Tissue-Specific Regulation of Renin Expression in the Mouse

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SUMMARY Increasing biochemical evidence suggests that the renin-angiotensin system may be present in many extrarenal tissues. We have employed the mouse submandibular gland renin complementary DNA (pDD-ID2) and the rat liver angiotensinogen complementary DNA (pRang 3) to demonstrate that renin and angiotensinogen messenger RNAs are expressed in the mouse kidney, submandibular gland, heart, adrenal, brain, and testis. To elucidate the factors that influence local tissue renin-angiotensin expressions, we studied tissue renin messenger RNA and enzymatic levels of male mice in response to sodium depletion and castration. Sodium depletion resulted in increased renin expression in the kidney, heart, and adrenal, but not in the submandibular gland and testis. Castration lowered renin levels in all extrarenal tissues but appeared to increase renin level in the kidney. Taken together, the above data demonstrate tissue-specific regulation of renin expression and imply different functions for the sodium responsive and nonresponsive systems.

KEY WORDS • renin gene • gene expression • tissue differential regulation • sodium • androgen

THE conventional concept that the renin-angiotensin system is a circulating endocrine system has been challenged recently by the observations that components of the renin-angiotensin system can be detected in many tissues.1 The hypothesis that angiotensin may be produced locally in peripheral tissues is gaining increasing support. It has been proposed that the local generation of angiotensin may be independent of the circulating system and may contribute to the regulation of local tissue function.2,3 However, direct evidence that the tissue renin-angiotensin systems are influenced by factors independent of the circulating system has not been reported. The purpose of this study is to examine whether tissue-specific regulation of renin gene expression occurs in various tissues of the mouse. Such data will support the notion of independent tissue renin-angiotensin systems and may provide insight into the function(s) of the tissue renin-angiotensin system(s).

Materials and Methods
Outbred Swiss Crl:CD-1 (ICR) BR male mice aged 40 days or older (Charles River Breeding Laboratories, North Wilmington, MA, USA) were studied. Animals were killed by cervical dislocation. For RNA analysis, various organs were removed within 3 minutes of death, immediately snap-frozen in liquid nitrogen, and stored at −70°C until use.

Sodium depletion was achieved by feeding the mice on Teklad low sodium chow (Teklad Test Diets, Madison, WI, USA) containing 0.02% (wt/wt) sodium chloride for 2 weeks. In addition, on Days 1 to 3, 1 mg of furosemide was administered subcutaneously each day to ensure sodium depletion. For the high sodium diet, animals were fed the same chow to which 3% sodium chloride (wt/wt) had been added for 2 weeks.

In separate experiments, adult CD-1 male mice were castrated. The animals were killed 10 days later and various tissues were obtained for analysis.
Renin Activity and Protein Assay

Tissues were disrupted with a cell sonicator (Heat Systems Ultrasonics, Farmingdale, NY, USA) or a Polytron homogenizer (Brinkman Instruments, Westbury, NY, USA) in 10 volumes of 0.1 molar Tris HCl, pH 7.4, containing 0.25% (vol/vol) Triton X-100, 0.5 mM EDTA, 0.5 mM sodium tetraethionate (NaTT), and 0.1 mM of phenylmethylsulfonyl fluoride (PMSF). After gentle rocking for 2 to 3 minutes, homogenates were centrifuged at 3000 g for 5 minutes and supernatants assayed for renin activity by using plasma from nephrectomized sheep with no detectable renin activity as the source of renin substrate. Reaction mixtures contained the angiotensinase inhibitors 8-hydroxyquinoline (5 mM) and 2,3 dimercapto-1-propanol (1.6 mM) in 0.1 mM Tris HCl, pH 7.4. Angiotensin I generated was quantitated by radioimmunoassay. To document that the angiotensin I-generating activity of various mouse organs was due to authentic renin, tissue homogenates of adult male mice were first incubated with renin-specific antisera R1720 at 37°C for 1 hour prior to the addition of sheep anephric plasma. This antisera is specific for renin and does not recognize nonrenin proteins, including cathepsin D.

Protein concentration was measured by Bio-Rad assay, colorimetrically determined by absorbance at 595 nm (Bio-Rad Laboratories, Richmond, CA, USA).

