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Divergent Pathways for the Angiotensin-(1-12) Metabolism in the Rat Circulation and Kidney

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Abstract

Evidence of endogenous angiotensin-(1-12) [Ang-(1-12)] may necessitate revision of the accepted view that Ang I is the immediate peptide product derived from the precursor protein angiotensinogen. As the processing of this peptide have not been fully elucidated, we characterized Ang-(1-12) metabolism in the serum and kidney of the mRen2.Lewis rat, a model of high circulating renin and ACE expression. A sensitive HPLC-based method to detect the metabolism *ex vivo* of low concentrations of ¹²⁵I-labeled Ang-(1-12) was utilized. Ang-(1-12) processing to serum did not reveal the participation of renin; however, serum ACE readily converted Ang-(1-12) to Ang I with subsequent metabolism to Ang II. Ang I and Ang II forming activities for serum ACE were 102 ± 4 and 104 ± 3 fmol/ml/min serum (n = 3), respectively and both products were abolished by the potent ACE inhibitor lisinopril. The metabolism of Ang-(1-12) in renal cortical membranes also revealed the formation of Ang I; however, the main products were Ang-(1-7) and Ang-(1-4) at 129 ± 9 and 310 ± 12 fmol/mg/min protein (n = 4), respectively. Neprilysin inhibition abolished these products and substantially reduced the overall metabolism of Ang-(1-12). Incubation of Ang-(1-12) with either human or mouse neprilysin revealed identical products. We conclude that endogenous Ang-(1-12) may contribute to the expression of biologically active angiotensins through a renin-independent pathway. The preferred route for Ang-(1-12) metabolism likely reflects the relative tissue content of ACE and neprilysin.

Keywords

Angiotensin-(1-12); renin; ACE; neprilysin; Ang-(1-7); Ang-(1-4); HPLC

1. Introduction

The increasing complexity of the renin-angiotensin system (RAS) may now necessitate revision of the well-accepted tenet that angiotensin I (Ang I) is the sole peptide product derived from the precursor protein angiotensinogen [9,12]. Nagata and colleagues have recently provided compelling evidence for the expression of the novel peptide Ang-(1-12) [Asp¹-Arg²-Val³-Tyr⁴-Ile⁵-His⁶-Pro⁷-Phe⁸-His⁹-Leu¹⁰-Leu¹¹-Tyr¹²] in plasma, heart, kidney and other tissues of the normotensive Sprague Dawley rat [20]. The endogenous

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Conflict of interests

The authors declare that there are no competing financial interests in the work described.

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expression of Ang-(1-12) was confirmed in heart and kidney of both the Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats, although Ang-(1-12) content varied between the two strains [14]. The immunocytochemical distribution of Ang-(1-12) was primarily localized to the proximal tubules of the renal cortex, consistent with evidence for a local RAS within the tubular epithelium [14,18]. Moreover, Nagata and colleagues found that peripheral administration of Ang-(1-12) resulted in an immediate increase in blood pressure and that the pressor response was blocked by administration of either the ACE inhibitor captopril or the AT₁ receptor antagonist candesartan [20]. Similarly, Arnold et al [5] reported that an ACE inhibitor or AT₁ antagonist blocked the actions of Ang-(1-12) microinjected into the NTS region of rat brain. In contrast, Prosser and colleagues reported that cardiac actions of Ang-(1-12) were attenuated by serine protease inhibitor chymostatin but not by the ACE inhibitor captopril in an isolated heart preparation from rat [23]. Moreover, the actions of Ang I were abolished by the ACE inhibitor suggesting the selective processing of Ang-(1-12) and Ang I to Ang II by multiple enzymes at least in the rat heart [23]. These reports clearly demonstrate that biological actions Ang-(1-12) are likely through the conversion to Ang II, although the enzymatic pathways for this peptide have not been entirely elucidated. Therefore, we characterized the metabolism of Ang-(1-12) in the serum and cortical membranes from the kidney of the hypertensive mRen2.Lewis rat, a model that contains particularly high circulating levels of renin activity, as well as significant levels of ACE and ACE2 [22,29]. Our studies show that Ang-(1-12) is a substrate for serum ACE but not for circulating renin. ACE converts Ang-(1-12) to Ang I and Ang II in a sequential fashion consistent with the catalytic properties of the enzyme as a dipeptidyl carboxypeptidase cleaving two residues at a time. In contrast, the processing of Ang-(1-12) in the renal cortex revealed that Ang-(1-4) and Ang-(1-7) were the major peptide products formed exclusively by the endopeptidase neprilysin. The present results provide compelling evidence that Ang-(1-12) may serve as a precursor in the formation of the biologically active angiotensins which in the case of the kidney may cause preferential formation of Ang-(1-7).

