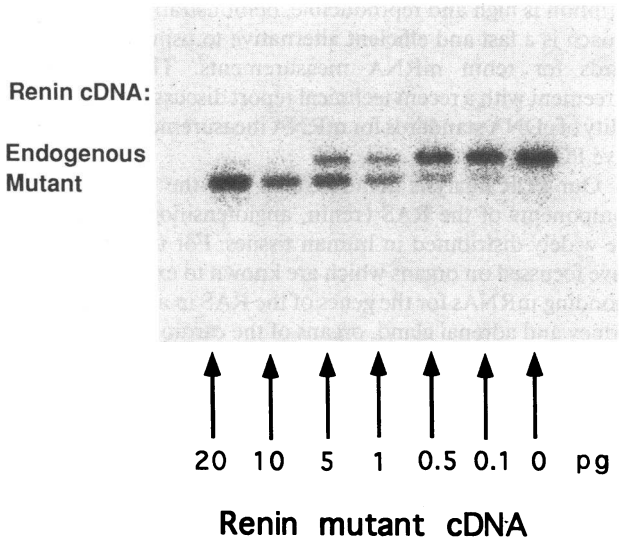


Figure 4. Increasing concentrations of endogenous or mutant renin-cDNA compete for the primers. Raising amounts of mutant renin-cDNA from 0.01 to 10 pg progressively inhibit coamplification of endogenous renin-cDNA.

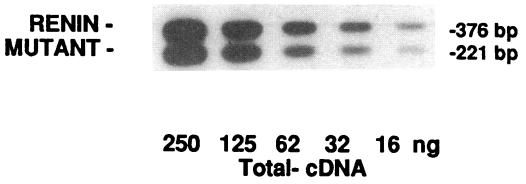
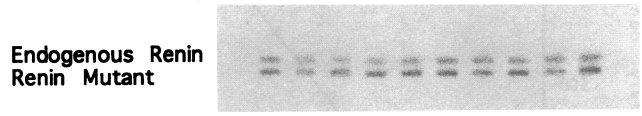


Figure 5. Serial 1:2 dilution of a reaction mixture containing both mutant and endogenous renin-cDNA. The decrease in signal intensities reflects the lowering amounts of renin-cDNA through the dilutional steps. Southern blot of renal renin-mRNA after reverse transcription.

showed low sample to sample variability and the coefficient of variance between the samples was found to be 0.15 (Fig. 6).

In that the concentrations of the mutant renin-cDNA as well as the amount of total RNA is known for each sample, the corresponding signal intensities can be obtained from autoradiographs. The serial dilutions were used to create regression curves for the endogenous and the mutant renin genes. The increasing amounts of renin mRNA were then calculated from the regression equations, thereby minimizing sample-to-sample variations between individual PCR vials (Fig. 7). By this procedure, numerical data of the proportion of specific renin-mRNA per microgram of total RNA could be obtained for all tissues with the exception of heart samples, where renin gene expression was extremely low.

To ensure that quantification occurs within the exponential phase of the PCR, amplification products of the same reaction mixture were amplified for up to 40 cycles and aliquots were taken out at different time points of the amplification process. Results indicated that at 26 cycles, where the quantitative analysis was carried out, amplification was linear, whereas higher cycles showed a decrease in amplification efficiency (Fig. 8). Increasing reverse transcriptase concentrations (from 200 to 1,000 U) or reverse transcription times did not affect the ratio of endogenous to mutant renin, indicating stable cDNA levels after reverse transcriptase procedure (data not shown). Renin-mRNA expression was quantitated in the kidney cortex (1.74±0.2 pg renin/μg total RNA), adrenal cortex (1.15±0.15 pg renin/μg total RNA), cross-sectional samples of the placenta (0.7±0.1 pg renin/μg total RNA), and saphenous vein (0.02±pg renin/μg total RNA).



Renin/mutant - ratio:	x = 1.57
Standard deviation:	0.23
Coefficient of variance:	0.15

Figure 6. Determination of interassay variability of the PCR reaction for renin mRNA detection. Ten experiments were performed of an individual sample of human kidney RNA. Signals obtained after reverse transcription and amplification were analyzed by a computer imaging system (Fuji Bas 2000).

