Does the Angiotensin-Converting Enzyme (ACE)/ACE2 Balance Contribute to the Fate of Angiotensin Peptides in Programmed Hypertension?

K. Bridget Brosnihan, Liomar A.A. Neves, Mark C. Chappell

The renin-angiotensin system (RAS) plays a key role in blood pressure regulation, fluid and electrolyte balance, cellular growth, thirst, and cardiac/renal function. The classic system primarily involves 2 enzymes: renin, which cleaves angiotensinogen to the inactive decapeptide angiotensin (Ang) I; and Ang-converting enzyme (ACE), a dipeptidyl carboxypeptidase that hydrolyzes Ang I to the octapeptide Ang II. The elucidation of the ACE pathway in parallel with potent and selective ACE inhibitors is clearly a pivotal achievement in our understanding of the RAS and in attaining effective therapies for hypertension and end organ damage. Indeed, ACE inhibitors attenuate Ang II formation and augment the levels of the heptapeptide Ang (1–7), a peptide that counterbalances the actions of Ang II on blood pressure and cellular growth through a unique receptor system. Ang II mediates the majority of its actions at the Ang II type 1 (AT1) receptor, including the stimulation of vasoconstriction, sodium retention, cellular growth, and oxidative stress, whereas recent studies show that Ang (1–7) at the AT1<sub>C</sub> or mas receptor and Ang II via the AT<sub>2</sub> receptor subtype counterregulate the actions of Ang II at the AT<sub>1</sub> receptor.

The discovery of the ACE homolog ACE2 provides further evidence that the RAS is far more complex than originally thought. The enzymatic cascade of the RAS should now encompass the ACE2-dependent pathways that directly yield Ang (1–7) from Ang I and degrade Ang II to Ang (1–7) (Figure). The interplay between ACE and ACE2 that may govern the formation and metabolism of Ang effector peptides warrants the simultaneous study of both enzymes to ultimately assess their contribution to blood pressure regulation and cardiac/renal function.

ACE is a zinc metalloendopeptidase that functions as a C-terminal peptidyl dipeptidase and acts to convert Ang I to Ang II and inactivate the vasodilator bradykinin. The 2 forms of ACE, somatic and testicular, are encoded from a single gene. Somatic ACE has 2 independent catalytic sites with distinct properties, and it exists as soluble and membrane-bound forms. The germinal form, which is found exclusively in the testes, has 1 catalytic site and is involved in fertility. Recently, it has been shown that ACE hydrolyzes Ang (1–7) to Ang (1–5), thus facilitating the breakdown of Ang (1–7).<sup>1,2</sup> Under conditions of ACE inhibition, not only is there an increase in the substrate Ang I, which can be converted to Ang (1–7) through the action of endopeptidases such as nephrilysin and prolylendopeptidase, but there is also prevention of the breakdown of Ang (1–7). Acting at multiple sites in the processing scheme, ACE inhibition results in increased plasma, tissue, and urine levels of Ang (1–7).<sup>3,4</sup> Blockade of Ang (1–7) by its specific receptor antagonist D-alanine<sup>7–Ang</sup> (1–7) reverses the blood pressure–lowering actions of ACE inhibitor lisinopril,<sup>5</sup> illustrating that the increased levels of Ang (1–7) account for a part of the antihypertensive actions of ACE inhibition.

ACE2 is the first known human homologue of ACE, which was isolated from 2 human cDNA libraries prepared from ventricular and lymphoma tissues.<sup>8,9</sup> The enzyme exhibits 42% sequence identity and 61% sequence similarity to ACE. ACE2 contains a single zinc-binding domain HEXXH, which is homologous to the active sites of ACE; however, it is not inhibited by ACE inhibitors.<sup>10</sup> ACE2 exhibits carboxypeptidase activity cleaving a single amino acid residue at the carboxyl terminus. Although ACE2 was first shown to hydrolyze Ang I to release Ang (1–9) with subsequent hydrolysis by ACE to Ang (1–7) in cardiomyocytes, the kinetic data by Vickers et al<sup>11</sup> with recombinant ACE2 revealed a relatively low catalytic constant (k<sub>c</sub>) for Ang (1–9) formation. In contrast, the hydrolysis of Ang II to Ang (1–7) by ACE2 exhibited a very high catalytic efficiency (k<sub>c</sub>/K<sub>m</sub>) of 240-fold greater than that for Ang I to Ang (1–9). In this scheme, the formation of the vasodilator peptide Ang (1–7) occurs at the expense of the vasoconstrictor hormone Ang II. Despite the kinetic data favoring Ang II as the substrate for ACE2, Li et al<sup>12</sup> found that Ang I was the preferred substrate for ACE2 in proximal tubules with no evidence for ACE2-dependent generation of Ang (1–7) from Ang II. These data emphasize that both Ang I and Ang II should be used as substrates to characterize ACE2 activity.

