Ontogeny of Somatic Angiotensin-Converting Enzyme

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Abstract Angiotensin-converting enzyme or kininase II (ACE-KII) plays a central role in the control of circulating and tissue levels of angiotensin II and kinins. Both peptides have been implicated in the regulation of renal function and growth during normal development. We tested the hypothesis that the developing rat kidney expresses ACE-KII mRNA transcripts and the active enzyme and evaluated whether the developmental expression of the ACE-KII gene is related to changes in circulating angiotensin II and tissue kallikrein. ACE-KII mRNA and enzymatic activity were low in the newborn kidney; peak expression occurred on days 15 and 20 of postnatal life (16-fold versus day 1). In extrarenal tissues, ACE-KII activity and mRNA levels were also low during the newborn period in the following order of abundance: lung > kidney > aorta > heart. The lung showed a higher age-related increase in active ACE-KII and mRNA abundance (15-fold) than heart and aorta (activity, 3- to 4-fold; mRNA, 6- to 10-fold). The developmental profile of ACE-KII correlated temporally with changes in circulating angiotensin II and tissue kallikrein. Plasma angiotensin II levels were 2.5-fold higher in newborn than adult rats, whereas renal and extrarenal kallikrein-like activity increased twofold to fivefold from birth to adulthood. These results demonstrate that the ACE-KII gene is developmentally regulated in a tissue-specific manner. Tissue kinin generation and degradation, reflected by kallikrein and ACE-KII activities, are coordinately regulated during development, whereas circulating angiotensin II and tissue ACE-KII change in a reciprocal manner. Based on these results, we hypothesize that kinins and angiotensin II influence the developmental regulation of ACE-KII. (Hypertension. 1994;23:369-374.)

Key Words • kininase II • kallikrein-kinin system • gene expression • renin-angiotensin

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whether such a reciprocal relation between Ang II and ACE-KII exists in the developing rat. Furthermore, we have previously demonstrated that renal kallikrein gene transcription and enzymatic activity are developmentally regulated.18,19 Because ACE-KII is the major kallikrein-degrading enzyme in the rat, the fourth and final aim of this study was to test whether the developmental changes in tissue kallikrein (and presumably kinas) and ACE-KII enzymatic activity are temporally linked during ontogeny.

Methods

Newborn (1, 5, 10, 15, and 20 days of postnatal life) and adult male (150 to 250 g) Sprague-Dawley rats (Charles River Laboratories) were studied. These age groups were chosen to encompass the developmental periods preceding the completion of nephrogenesis (days 7 through 10 in the rat) as well as maturing and fully mature kidneys.

Measurement of ACE-KII Activity

Organs (heart, aorta, lungs, liver, kidneys) were dissected free of connective tissue and immediately homogenized mechanically in ice-cold Tris-HCl buffer (0.1 mol/L, pH 8.2). Samples were centrifuged for 10 minutes at 3000g at 4°C, and the supernatant was dried in a vacuum centrifuge for 6 hours at 4°C, redissolved in 0.1 mL deionized distilled water, centrifuged for 10 minutes at 3000g at 4°C, and kept at -20°C until use. Tissue ACE-KII activity was measured by fluorometric assay of the enzymatic cleavage of hippurate from hippuryl-histidyl-leucine as described by Friedland and Silverstein,20 with modifications for application to tissue homogenates.21 Protein concentrations were determined as described by Lowry et al,22 and ACE-KII activity was expressed as nanomoles 1-histidyl-l-leucine formation per minute per milligram protein.