2. Materials and methods

2.1 Animals

Hemizygous male mRen2.Lewis (mRen) rats were obtained from the WFU Hypertension Center Colony. Serum and kidney tissues were from 15 week old hypertensive males rats that exhibited a mean systolic blood pressure of 205 ± 12 mmHg (n=3).

2.2 Biochemical Assays

To characterize the metabolism of the peptide *ex vivo*, ¹²⁵I-labeled rat Ang-(1-12) [0.5 nM] was incubated with either serum (0.02 ml), mouse and human recombinant neprilysin (0.015 µg) or the solubilized membranes (10 µg protein) obtained from the renal cortex as previously described [25]. ACE and neprilysin activities were also derived for ¹²⁵I-Ang I [0.5 nM] in serum and cortical membranes under identical conditions for Ang-(1-12). The concentration of the recombinant neprilysin was derived from the similar extent of Ang-(1-12) metabolism observed for the rat cortical membranes (~40%). In brief, metabolism assays (0.5 ml final volume) were conducted at 37°C [20 and 60 minutes for serum; 30 minutes for tissue or recombinant enzymes] in 10 mM HEPES, 125 mM NaCl, 10 µM ZnCl₂ pH 7.4 in 0.5 ml. The reaction was stopped by addition of ice-cold 1.0 % phosphoric acid, centrifuged at $16,000 \times g$ and the supernatant stored at 4°C. Samples were filtered prior to separation by reverse-phase high performance liquid chromatography (HPLC) and the ¹²⁵I products monitored by a Bioscan flow-through γ detector. Products were identified by comparison of retention times to ¹²⁵I-standard peptides. The peptidase inhibitors for serum included amastatin (AM, 2 µM); bestatin (BS, 10 µM); chymostatin (CHYM, 10 µM) benzyl succinate (BSC, 10 µM) and *para*-chloro-mercuribenzoic acid (PCMB, 500 µM). We

subsequently added the renin inhibitor acetyl-His-Pro-Phe-Val-Sta-Leu-Phe-amide to block rat and mouse renin activity or lisinopril to block ACE activity (both at 10 μ M). For the cortical membranes, only the inhibitor SCH39370 (10 μ M) was added to block neprilysin activity. The enzymatic activities were expressed as fmol product per ml of serum, per μ g recombinant neprilysin or per mg protein per minute. All values are reported as the mean $\pm$ standard error of the mean (SEM).

2.3 Modeling

We utilized the current enzymatic data with the published endogenous concentrations of Ang-(1-12) and Ang I from Nagata et al [19] to model the processing of the Ang (1-12) in the serum or circulation (circ) and renal cortex (kid). Specifically, we modeled the production of Ang I in serum from angiotensinogen (Aogen) by renin to Ang-(1-12) by ACE and in the kidney from Ang-(1-12) by neprilysin. Assuming steady state, the equation for the Ang I mass balance in the circulation was expressed as the change in Ang I over time equivalent to the renin-dependent hydrolysis of Aogen and the ACE-dependent hydrolysis of Ang-(1-12) subtracted from the ACE-dependent hydrolysis of Ang I:

$$[\text{Ang I}_{\text{circ}}/\text{dt}] = \text{Renin}_{\text{circ}}[\text{Aogen}_{\text{circ}}] + \text{ACE}_{\text{circ}}[\text{Ang}(112)_{\text{circ}}] - \text{ACE}_{\text{circ}}[\text{Ang I}_{\text{circ}}] = 0. \quad (1)$$

