Published in final edited form as:

Hypertens Res. 2010 February ; 33(2): 107–109. doi:10.1038/hr.2009.207.

Does ACE2 Contribute to the Development of Hypertension?

Mark C. Chappell, PhD, FAHA[Professor]

The Hypertension and Vascular Research Center, Wake Forest University School of Medicine,
Winston-Salem, NC 27157-1032

It is now a decade since the discovery of the angiotensin I converting enzyme homolog ACE2 [EC 3.4.15.1]. In that time period, efforts towards the characterization of this enzyme have provided greater insights into the renin-angiotensin-aldosterone system (RAAS). The ongoing studies provoked more questions particularly regarding role of the enzyme in the development and progression of hypertension and renal injury, as well as other pathologies including diabetes, heart failure, liver failure and pulmonary injury. In this issue of *Hypertension Research*, Kamilic et al. determined the distribution, expression and activity of ACE2 within the kidney of two rat models of established hypertension and their normotensive controls¹. Although the reported localization of ACE2 within the kidney differs to some extent with other reports in rat, the current study reveals no difference in ACE2 expression between the hypertensive and normotensive rats suggesting that the enzyme may not contribute to the development of the hypertensive phenotype.

Although both ACE and ACE2 are chloride-activated metallopeptidases that are primarily membrane bound and ubiquitously distributed in various tissues, ACE2 is a carboxypeptidase that hydrolyzes a single amino acid from the carboxyl end of peptides as opposed to the dipeptidylcarboxypeptidase properties of ACE. ACE inhibitors do not attenuate ACE2 activity but may in certain circumstances increase expression of the enzyme². Early studies identified angiotensin I (Ang I) as the substrate for ACE2 likely given the similar homology to ACE and the existing evidence for ACE-independent pathways. Indeed, Ang I was metabolized by the enzyme to the nonapeptide Ang-(1–9), but not directly to Ang II. Subsequently, kinetic studies revealed that the conversion of Ang II to Ang-(1–7) was the preferred pathway with a 500 fold greater efficiency (kcat/Km ratio) than that for hydrolysis of Ang I to Ang-(1–9). Indeed, ACE2 exhibits a higher catalytic efficiency in comparison to other peptidases that generate Ang-(1–7) (Figure 1).

Various studies have revealed the importance of ACE2 to influence expression of Ang II and Ang-(1–7) particularly within the kidney. Transgenic mice with total knockout of ACE2 exhibit higher tissue levels of Ang II^{3,4}. Tikellis and colleagues recently showed that in addition to enhanced Ang II levels in ACE2^{-/-} transgenic mice, the renal content of Ang-(1–7) was markedly lower which clearly demonstrates the direct utilization of Ang II by ACE2 as the precursor for Ang-(1–7) within the kidney⁵. Induction of type 1 diabetes by streptozotocin (STZ) is associated with lower ACE2 activity in the kidney which primarily reflects reduced expression in proximal tubules and glomerulus^{5,6}. Induced diabetes in the ACE2 deficient mice reveals an exaggerated phenotype of renal injury (increased albuminuria) in comparison to the STZ-treated wildtype mice. In this regard, RAS blockade by either an ACE inhibitor or an AT1 receptor antagonist is less effective in the diabetic ACE2 deficient mice or following chronic treatment with an ACE2 inhibitor than the

Address for Correspondence: Mark C. Chappell, Ph.D., Hypertension and Vascular Disease Center, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27015, Telephone: (336)-716-9236, FAX: (336)-716-2456, mchappel@wfubmc.edu.

diabetic control mice^{5,6}. Moreover, Benter et al find that Ang-(1–7) or its non-peptide receptor agonist AVE0991 attenuates proteinuria and oxidative stress in hypertensive diabetic rats⁷. These studies clearly implicate the importance of renal ACE2 and its product Ang-(1–7) in diabetes-induced renal injury.