Extraction, Electrophoresis, and Hybridization of RNA to cDNAs

Total RNA from heart, aorta, lungs, kidney, and liver was extracted using the guanidinium isothiocyanate–phenol–chloroform method of Chomczynski and Sacchi.23 Total RNA (30 μg per sample) was pooled from newborns at postnatal day 1 (n=8), day 5 (n=8), day 15 (n=7), and day 20 (n=7) and from adult male (n=6) rats. Denatured RNA was resolved by electrophoresis on 1.2% agarose gels containing 2.2 mol/L formaldehyde followed by a transfer into a positively charged membrane (Zetabind; AMP Cuno, Inc.). In addition, slot blots were prepared by dissolving 5 to 1.25 μg of total RNA from individual animals in 0.5 mL sterile 25 mmol/L sodium phosphate (pH 7.2) and applying this RNA to nylon filters as described by Lowry et al,22 and ACE-KII activity was expressed as nanomoles 1-histidyl-l-leucine formation per minute per milligram protein.

Extraction, Electrophoresis, and Hybridization of RNA to cDNAs

To ensure that comparisons were performed on RNA of similar levels, three dilutions of RNA were obtained. The dilutions to be analyzed were chosen to ensure that comparisons were performed on the linear portion of the dose-response curve. To correct for differences in RNA blotting on the filter, the densitometric signals obtained from GAPDH were quantified, and results from the ACE-KII signal were expressed relative to the GAPDH signal. Duplicate samples of RNA from each animal were analyzed for ACE-KII and GAPDH. Data were analyzed by ANOVA, and individual comparisons among groups were further analyzed by the Scheffé test. A probability value of less than .05 was considered significant. All data are reported as mean±SEM.

Results

ACE-KII mRNA transcripts were expressed in the kidney, lung, heart, and aorta as early as day 1 of postnatal life. Active ACE-KII was also present in these tissues and in the serum of the newborn rat. Serum and tissue ACE-KII activity and tissue ACE-KII gene expression were all upregulated during development. The time of weaning (days 20 to 21 in the rat) was temporally associated with activation of the ACE-KII gene. However, the timing and magnitude of peak enzymatic activity and gene expression were different among the various tissues studied.

As shown in Fig 1A, serum ACE-KII activity peaked on day 10 of postnatal life (P<.05 versus days 1 and 5) and remained significantly elevated until adulthood, when it decreased toward levels observed during the first week of life.

Pulmonary ACE-KII activity (Fig 1B) and mRNA (Fig 2A) were detectable at low levels immediately after birth and increased significantly (8- to 12-fold) near the time of weaning (P<.005). Pulmonary ACE-KII activity and gene expression were further upregulated in adult rats. Overall, ACE-KII enzymatic activity and mRNA levels increased 25- and 15-fold, respectively, during the transition from newborn to adult life (P<.0001).

Kidney ACE-KII activity and mRNA expression were relatively low during the early newborn period, in amounts similar to those found in other tissues (Figs 1C and 2B). This was followed by a peak in both activity and gene expression (10- and 16-fold, respectively, P<.001) on day 15 of postnatal life. ACE-KII mRNA levels were significantly lower in the adult than in the kidneys of 15- and 20-day-old rats (P<.05). Kidney ACE-KII activity was also lower in the adult than in weanling rats, but the differences did not reach statistical significance (P=.09).

ACE-KII was expressed in the newborn rat hearts and aorta at approximately one tenth its concentrations in the lung (Figs 1D, 1E, 2C, and 2D). Cardiac and aortic ACE-KII dissociated during the newborn period; only the aortic ACE-KII displayed a significant rise in activity and gene expression around the time of weaning.
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Fig 1. Bar graphs show ontogeny of serum and tissue angiotensin-converting enzyme/kininase II (ACE-KII) activity (n=4 to 6 per age group) in serum (A), lung (B), kidney (C), heart (D), and aorta (E). + indicates after birth. Aortic samples were pooled for the newborn age groups because of the small amount of tissue. ACE-KII activity in serum was highest during the preweanling period (P<.05 vs newborn and adult), whereas tissue ACE-KII activity peaked in adulthood (P<.01 vs newborn groups in all tissues).

(10-fold, P<.001). A sixfold increase in cardiac ACE-KII expression was observed during postnatal maturation (P<.01).