In the current issue of Hypertension, Rivière et al<sup>13</sup> provide an important study by examining the regulation and distribution of ACE2 mRNA and ACE mRNA in a rat model of fetal programming. In their study, adult offspring (4 months old) (FR30) of dams food-restricted throughout gestation showed mild hypertension, reduced nephron number, and elevated...
ACE/ACE2 contribution to the formation of angiotensin peptides. In the classic pathway, Ang I is converted by ACE to Ang II. Ang (1–7) can be formed from Ang II by the action of ACE2 in a highly efficient manner. The pathway from Ang I to Ang (1–9) by ACE2 and then Ang (1–7) by ACE or other endopeptidases represents alternative steps in this pathway. Ang I conversion to Ang (1–7) by the endopeptidase nephrilysin is a likely pathway in the circulation. ACE inactivates Ang (1–7) to Ang (1–5). The density of the arrows for ACE/ACE2 indicates the more favored pathway.

plasma Ang II and aldosterone, the latter changes suggesting a key role of systemic RAS to contribute to the development of hypertension. The FR30 animals also exhibited increased ACE (1.5-fold) and ACE2 (2-fold) activity in the lung, but increased mRNA content of either enzyme did not accompany the changes in activity. Interestingly, these data also reveal the tissue-dependent regulation of ACE and ACE2, whereas other important cardiovascular areas including the kidney and heart did not show changes in either mRNA or activity for ACE and ACE2. Although the current view has proposed that ACE and ACE2 act to counterregulate one another, the study by Riviére et al reveals a similar upregulation of the activities of the 2 enzymes that may occur posttranslationally in the food-restricted offspring. From the data presented, it is not possible to determine the relative contribution of each enzyme to the elevated blood pressure although the findings are more consistent with the elevated lung ACE activity being responsible for the elevated plasma Ang II and hypertension. In this regard, the relative magnitude of ACE and ACE2 activities is an important consideration to the generation of Ang II and Ang (1–7) although the latter peptide was not measured in the current study. In the lung, ACE was shown to be the more abundant enzyme with 100-fold higher activity in the offspring of both the food-restricted dams and the control group. Therefore, even though ACE2 increased to a greater extent than ACE with undernutrition, the greater abundance of ACE may be the determining factor for the expression of the 2 peptides.

The presence of hypertension and reduced nephron number in the FR30 rats supports the fetal programming phenomenon resulting from undernutrition of pregnant mothers. The elevation of plasma glucocorticoids in the mothers at day E14 is also consistent with the proposed role of steroids in fetal programming. Finally, upregulation of ACE activity in the lungs of the FR30 rats, together with increased plasma Ang II and aldosterone, supports their participation in the hypertension of the offspring. The exact role for ACE2 in blood pressure regulation in this model is unknown; however, there are a number of observations that have linked ACE2 gene expression to blood pressure regulation. If we hypothesize that ACE2 generates Ang (1–7) and hydrolyzes Ang II, then an upregulation of ACE2 would be consistent with an attenuation of the elevated blood pressure (ie, a protective effect of ACE2 that provides resistance to the development of hypertension). Crackower et al reported that ACE2 gene expression was decreased in the hypertensive Sabra rat, a salt-sensitive strain when compared with the hypertensive resistant strain maintained on a normal salt diet. With salt loading, ACE2 levels were reduced and accompanied by a further augmentation of the hypertension. On the other hand, the increased ACE2 in the salt resistant rats is consistent with their maintenance of normal blood pressure. Additional findings in spontaneously hypertensive rats and spontaneously hypertensive-stroke prone rats showed that ACE2 gene expression is consistently lower than the normotensive Wistar Kyoto control. Future studies to overexpress ACE2 or induce its activity are required to decipher the extent that hypertension in these models is dependent on the reduced expression of this enzyme.

Of particular interest, the Riviére et al study found a high level of ACE2 expression in the placenta. In agreement with their finding of no in situ labeling of ACE2 on the maternal side of the rat placenta, we found no immunocytochemical staining for ACE2 and Ang (1–7) in the trophospongium of the rat placenta (unpublished data, 2005), but ACE2 and Ang (1–7) were highly expressed in the fetal placenta/labyrinth. In human placenta we showed pronounced immunocytochemical expression of ACE2 and Ang (1–7) and specific staining in cytrophoblast, sincyciotrophoblast, and the endothelium of the blood vessels of the primary and secondary villi. In addition, we found that ACE2 and Ang (1–7) were expressed in the maternal stromal in the invading and intravascular trotophoblast and the decidual cells. The similar localization of Ang (1–7) and ACE2 in the human and rat placenta strongly suggests that ACE2 is involved in the processing of Ang (1–7) in local tissue expression of the peptide. Our most recent study showed the upregulation of ACE2 mRNA in normal pregnant uterus and downregulation of ACE2 mRNA in a model of preeclampsia in the rat (reduced uterine perfusion pressure) with no change in the level of ACE2 mRNA in the placenta of normal and preeclamptic pregnant rats. Taken together, these studies suggest an important role for ACE2 in the utero-placenta unit, which can be regulated with placenta ischemia. Further studies are warranted to evaluate the role of ACE2/Ang (1–7) and ACE in models of intrauterine growth restriction.

In conclusion, the RAS has become a “system within a system” characterized by counterregulatory effective peptides acting at multiple receptor subtypes. The discovery of ACE2 has added a new dimension to the system, whereby not only the traditional ACE-mediated arm of the RAS must be considered, but the ACE2 pathway may also be affected and counterbalance ACE, giving rise to the vasodilatory and antiproliferative peptide Ang (1–7).

References


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