We defined the contribution of the Renin-Aogen and ACE-Ang-(1-12) pathways to the generation of Ang I in the circulation compartment as the ratio of $\text{Renin}_{\text{circ}}[\text{Aogen}_{\text{circ}}]/\text{ACE}_{\text{s}}[\text{Ang-(1-12)}_{\text{p}}]$ or f_{circ} . Compartment substitution into equation (1) yielded the following equation for the circulation:

$$\text{ACE}_{\text{circ}}[\text{Ang I}_{\text{circ}}]/(\text{ACE}_{\text{circ}}[\text{Ang}(112)_{\text{circ}}] + 1) = f_{\text{circ}} \quad (2)$$

The mass balance of renal Ang I was similarly expressed as the change in Ang I over time equivalent to the renin-dependent hydrolysis of Aogen and the neprilysin-dependent hydrolysis of Ang-(1-12) subtracted from the neprilysin (NEP) hydrolysis of Ang I:

$$[\text{Ang I}_{\text{kid}}/\text{dt}] = \text{Renin}_{\text{kid}}[\text{Aogen}_{\text{kid}}] + \text{NEP}_{\text{kid}}[\text{Ang}(112)_{\text{kid}}] - \text{NEP}_{\text{kid}}[\text{Ang I}_{\text{kid}}] = 0. \quad (3)$$

We defined the renal contribution of the Renin-Aogen and NEP-Ang-(1-12) pathways to the formation of Ang I as the ratio of $\text{Renin}_{\text{kid}}[\text{Aogen}_{\text{kid}}]/\text{NEP}_{\text{kid}}[\text{Ang-(1-12)}_{\text{kid}}]$ or f_{kid} . Compartment substitution into equation (3) yields the following equation for the kidney:

$$\text{NEP}_{\text{kid}}[\text{Ang I}_{\text{kid}}]/\text{NEP}_{\text{kid}}[\text{Ang}(112)_{\text{kid}}] + 1 = f_{\text{kid}} \quad (4)$$

2.4 Reagents

The mouse and human recombinant neprilysin was obtained from R&D Systems (Minneapolis, MN). Rat Ang-(1-12) [Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Tyr] was synthesized (Genscript, Piscataway NJ), iodinated by the chloramine T method and purified by HPLC as described [10]. The rat/mouse renin inhibitor acetyl-His-Pro-Phe-Val-Sta(tine)-Leu-Phe-amide was obtained from Anaspec (Fremont, CA). The recombinant mouse and human neprilysin were obtained from R & D System (Minneapolis, MN).

3. Results

As shown in the chromatographs from Figure 1, absence of the ACE and renin inhibitors revealed radiolabeled peaks corresponding to the retention times for Ang II and Ang I as the main products of Ang-(1-12) metabolism in rat serum of the mRen2.Lewis rat (panel A).

The addition of the potent ACE inhibitor lisinopril essentially abolished the peaks for Ang II and Ang I (Figure 1B) while the subsequent addition of the rat renin inhibitor had no effect (panel C). These data suggest that Ang-(1-12) is initially processed to Ang I by serum ACE with the subsequent removal of the dipeptide His⁹-Leu¹⁰ to form Ang II. The metabolism studies from the serum of three rats revealed a similar degree of conversion for Ang I and Ang II (Figure 2) The increasing fractional conversion for Ang II as compared to Ang I suggests that Ang I may be the more preferred substrate for serum ACE activity rather than Ang-(1-12) under the present experimental conditions (Figure 2).