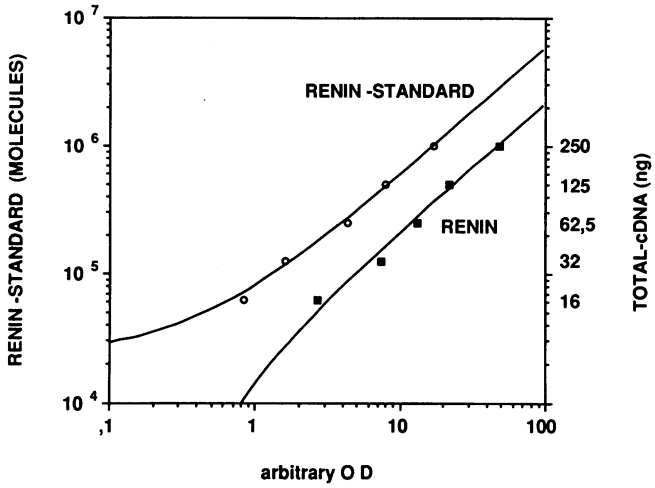


Figure 7. Quantification of the signal intensities obtained from laser densitometry of serial dilution of endogenous and mutant renin-cDNA. At the same optical density (OD), a defined number of renin mutant molecules corresponds to the amount of total RNA necessary to yield the same signal strength for endogenous renin-mRNA.

Discussion

To our knowledge, this is the first systemic analysis that documents the presence of tissue RAS gene expression in humans. The finding of human tissue RASs undoubtly will have important implications in human physiology and pathophysiology. In this study we have used qualitative and quantitative PCR analysis to study the expression of genes of the RAS in human tissues.

For qualitative PCR we have used reverse transcription of total RNA for cDNA first-strand synthesis, followed by PCR amplification of specific target sequences as described (15).

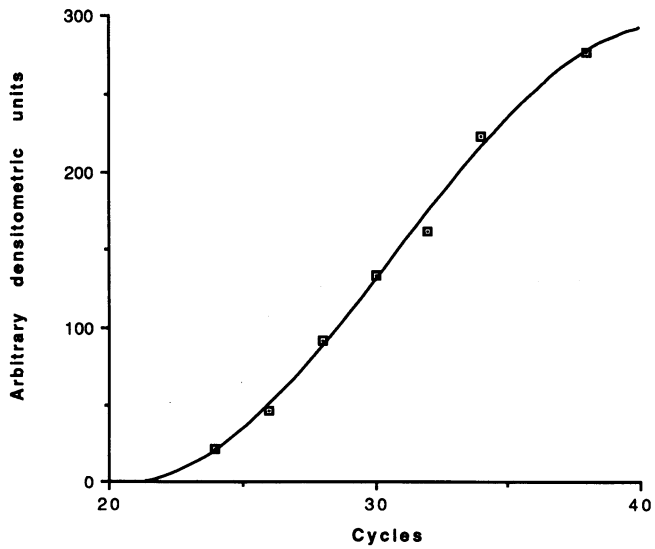


Figure 8. Linearity of the PCR-amplification depending on the PCR cycle number. Note that linearity is given at the 26th cycle, where quantitative PCR is performed, but that amplification efficiency decreases at higher cycles.

Several methods for mRNA quantitation by PCR have been established. The coamplification of control sequences of a different gene (control gene), such as the β-actin or aldolase cDNAs (16), have not been effective, in that these use different primers other than the targeted sequence. Therefore, kinetics for primer hybridization may be rather variable, thus influencing amplification efficiency and impairing accurate comparison. Furthermore, the use control genes only allows quantification by the ratio of the control gene to the gene of interest. This becomes a problem when the control gene expression itself is affected by the physiological condition. An alternative approach, which is used here, is the competitive coamplification of defined amounts of specific cDNA or mRNA fragments which contain identical primers but carry a sequence alteration, such as an additional restriction site, sequence deletions or insertions (8, 9). The use of the same primer hybridization kinetics secures identical amplification efficiencies. Co-amplification of the endogenous and mutant genes yields amplification products, which can be easily size-separated by gel electrophoresis. Additionally, using dilutional series of the coamplification products to create standard curves allows the calculation of the specific mRNA concentration from the regression equations rather than an individual PCR sample, thus minimizing the effect of sample-to-sample variations and thereby improving the accuracy of the method.