RNA Isolation and Northern Blot Hybridization Studies

Tissues were stored at −70°C until homogenized in 4 M guanidine thiocyanate (GTC), 0.5% Na-N-lauryl sarcosine, 25 mM Na citrate, and 0.1 M β-mercaptoethanol. Homogenate (1 g tissue/10 ml GTC) was applied to 5 ml of autoclaved 5.7 M CsCl2, 25 mM Na acetate, pH 5.5. Preparations were subjected to ultracentrifugation in a Ti 70.1 rotor (Beckman, Palo Alto, CA, USA). RNA from a kidney or submandibular gland from each individual animal (n = 4) was separately extracted and analyzed. In organs with low quantities of renin messenger RNA (mRNA) (e.g., heart and testis), the organs of 6 to 8 animals were pooled and 3 to 4 separate RNA extractions were performed. Regression lines were calculated from the densitometric integral values and the relative mRNA levels were estimated from the slope of the regression line. When the linearity for each series of dilutions was analyzed, only r values ≥0.90 were accepted. Comparison of mRNA levels of each tissue in response to various perturbations was made by comparing the respective slopes of the densitometric signals. When comparisons were made between autoradiograms, an interblot standard curve from a standard pool of kidney RNA was included in each blot. The interblot and intrablot coefficients of variation are 9 and 10%, respectively. If multiple exposures were necessary, the data were corrected for exposure time and, if necessary, radioisotopic decay.

Renin and Angiotensinogen Complementary DNA

We used the angiotensinogen probe pRang 3, cloned by Lynch et al. into the BamHI site of pUC9. This partial length cDNA corresponds to nucleotides 650 to 1140 of rat angiotensinogen cDNA sequence. The renin cDNA pDD-1D2 is a full-length mouse submandibular gland renin cDNA cloned by Field et al. into the PstI site of pBR 322.

Quantitation of Messenger RNA

Serial quantities of total RNA from each organ were used for hybridization, and autoradiographs were scanned with an LKB microdensitometer (Paramus, NJ, USA). RNA from a kidney or submandibular gland from each individual animal (n = 4) was separately extracted and analyzed. In organs with low quantities of renin messenger RNA (mRNA) (e.g., heart and testis), the organs of 6 to 8 animals were pooled and 3 to 4 separate RNA extractions were performed. Regression lines were calculated from the densitometric integral values and the relative mRNA levels were estimated from the slope of the regression line. When the linearity for each series of dilutions was analyzed, only r values ≥0.90 were accepted. Comparison of mRNA levels of each tissue in response to various perturbations was made by comparing the respective slopes of the densitometric signals. When comparisons were made between autoradiograms, an interblot standard curve from a standard pool of kidney RNA was included in each blot. The interblot and intrablot coefficients of variation are 9 and 10%, respectively. If multiple exposures were necessary, the data were corrected for exposure time and, if necessary, radioisotopic decay.

Statistical Analysis

The results are expressed as means ± standard error. Data were subjected to one-way analysis of variance or to the Wilcoxon rank sum test.

Results

We observed that angiotensinogen mRNA is readily detected in the mouse liver, kidney, adrenal, testis, brain, and submandibular gland. It is absent in the pancreas and spleen. Renin mRNA is detected in the mouse submandibular gland, kidney, adrenal, and testis. In the brain and heart, renin mRNA levels are low, requiring large amounts of total RNA for detec-
We examined the influence of high and low sodium diets on mouse tissue reninlike activities as well as renin mRNA levels. Sodium depletion resulted in significantly higher reninlike activities in the heart, kidney, and adrenal compared to the reninlike activities in these tissues of mice fed a high sodium diet (Figure 1). In contrast, submaxillary gland and testicular reninlike activities did not change significantly in response to the alteration in dietary sodium. The tissue reninlike activities were completely neutralized by renin-specific antibody, demonstrating their homology with renal renin (data not shown). The low sodium diet resulted in 2.5-fold ($p<0.01$) higher renal and 4.5-fold ($p<0.05$) higher cardiac renin mRNA levels when compared to the mRNA levels in these tissues of mice fed a high sodium diet, but neither sodium diet influenced mouse submandibular gland or testicular renin mRNA concentrations (Figure 2). Due to the small amount of RNA extracted from the adrenals, accurate quantitation of the changes in adrenal renin mRNA level were not made.

We have previously observed that the male mouse submandibular gland, testicular, and adrenal renin activities increase markedly at puberty, whereas kidney renin levels do not change during ontogeny. These patterns of renin activity during development were supported by parallel changes in tissue renin mRNA levels. The increases in extrarenal renin expressions at puberty may be androgen-mediated, since such increases were not observed in the female mice. To examine further the hypothesis of androgen-regulated tissue renin expression, we performed castration on CD-1 male mice and compared their tissue renin expressions to those of normal controls. Submandibular gland, cardiac, and adrenal renin activities fell significantly 10 days after castration (Figure 3). In contrast, kidney renin activity appeared to increase in the castrated animals. Submandibular gland renin mRNA levels fell significantly in the castrated male mice (threelfold reduction; $p<0.01$). Kidney renin mRNA
and co-workers have demonstrated that renin levels present in the adrenal cortex correlate closely with angiotensin is produced locally in these tissues. It has exerted important influences on tissue function. Doi been proposed that locally generated angiotensins may be of cardiovascular significance. The possible influences of locally generated angiotensin on central nervous system, pituitary, cardiac, and renal functions have been reviewed.