As shown in Figure 3, the chromatographs reveal that Ang-(1-12) is also processed to multiple peptide products in the renal cortex of the mRen2.Lewis rat (3A). In this case, Ang-(1-7) and Ang-(1-4) were the major products from Ang-(1-12); the addition of the neprilysin inhibitor SCH39378 significantly attenuated both peaks (3D). The peak corresponding to Ang I was also reduced by the neprilysin inhibitor suggesting that Ang I may be an intermediate product to the formation of Ang-(1-7) and Ang-(1-4). As we did not expect formation of Ang I from Ang-(1-12) by neprilysin, we then assessed the metabolism of Ang-(1-12) with recombinant mouse and human neprilysin. Similar to the data obtained in rat renal cortex, Ang-(1-12) was processed to Ang I, Ang-(1-7) and Ang-(1-4) by mouse and human neprilysin (3B and 3C, respectively). Moreover, addition of the neprilysin inhibitor abolished all three peaks and preserved the peak for Ang-(1-12) (3E and 3F). It should be noted while the sequences of rat and mouse Ang-(1-12) are identical, human Ang-(1-12) contains an isoleucine for leucine at the 11th position. The velocity data from four rat kidneys is shown in Figure 4 and reveal that Ang-(1-7) and Ang-(1-4) are the major products that arise from neprilysin in the rat kidney.

From the proceeding data, the existence of multiple enzymatic pathways for the metabolism of Ang-(1-12) in the serum and the renal cortex was evident. Both pathways apparently utilize Ang I as an intermediate for the subsequent formation of Ang II (serum) and Ang-(1-7)/Ang-(1-4) in renal cortical membranes. To compare the efficiency of Ang-(1-12) processing to the renin-Ang I pathway, we modeled the processing of both Ang I and Ang-(1-12) using the current enzyme velocity data, as well as the endogenous angiotensin levels reported by Nagata and colleagues for the plasma and kidney [19]. These equations were solved for the conservation of Ang I $d[AI]/dt = 0$ in the circulation where f_{circ} is the ratio of Ang I [Renin-Aogen] to Ang I [ACE-Ang-(1-12)] and in the kidney where f_{kid} is the ratio of Ang I [Renin-Aogen] to Ang I [NEP-Ang-(1-12)]. The calculated f values suggest that renin-dependent conversion of Aogen to Ang I is markedly favored over the ACE to Ang I conversion from Ang-(1-12) in serum ($f_{circ} = \text{Ren}_{circ}[\text{Aogen}_{circ}]/\text{ACE}_{circ}[\text{Ang-(1-12)}_{circ}] = 39.4 \pm 0.3$ (Table 1). However, the renal pathways for Ang-(1-12) conversion are more comparable where $f_{kid} = \text{Ren}_{kid}[\text{Aogen}_{kid}]/\text{NEP}_{kid}[\text{Ang-(1-12)}_{kid}] = 1.5 \pm 0.2$ (Table 1).

4. Discussion

The present findings reveal that Ang-(1-12) is a substrate for both ACE and neprilysin that culminate in the formation of the functionally active peptides Ang II and Ang-(1-7). In the serum, soluble ACE activity readily converted Ang-(1-12) to Ang I and Ang II suggesting that Ang I is a necessary intermediate in the formation of Ang II. Similarly, the renal processing of Ang-(1-12) also involves Ang I as an intermediate, however, the neprilysin-dependent processing forms Ang-(1-7) and Ang-(1-4). Demonstration of the conversion Ang-(1-12) to active products clearly supports the functional actions of exogenous Ang-(1-12), as well as the potential contribution of endogenous tissue sources of the peptide to the local expression of Ang II or Ang-(1-7) [9,12].