Although the influence of ACE2 in renal and cardiac injury has become more evident, the role of the enzyme in the regulation of blood pressure, particularly in hypertensive animals is equivocal at this point. Crackower et al. first reported that the ACE2 transgenic mice exhibited *lower* blood pressure than the wildtype mice; however, the reduced pressure likely reflects reduced cardiac output due to impaired cardiac function in older transgenic mice³. Subsequent reports in other ACE2^{-/-} transgenic strains have revealed either no change or a slight increase in blood pressure with no evidence of cardiac ventricular dysfunction in younger mice^{4,8}. The Crackower study also demonstrated reduced protein expression of ACE2 in the kidneys of several hypertensive models including the spontaneously hypertensive rat (SHR), the stroke-prone SHR (SP-SHR), and the Sabra salt sensitive rat³. Moreover, in a sheep model of fetal programmed hypertension sensitive to AT1 receptor blockade, ACE2 activity in the proximal tubules and urine was markedly reduced without changes in renal ACE⁹. Tikellis and colleagues confirmed the findings in SHR in their study of the developmental expression of ACE2 in the SHR and WKY¹⁰. ACE2 mRNA expression and enzyme activity were significantly reduced in the SHR kidneys at 6 weeks and 12 weeks as compared to WKY. However, systolic blood pressures were markedly higher in the SHR by 6 weeks of age and the reduction in ACE2 may reflect renal damage. Moreover, ACE expression and activity were reduced in the kidney of the SHR at the same age as for ACE2¹⁰. The reduction in both ACE2 and ACE in the SHR raises the issue of whether the ratio of Ang II to Ang-(1–7) is actually changed.

In the present findings by Kamilic and colleagues, these authors find no change in ACE2 expression or activity in the kidneys of adult male SP-SHR and transgenic (mRen2)27 rats with established hypertension in comparison to their respective normotensive controls. Interestingly, the (mRen2)27 and Sprague Dawley rats exhibited 3-fold higher renal ACE2 activity than the WKY or SP-SHR rats emphasizing the strain differences in enzyme expression. It is not clear why the present findings in SHR-SP contrast with previous studies in this strain that report reduced in ACE2³. The Crackower study determined ACE2 mRNA and protein expression, but not enzyme activity in renal tissue³. In addition to gene and protein expression, the Kamilic study assessed ACE2 activity in the kidney by a widely-used method that measures the hydrolysis of the quenched fluorescent substrate Mca-Ala-Pro-Lys-DNP¹. This substrate is not completely specific for ACE2, however, and the addition of an ACE2 inhibitor (and possibly other peptidase inhibitors) is necessary to verify activity. In this regard, Tikellis et al include an inhibitor of the enzyme prolyl oligopeptidase to prevent the hydrolysis of the substrate by this peptidase which cleaves the Pro-X bond of various peptides¹⁰. The two most widely used ACE2 inhibitors are MLN4760, a non-peptide inhibitor no longer available from Millennium Pharmaceuticals and DX600, a large peptide inhibitor that may not be completely resistant to proteolytic degradation in tissue. To our knowledge, the studies by Kamilic are first to assess ACE2 expression and activity in the kidney of the homozygous (mRen2)27 rat. This transgenic strain expresses the renin 2 (Ren2) gene and is considered a hypertensive model of an elevated tissue RAS. Despite the reduced expression of renal renin in the (mRen2)27, the kidney exhibits higher Ang II content¹¹. We assessed renal ACE2 in hemizygous mRen2.Lewis rats originated from the backcross of the (mRen2)27 into the Lewis background¹². The male mRen2.Lewis rats also exhibit lower renin and higher Ang II in the renal cortex, but lower ACE2 activity as compared to the Lewis control rats¹¹. Indeed, the difference in the Ang II to Ang-(1–7) ratio between the Lewis and mRen2.Lewis rats reflects a similar change in the ACE to ACE2 ratio (Figure 2). Moreover, chronic RAS blockade with an ACE inhibitor or AT1 antagonist

that markedly reduces blood pressure in male mRen2.Lewis rats was associated with increased ACE2 mRNA expression and enzyme activity in the renal cortex¹². However, Ang II-dependent activation of AT1 receptor reduces the expression of ACE2¹³, thus the issue remains as to whether lower ACE2 activity increases Ang II or whether Ang II down regulates ACE2 in the kidneys of the mRen2.Lewis.