An important issue to be addressed is whether local tissue renin expression is regulated independent of kidney renin. Accordingly, we examined the influence of dietary sodium on the renin mRNA levels and enzymatic activities of various tissues in the mouse. We studied the CD-1 mouse, since much is known about its molecular genetics and we have the molecular probe for renin mRNA analysis. Our results demonstrated that sodium depletion stimulated renin expressions in the mouse kidney, heart, and adrenal, but not in the submandibular gland and the testis. This observation demonstrates the existence of tissue-specific regulation. The exact mechanism by which sodium depletion stimulated kidney, cardiac, and adrenal renin expressions has not been determined. To understand further the regulation of local renin gene expression, we examined tissue renin activities and renin mRNA levels in the male CD-1 mouse during growth and development. We observed that the submandibular gland, cardiac, testicular, and adrenal renin levels increased significantly at puberty. In contrast, kidney renin concentration and mRNA level were relatively constant throughout ontogeny. The influence of puberty on the expression of extrarenal (submandibular, testicular, cardiac, and adrenal) renins may be mediated by androgen. To document androgen regulation of extrarenal renin expression, we examined the effect of castration on tissue renin activities of the adult male CD-1 mouse. Submandibular gland, cardiac, and adrenal renin expressions were suppressed in the castrated male mice, whereas kidney renin expression appeared to be stimulated. The mechanism of androgen-mediated tissue-specific expression is unknown. The modest increase in kidney renin activity in response to castration is also interesting. It is possible that castration led to a reduction in the plasma angiotensin level as a result of a decrease in extrarenal production of renin or angiotensin or both, which in turn caused an increase in kidney renin level. We were unable to address this possibility directly in our study because we did not measure plasma angiotensin levels. A similar hypothesis has been suggested by Catanzaro et al., who have shown that renin mRNA and renin enzymatic activity are higher in female kidney than in male kidney. Regardless of the mechanism, our data taken together demonstrate that renin gene expressions in various tissues may be differentially regulated. Although this study did not examine tissue-specific regulation of angiotensinogen expression, our previous observation that sodium depletion stimulated rat kidney angiotensinogen mRNA levels but not hepatic angiotensinogen mRNA levels is consistent with this hypothesis.

Tissue-specific regulation of renin and angiotensinogen may have important functional implications.
Renin and angiotensinogen expressions in tissues that are known to be involved with cardiorenal homeostasis (i.e., kidney, heart, and adrenal) appear to be influenced by sodium levels in the animal. Viewed teleologically, increased local angiotensin production in these tissues in response to sodium depletion should be expected to activate angiotensin-mediated responses that are functionally important for blood pressure and fluid and electrolyte regulation. On the other hand, the tissues whose renin-angiotensin activities are not influenced by sodium status (i.e., submandibular gland and testis) are not traditionally thought to be involved in cardiovascular regulation. The influences of androgen, estrogen, or gonadotropin hormones on submandibular gland and testis functions have been well documented. That responses to renin and angiotensin in these tissues parallel those of androgen, estrogen, or gonadotropin hormones suggest that the local renin-angiotensin system plays a role in modulating these hormonal functions. Castration resulted in decreases of renin expression in these tissues. Interestingly, adrenal and cardiac renin levels also fell with castration. This suggests that, in contrast to renal renin, all extrarenal renin expressions are androgen-regulated. We speculate that androgen may exert a tonic or long-term influence on the renin activity and angiotensin-mediated function of extrarenal tissues.

The present study was performed in the CD-1 mouse, which contains two renin genes (Ren-1 and Ren-2). Our preliminary observation in the C57 mouse, which contains only one renin gene (Ren-1), suggests that the extrarenal tissue renins are expressed in small amounts compared to amounts expressed by the two-gene strain. On the other hand, renal renin levels are the same in the two strains. One explanation for this similarity is that androgen selectively regulates Ren-2 gene expression; whereas sodium status selectively influences Ren-1 gene expression. In other words, Ren-1 gene is predominately expressed in the kidney of the two-gene strain, whereas Ren-2 gene is predominantly expressed in extrarenal tissues. This hypothesis is supported by our recent immunohistochemical study using monoclonal antibody selective for kidney renin. However, this hypothesis is not consistent with the data of Field et al. Using primer extension analysis, these investigators reported that both Ren-1 and Ren-2 transcripts are present in approximately equal quantities in the kidney of the mouse with two renin genes. It should be pointed out that the data of Field et al. only examined renin transcripts and not the enzyme itself. Therefore, sodium diet or androgen may additionally influence translational efficiency and posttranslational processing. The basis of these discrepancies remains to be elucidated.

In summary, our current and previous data demonstrate the widespread expression of both renin and angiotensinogen genes in many tissues in the CD-1 mouse. Tissue-specific regulation of renin gene expression was observed. Our data may provide insight into the possible role of the tissue renin-angiotensin system. The mechanisms underlying differential regulation of renin genes in various tissues is an important area of future research. It remains to be determined whether tissue-specific regulation of renin genes also exists in other species.

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