The initial findings by Nagata and colleagues revealed that Ang-(1-12) evoked an immediate and significant pressor response following bolus administration of the peptide in normotensive rats that was blocked by either an ACE inhibitor or an AT₁ antagonist [20]. Moreover, infusion of Ang-(1-12) increased perfusion pressure in the isolated rat heart of multiple strains that was associated with the formation of both Ang I and Ang II in the perfusate, although the Ang II-forming enzymes were unfortunately not identified in this study [28]. ACE was the predominant pathway for Ang II formation from Ang-(1-12) in neonatal myocytes obtained from spontaneously hypertensive (SHR) and normotensive (WKY) rats [1]. In the rat brain, the actions of Ang-(1-12) were also abolished by an ACE inhibitor or an AT₁ antagonist [5]. However, Prosser et al demonstrated an ACE-independent pathway for the conversion of Ang-(1-12) to Ang II in the perfused heart isolated from Sprague-Dawley rats [23]. The formation of Ang II from Ang-(1-12) was inhibited by chymostatin, a general inhibitor of chymotrypsin-like proteases, while Ang II generation from Ang I was blocked by the specific ACE inhibitor captopril [23]. The Prosser study is of particular interest as it suggests the preferential processing of Ang-(1-12) and Ang I in the rat heart by distinct enzymes. These data markedly contrast with the current study where serum ACE readily converts Ang-(1-12) to Ang II, as well as other functional studies on the exogenous administration of the peptide [4,5,20]. Indeed, Nagata and colleagues find that the acute pressor effects of Ang-(1-12) were abolished by the ACE inhibitor captopril suggesting the ACE-dependent generation of Ang II in the circulation [20]. We do not suggest that ACE exclusively processes Ang-(1-12) to Ang II as Ahmad and colleagues described a chymostatin-sensitive peptidase, likely chymase, in the atrial appendage of the human heart that metabolized Ang-(1-12) to Ang II [2]. Moreover, cardiac ACE activity was essentially undetectable in the latter study and the lack of ACE may promote greater participation of other enzymes such as chymase in the generation of Ang II particularly in damaged or injured tissues [2]. These studies also emphasize the apparent species difference in the processing enzymes that contribute to the generation of angiotensin peptides.

In the serum, the processing of Ang-(1-12) by ACE resulted in the formation of Ang I and subsequent metabolism to Ang II. The processing is consistent with the catalytic function of ACE to sequentially cleave two residues from the carboxyl terminus of various peptides including bradykinin and Ang-(1-7) [29]. We find that addition of the rat renin inhibitor did not influence the processing of Ang-(1-12) in the serum of the mRen2.Lewis rat even in the presence of the ACE inhibitor lisinopril. We reported that renin activity in the heterozygous mRen2.Lewis rat is approximately 4-fold higher than the background Lewis strain and is composed of both rat and mouse renin isoforms [22]. In this case, Ang-(1-12) apparently lacks the minimum number of residues on the carboxy terminus recognized and cleaved by renin. Indeed, the Ang-(1-14) substrate is considered to contain the minimum sequence for renin to cleave the His-Leu in the formation of Ang I [11, 26]. Although our results do not support a role for renin to process Ang-(1-12), enzymatic pathway(s) for generation of the peptide in the circulation is not known. Bujak-Gizycka et al [7] demonstrated peptidase activity in rat aortic segments that processed the synthetic tetradecapeptide [Ang-(1-14)] substrate primarily to Ang-(1-12) with lesser amounts of Ang I and Ang II formed. It is not known whether Ang I and Ang II were derived directly from Ang-(1-12) or Ang-(1-14), nor was the Ang-(1-12)-forming activity characterized by sensitivity to various classes of enzyme inhibitors [7]. Moreover, the study exclusively utilized Ang-(1-14) to demonstrate Ang-(1-12) formation rather than the endogenous substrate angiotensinogen. The substrate properties of the 14 amino acid peptide may potentially be quite different than the >350 residue protein and use of the precursor protein is the preferred approach to demonstrate a putative role of an enzyme in the processing of angiotensins [11, 26]. Renin specifically cleaves the Leu¹⁰-Leu¹¹ bond of rat angiotensinogen to form Ang I, while the hydrolysis between the two aromatic residues Tyr¹²-Tyr¹³ is required for the direct generation of Ang-

(1-12) [11,26]. The processing pathway we modeled in the circulation apparently favors the initial renin-dependent formation of Ang I rather than the formation of Ang I from Ang-(1-12) by ACE. Indeed, if Ang-(1-12) was the requisite precursor to Ang I in the circulation we would expect Ang I levels to be *reduced* following ACE inhibition, and typically both circulating renin and Ang I are markedly increased with this regimen [17].