Before this report, several investigators have attempted to analyze renin gene expression by PCR. Lou et al. (17) used qualitative PCR analysis to study renin expression in rat tissues and found signals in the kidney and several extrarenal tissues but did not show quantitation. Recently, Iwai and Inagami (18) presented such an approach using an mRNA standard carrying a deletional mutation for the analysis of renin mRNA in various tissues of genetically hypertensive and normotensive rats. In contrast to these reports, our study focuses on the distribution of mRNAs of the components of the RAS in human tissues, and the quantification of human renin mRNA by competitive PCR using a cDNA standard. Although this approach does not control for the efficiency of the RT reaction, we present data demonstrating that the effectiveness of reverse transcription is high and reproducible, demonstrating that this approach is a fast and efficient alternative to using mRNA standards for renin mRNA measurements. This is also in agreement with a recent technical report discussing the applicability of cDNA standards for mRNA measurements by competitive PCR (19).

Our PCR analysis demonstrated that the mRNAs of the components of the RAS (renin, angiotensinogen, and ACE) are widely distributed in human tissues. For these studies we have focussed on organs which are known to express the corresponding mRNAs for the genes of the RAS in animals, namely kidney and adrenal gland, organs of the cardiovascular system (saphenous vein, aorta, heart) and of the fetal-maternal circulation such as placenta and umbilical vein. The mRNAs for renin, ACE, and angiotensinogen were found in nearly all tissues, demonstrating that the requirements for local ANG II formation in these tissues are fulfilled.

All components of the RAS were present in the human kidney. Their expressions could be detected both in the cortex and medulla. As determined by quantitative PCR, renal renin-mRNA expression was high with 1.74±0.2 pg renin-mRNA/μg total renin RNA. This level is comparable to that reported

in the literature with a range of 2.5–5.2 pg renin/μg total RNA for rats and mice, respectively (20, 21). In that PCR is extremely sensitive and allows quantification of specific mRNA expression from small tissue samples such as biopsy specimens, it may be of use to study changes in renal RAS gene expression in man under various in vivo conditions.

The adrenal gland showed high levels of mRNA expression for renin as well as for the other components of the RAS. Quantitative PCR analysis, however, revealed that concentrations of renin were lower (1.15±0.15 pg renin/μg total RNA) than those in the kidney. The significance of adrenal renin mRNA expression has not completely been elucidated in man. Because adrenal renin expression is very high in the transgenic rat line TGR(mRen2)27 (22), which develops fulminant hypertension due to integration of the mouse Ren-2 gene as transgene, adrenal renin could possibly play a role for blood pressure regulation in humans.

Increased activity of the cardiac RAS in cardiovascular disease has been described in animal studies. Expression of ACE, for example, is elevated in cardiac hypertrophy (23). The presence of renin mRNA in cardiac tissue, however, is still controversial. Whereas several investigators (20, 21, 24) have described specific mRNA expression by Northern blot and solution hybridization analysis in mouse and rat hearts, studies by Ekker et al. (25) and Iwai and Inagami (18) were unable to detect cardiac renin mRNA. It is unclear at present if the apparent discrepancy is due to species differences, experimental conditions or differential regulation of renin. It is evident from our studies that renin is present only in very low concentrations in the human heart. This may be due to a low overall expression or to expression in very selective regions.

The vascular RAS is considered an important autocrine-paracrine system for the regulation of vascular tone and structure (24, 26). ACE is localized mainly on endothelial cells and angiotensinogen is synthesized in the media and adventitial layers (27). A role for the vascular RAS in restenosis and vessel wall hypertrophy has been suggested by recent animal studies. Like in the heart, conflicting results have been published as to the localization of renin in the rat aorta. A recent report failed to detect significant amounts of renin mRNA in rat aortic tissue (28). In our study, however, renin gene expression could readily be demonstrated in both aortic and venous vasculature. Levels of renin mRNA expression in the saphenous vein as determined by quantitative PCR were about one hundred times lower than in the kidney.

Analysis of mRNA expression by PCR offers new and exciting opportunities to study human diseases. Its particular strength for the analysis of the components of the human RAS lies in the possibilities to evaluate gene expression and to analyze the result in the context of in vivo parameters. It will now possible to correlate analysis of extremely small amounts of mRNA with plasma parameters such as plasma renin activity and clinical signs. This will be of increasing importance for the investigation of the molecular basis of diseases such as primary or renoparenchymatous hypertension or cardiovascular hypertrophy, conditions in which the tissue RASs seem to play an important role. Although mRNA measurements alone do not provide insight into a functional role of this system they are an important precondition for the investigation of such a role. Further studies will also focus on the quantitative analysis of other components of the RAS, since recent genetic data suggest that ACE and angiotensinogen are important candidate genes for genetic hypertension (29–31). The analysis of the expression of these genes in human tissues will very likely provide important insight into their contribution to the molecular and genetic basis of human hypertension.

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