Hepatic ACE-KII mRNA was not detectable in the liver at any age, even when larger amounts of RNA were loaded onto the gel (up to 50 μg). ACE-KII activity was extremely low in the liver (0.02±0.0016 on day 1 and 0.06±0.005 nmol/mg per minute in the adult, P<.001) and probably represented contamination by circulating ACE-KII.

The quantitative changes in ACE-KII mRNA levels in the lung, heart, aorta, and kidney as determined by densitometric analysis of the slot blots are shown in Fig 3. In each tissue, the relative levels of ACE-KII mRNA on day 1 of postnatal life were arbitrarily assigned a value of 1. During the first week of postnatal life, ACE-KII mRNA expression was low in all the tissues studied but increased significantly thereafter. However, the temporal changes in ACE-KII mRNA differed with each organ. In the lung, the levels of ACE-KII mRNA continued to increase after the time of weaning (day 21) and peaked in adulthood. In contrast, cardiac, aortic, and renal ACE-KII gene expression peaked between days 15 and 20 of postnatal life.

Tissue ACE-KII is a membrane-bound enzyme, and our extraction method did not involve the use of a detergent; therefore, we determined the amount of ACE-KII liberated in the presence or absence of 0.4% deoxycholate in adult rat kidneys (n=4). ACE-KII activity was slightly higher in deoxycholate-treated than nontreated samples (1.09±0.2 versus 0.77±0.07 nmol/min per milligram protein, P=.18). The method used in this study liberates the majority (75%) of cell-associated ACE-KII.

Unlike ACE-KII, which increased with age, we found that plasma Ang II levels were high in the newborn and decreased with age (115±15 versus 46±10 fmol/g, P<.05) (Fig 4).

Tissue kallikrein-like activity in the newborn rat was low but detectable in all the organs studied (Fig 5). During the immediate postnatal period, the kidney contained 3- to 10-fold higher levels of active kallikrein than other organs. Only the kidney manifested a surge in active kallikrein on day 1 after birth. Like ACE-KII, tissue kallikrein activity increased significantly with age and was not expressed in the liver during postnatal development.

Discussion

ACE-KII is a zinc metalloprotease involved in the posttranslational processing of many polypeptides, the most notable of which are Ang I and bradykinin. The present study demonstrates that ACE-KII activity is present in the developing kidney and that intrarenal synthesis from precursor mRNA is the likely source of renal ACE-KII activity. Furthermore, the upregulation
of renal ACE-KII activity during development appears to result at least in part from enhanced gene expression at the pretranslational level. The ACE-KII gene is expressed in several extrarenal organs, including the lung, heart, and aorta, during early development. Importantly, the somatic (endothelial) ACE-KII gene is not ubiquitous, as the hepatic cells did not express ACE-KII mRNA at any stage of postnatal maturation.

Ontogeny studies have clearly demonstrated that renal renin synthesis is highly activated during early postnatal development in several species.\textsuperscript{8-10} In contrast, angiotensinogen gene expression in the rat is low at birth and increases with age.\textsuperscript{25} Because kidney Ang II contents are significantly higher in the newborn compared with the adult (see Reference 26 and I.Y., S.S.Eli-D., unpublished observations), renin is considered to be the rate-limiting factor for Ang II generation during the preweaning period. It is possible that the age-related increase in ACE-KII activity serves to maintain intrarenal Ang II production in the face of declining renin synthesis and activity. The reasons for the relatively sustained ACE-KII activity in the adult...
kidney compared with its mRNA are not clear. Possible explanations include differential changes in mRNA stability, translational efficiency, enzyme activation, or trapping of circulating enzyme.

The role of ACE-KII in the developing kidney is illustrated physiologically by the remarkable effects of ACE-KII inhibition on renal growth and hemodynamics in the developing animal. ACE-KII inhibition decreases intrarenal Ang II and increases kinin concentrations and is associated with a significant decrease in fetal renal vascular resistance. Furthermore, chronic ACE-KII inhibition results in glomerular growth retardation in growing rats.