In the kidney, the present study reveals that Ang-(1-12) is a substrate for neprilysin also forming Ang I as a likely intermediate to the generation of Ang-(1-7) and Ang-(1-4). A similar pattern of metabolism for Ang-(1-12) was demonstrated using purified preparations of both mouse and human neprilysin. In contrast to the serum, modeling of the renal processing revealed a comparable contribution of the Ang-(1-12)-neprilysin pathway to that of renin-angiotensinogen in the formation of Ang I. The greater contribution of Ang-(1-12) in the renal pathway for Ang I formation likely reflects the high levels of neprilysin in the rat kidney as compared to other peptidases in the renal cortex, particularly the activity found in the apical region or brush border of the proximal tubules [3,19]. The present studies did not distinguish whether Ang I is a required intermediate in the formation of Ang-(1-7) or Ang-(1-4) or whether the latter peptides are directly generated from Ang-(1-12). The direct processing of rat Ang-(1-12) to Ang I, Ang-(1-7) and Ang-(1-4) would involve hydrolysis of the Leu¹⁰-Leu¹¹, Pro⁷-Phe⁸, and Tyr⁴-Ile⁵ bonds, respectively by neprilysin. Indeed, this metalloendopeptidase exhibits a preference for hydrophobic or aromatic residues in the *PI* – *P'I* positions [24]. Moreover, we did not assess earlier time points in the metabolism study to determine whether the ratio of Ang-(1-4) to Ang-(1-7) is predominant. However, we and others have described essentially an identical pattern of Ang-(1-7) and Ang-(1-4) formation from Ang I by neprilysin [3,24,27]. In this regard, neprilysin may contribute to the processing of Ang-(1-12) to Ang-(1-7) in the circulation provided the ACE pathway is significantly blocked; ACE inhibition prevents the metabolism of Ang-(1-7) to Ang-(1-5) and directs the processing of Ang I to neprilysin [9, 10]. Circulating levels of soluble neprilysin are essentially non-detectable; however, the endopeptidase is found on the vascular surface and neprilysin inhibitors significantly reduce circulating Ang-(1-7), particularly in the setting of ACE inhibition [8,15,16]. Although the renal RAS has been extensively studied for many years, the pathways that lead to the formation of Ang II or Ang-(1-7) within the kidney are still not completely resolved. This may reflect the processing by various cell types within the kidney, the differential expression of certain RAS components by these cells and the potential for the stable sequestration of circulating Ang II by the AT₁ receptor and potentially other transport mechanisms [6,13,20]. The demonstration of renal Ang-(1-12) and the processing to Ang-(1-7) add additional complexity to the intrarenal RAS, particularly if the generation of Ang-(1-12) occurs through a non-renin pathway. Indeed, if there are distinct compartments of Ang-(1-12) and angiotensinogen within the kidney, this may be the basis for the differential formation of Ang II and Ang-(1-7). The pathways for Ang-(1-12) formation and the compartmentalization of the peptide within the kidney, as well as in other cardiovascular tissues, await further studies.

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Glossary of terms

ACE	angiotensin converting enzyme
Ang-(1-12)	angiotensin-(1-12) [Asp ¹ -Arg ² -Val ³ -Tyr ⁴ -Ile ⁵ -His ⁶ -Pro ⁷ -Phe ⁸ -His ⁹ -Leu ¹⁰ -Leu ¹¹ -Tyr ¹²]
Aogen	angiotensinogen
RAS	renin-angiotensin system
Renin inhibitor	Acetyl-His ¹ -Pro ² -Phe ³ -Val ⁴ -Sta(tine) ⁵ -Leu ⁶ -Phe ⁷ -amide
Tetradecapeptide	Ang-(1-14) [Asp ¹ -Arg ² -Val ³ -Tyr ⁴ -Ile ⁵ -His ⁶ -Pro ⁷ -Phe ⁸ -His ⁹ -Leu ¹⁰ -Leu ¹¹ -Tyr ¹² -Tyr ¹³ -Ser ¹⁴]