Finally, the Kamilic study report that the localization of ACE2 in the rat kidney is predominantly localized to the glomerulus with high expression in the visceral epithelial cells with moderate expression in both endothelial and medial aspects of the renal vasculature, but no evidence for ACE2 in the proximal tubules¹. The authors utilized the Millennium antibody for ACE2 in which staining was blocked by the immunogenic peptide, as well as demonstrated comparable staining in both frozen and paraffin-embedded tissue sections. The absence of ACE2 in the tubular epithelium contrasts with other studies in human, mouse, sheep and rat that demonstrate ACE2 staining, mRNA expression or enzyme activity in the proximal tubules in addition to other cell types within the kidney^{5, 10, 14–16}. Indeed, Warner et al find that ACE2 expressed in the MDCK tubular cells preferentially localizes or traffics to the apical aspect of the cell membrane while ACE is found on both basolateral and apical areas consistent with *in situ* studies¹⁷. Localization of ACE2 on the apical surface of the tubular epithelium would certainly favor a regulatory role for the enzyme in the processing of Ang II to Ang-(1–7) in the tubular fluid. Moreover, the intracellular localization of the enzyme within the kidney may provide a mechanism to buffer Ang II-dependent formation of reactive oxygen species¹⁸. The absence of immunoreactive ACE2 on tubular epithelium of the rat kidney in the present study is not clear. Possibly, the various ACE2 antibodies may recognize different molecular forms of the enzyme which could be present in the kidney and other tissues. Alternatively, different incubation or permeabilization conditions may have a significant influence on antibody recognition of ACE2. As with other components of the RAAS, a standard antibody for ACE2 available for multiple species would be beneficial for comparative studies. Nevertheless, the present study emphasizes the need for continued research on ACE2 particularly in relation to ACE and perhaps other Ang II-forming enzymes (i.e., chymase) that generate the requisite substrate for ACE2. Indeed, it is evident that future studies require a more comprehensive inclusion of the components that comprise the distinct functional arms of the RAAS¹⁹.

Acknowledgments

Sources of Funding

These studies were supported in part by grants from the National Institute of Health grants (HL-56973, HL-51952)

Literature Cited

1. Kamilic J, Hamming I, Kreutz R, Bolbrinker J, Siems WE, Nassar I, Sluimer JC, Walther T, Navis GJ, van Goor H. Renal ACE2 expression and activity is unaltered during established hypertension in adult SHRSP and TGR(mREN2)27. *Hypert Res.* 2009
2. Ferrario CM, Jessup JA, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, Diz DI, Gallagher PE. Effect of angiotensin converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin converting enzyme 2. *Circulation.* 2005; 111:2605–10. [PubMed: 15897343]
3. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santo AJ, da Costa J, Zhang L, Pei Y, Scholey J, Bray MR, Ferrario CM, Backx PH, Manoukian AS, Chappell MC, Yagil Y, Penninger JM. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature.* 2002; 417:822–8. [PubMed: 12075344]