The demonstration of ACE-KII transcripts and activity in the developing cardiovascular system has important physiological implications. Ang II is a growth-promoting factor and is linked to hypertrophy of vascular smooth muscle cells. Concerning the developing heart, a recent study using neonatal rat cardiac fibroblasts demonstrated a remarkable Ang II–mediated activation of new protein and RNA and DNA synthesis ranging between 60% and 120% of basal levels. On the other hand, bradykinin, which is also produced locally in the heart and vessel wall, exerts antiproliferative effects in the injured vascular wall. Therefore, the upregulation of ACE-KII expression during the preweaning period would be expected to influence the ratio of vascular Ang II to bradykinin in favor of Ang II, thus promoting vascular growth.

An interesting finding of this study is that although there was a generalized increase in ACE-KII gene expression and activity with age, peak expression and activity of ACE-KII differed among the various tissues. Thus, whereas in the present study and that of Wallace et al lung ACE-KII activity and gene expression peaked in adulthood, renal, cardiac, and aortic ACE-KII gene expression peaked at around the time of weaning. It should be pointed out here that the ontogeny of ACE-KII in the rat may not reflect that of other species in which nephrogenesis is complete before birth (eg, human).

The factors involved in the tissue-specific expression of the ACE-KII gene are not known. One possible explanation for the regulation of tissue ACE-KII during postnatal development is the relation between ACE-KII gene expression and the endothelial cell proliferative state. In this regard, Shai et al have recently shown that ACE-KII mRNA expression is regulated in bovine aortic endothelial cells in an inverse relation to their growth state. Thus, confluent and postconfluent endothelial cell cultures express much higher levels of ACE-KII mRNA than subconfluent cells. In highly vascularized organs, such as the cardiovascular, pulmonary, and renal systems, the developmental upregulation of ACE-KII gene expression may therefore be related to the slower rate of vascular endothelial growth in mature than younger animals. Another factor that may contribute to the differential changes in tissue ACE-KII may be cellular trapping of the circulating enzyme.

Alternatively or additionally, the decreasing levels of circulating Ang II during postnatal maturation may be associated with induction of tissue ACE-KII synthesis, activity, or both. In support of this hypothesis is a study by Schunkert et al which showed that ACE-KII inhibition in rats was associated with a 140% increase in pulmonary ACE-KII mRNA compared with vehicle-
treated controls, whereas chronic (days) Ang II infusion caused a 50% to 60% decrease in lung ACE-KII mRNA, suggesting reciprocal regulation of Ang II and ACE-KII. The findings in the present study of higher plasma Ang II and low tissue ACE-KII levels in the newborn and the reciprocal changes between circulating Ang II and ACE-KII with age support the concept that Ang II may regulate ACE-KII gene expression during development.

Because kinins are important substrates for ACE-KII, we investigated the relationship between the ontogeny of tissue kallikrein and ACE-KII. Several ontological features are shared between the two enzymes: (1) Both enzymatic activities increase significantly during postnatal life; (2) the third week of postnatal life appears to coincide temporally with activation of both enzymes; and (3) neither enzyme is expressed in the liver. These results imply that local kinin generation and degradation are coordinately regulated during development and suggest a possible regulatory link between kallikrein-kinins and ACE-KII. The elevated levels of kallikrein-like activity in the kidney immediately after birth are consistent with our previous findings and suggest that perinatal factors (e.g., glucocorticoid surge) selectively induce renal prokallikrein activation.

In summary, the present study demonstrates that the ACE-KII gene is expressed in the developing rat kidney, lung, heart, and aorta but not the liver. The transcriptional and enzymatic activities of tissue ACE-KII are upregulated with maturation, particularly around the time of weaning. The data are also consistent with the hypothesis that circulating Ang II and tissue kallikrein activity may be important in the developmental regulation of tissue ACE-KII.

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