Highlights

Serum ACE processes Ang-(1-12) to Ang II through the generation of Ang I

Serum renin does not metabolize Ang-(1-12) to shorter angiotensin peptides

Rat, human and mouse neprilysin process Ang-(1-12) to Ang-(1-7) and Ang-(1-4)

Kinetic modeling does not favor Ang-(1-12) as the immediate precursor to Ang I

Figure 1.

Chromatograph reveals the metabolism of ^{125}I -Ang-(1-12) to Ang II in serum of male mRen2.Lewis rats. Panel A: Ang I and Ang II are the primary products of Ang-(1-12) metabolism in the absence of the ACE inhibitor lisinopril and the renin inhibitor WFML. Panel B: addition of the ACE inhibitor lisinopril and lisinopril/WFML respectively. ^{125}I -Ang-(1-12) was incubated in serum for 20 min at 37°C prior to separation by reverse phase-HPLC.

Figure 2. Fractional conversion of Ang-(1-12) by rat serum ACE. Incubation times for the substrate and serum were at 20 and 60 minutes at 37°C. Data are the mean $\pm$ SEM (n = 3)

Figure 3. Processing of ^{125}I -Ang-(1-12) by rat renal cortex or purified mouse and human neprilysin. Chromatographs reveal that the products of Ang-(1-12) were Ang-(1-4), Ang-(1-7) and Ang I in rat cortical membranes (A), and mouse (B) and human (C) recombinant neprilysin. The neprilysin inhibitor SCH39370 was added to the rat, mouse and human samples (D, E and F, respectively). Incubation time for the substrate and all enzyme preparations was 30 minutes at 37°C .

Figure 4. Comparison of enzymatic velocities for the products of Ang-(1-12) metabolism. Enzyme processing from solubilized membranes from rat cortical neprilysin (R NEP; fmol/mg protein/min), mouse (M) and human (H) recombinant neprilysin (fmol/ μ g protein/min), and rat serum ACE (R ACE; fmol/ml/min). Data are the mean $\pm$ SEM for rat cortex ($n = 4$), serum ($n = 3$) and mouse and human recombinant neprilysin ($n = 1$).

Circulation

Kidney

Figure 5.

Potential pathways for Ang I and Ang-(1-12) processing in the circulation and kidney. In the circulation, the renin-dependent generation of Ang I is preferred over Ang-(1-12) processing to Ang I from ACE. Circulating ACE converts Ang-(1-12) to Ang I and subsequently to Ang II. In the renal cortex, renin-dependent formation of Ang I is comparable to processing of Ang-(1-12) by neprilysin. Renal neprilysin may potentially convert Ang-(1-12) directly to Ang-(1-7) and Ang-(1-4) or indirectly through Ang I.

Table I

Peptidase activities, angiotensin content and f values for modeling equations of Ang-(1-12) metabolism.

	Mean	n	Reference
Cortical NEP - Ang I	291 ± 51	4	
Cortical NEP - Ang-(1-12)	228 ± 13	4	
Cortical Ang I	365 ± 30	8	8
Cortical Ang 1-12	380 ± 27	8	8
Serum ACE - Ang I	104 ± 3.4	3	
Serum ACE - Ang-(1-12)	102 ± 3.8	3	
Plasma - Ang I	383 ± 80	8	8
Plasma Ang-(1-12)	10.1 ± 2.7	8	8
f_{kid}	1.5 ± 0.2		
f_{circ}	39.4 ± 0.3		

Cortical neprilysin (NEP) activity from Ang I or Ang-(1-12), fmol/min/mg protein;

Cortical peptide content, fmol/mg protein;

Serum ACE activity from Ang I or Ang-(1-12), fmol/min/ml serum;

Plasma Ang I or Ang-(1-12) content, fmol/ml plasma.