4. Gurley SB, Allred A, Le TH, Griffiths R, Mao L, Philip N, Haystead TA, Donoghue M, Beitbart RE, Acton SL, Rockman HA, Coffman TM. Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. *J Clin Invest*. 2006; 116(8):2218–25. [PubMed: 16878172]
5. Tikellis C, Bialkowski K, Pete J, Sheehy K, Su Q, Johnston C, Cooper M, Thomas M. ACE2 deficiency modifies renoprotection afforded by ACE inhibition in experimental diabetes. *Diabetes*. 2008; 57:1018–25. [PubMed: 18235039]
6. Wysocki J, Ye M, Soler MJ, Gurley SB, Xiao HD, Bernstein KE, Coffman TM, Chen S, Battle D. ACE and ACE2 activity in diabetic mice. *Diabetes*. 2006; 55:2132–9. [PubMed: 16804085]
7. Benter IF, Yousif MH, Dhaunsi GS, Kaur J, Chappell MC, Diz DI. Angiotensin-(1–7) prevents activation of NADPH oxidase and renal vascular dysfunction in diabetic hypertensive rats. *Am J Nephrol*. 2008; 28:25–33. [PubMed: 17890855]
8. Yamamoto K, Ohishi M, Katsuya T, Ito N, Ikushima M, Kaibe M, Tatara Y, Shiota A, Sugano S, Takeda S, Rakugi H, Ogihara T. Deletion of angiotensin-converting enzyme 2 accelerates pressure overload-induced cardiac dysfunction by increasing local angiotensin II. *Hypertension*. 2006; 47:718–26. [PubMed: 16505206]
9. Shaltout HA, Figueroa JP, Rose JC, Diz DI, Chappell MC. Alterations in circulatory and renal angiotensin-converting enzyme and angiotensin-converting enzyme 2 in fetal programmed hypertension. *Hypertension*. 2009; 53:404–8. [PubMed: 19047579]
10. Tikellis C, Cooper ME, Bialkowski K, Johnston CI, Burns WC, Lew RA, Smith AI, Thomas MC. Developmental expression of ACE2 in the SHR kidney: A role in hypertension? *Kidney Int*. 2006; 70:34–41. [PubMed: 16710353]
11. Hartner A, Porst M, Klanke B, Cordasic N, Veelken R, Hilgers KF. Angiotensin II formation in the kidney and nephrosclerosis in Ren-2 hypertensive rats. *Nephrol Dial Transplant*. 2006; 21:1778–85. [PubMed: 16522658]
12. Pendergrass KD, Pirro NT, Westwood BM, Ferrario CM, Brosnihan KB, Chappell MC. Sex differences in circulating and renal angiotensins of hypertensive mRen(2).Lewis but not normotensive Lewis rats. *Am J Physiol Heart Circ Physiol*. 2008; 295:H10–H20. [PubMed: 18456730]
13. Gallagher PE, Ferrario CM, Tallant EA. MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides. *Am J Physiol Cell Physiol*. 2008; 295:C1169–C1174. [PubMed: 18768926]
14. Shaltout HA, Westwood B, Averill DB, Ferrario CM, Figueroa J, Diz DI, Rose JC, Chappell MC. Angiotensin metabolism in renal proximal tubules, urine and serum of sheep: Evidence for ACE2-dependent processing of Angiotensin II. *Am J Physiol Renal Physiol*. 2006; 292:F82–F91. [PubMed: 16896185]
15. Reich HN, Oudit GY, Penninger JM, Scholey JW, Herzenberg AM. Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. *Kidney Int*. 2008; 74:1610–6. [PubMed: 19034303]
16. Joyner J, Neves LAA, Granger JP, Alexander BT, Merrill DC, Chappell MC, Ferrario CM, Davis WP, Brosnihan KB. Temporal-spatial expression of angiotensin-(1–7) and angiotensin converting enzyme 2 in the kidney of normal and hypertensive pregnant rats. *Am J Physiol Regul Integr Comp Physiol*. 2007; 293:R169–R177. [PubMed: 17428896]
17. Warner FJ, Lew RA, Smith AI, Lambert DW, Hooper NM, Turner AJ. Angiotensin-converting enzyme 2 (ACE2), but not ACE, is preferentially localized to the apical surface of polarized kidney cells. *J Biol Chem*. 2005; 280:39353–62.
18. Gwathmey TM, Pendergrass KD, Reid SD, Rose JC, Diz DI, Chappell MC. Angiotensin-(1–7)-ACE2 attenuates reactive oxygen species formation to angiotensin II with the cell nucleus. *Hypertension*. 2009 in press.
19. Chappell MC. Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1–7) mas receptor axis; more than regulation of blood pressure? *Hypertension*. 2007; 50(4):596–9. [PubMed: 17785634]
20. Chappell MC. Role of ACE, ACE2 and Neprilysin in the Kidney. In: Leung, PS., editor. *Frontiers in Research of Renin Angiotensin System on Human Disease*. Vol. 7. Springer; New York: 2007. p. 1–20.

Figure 1.

Comparison of the efficiency constants for Ang-(1–7) forming enzymes. ACE2 exhibits the highest efficiency constant among the enzymes capable of forming Ang-(1–7) from the substrates Ang I, Ang II or Ang-(1–9). The efficiency constant is the ratio of the catalytic (kcat) constant to the Michelis-Menten (Km) value for prolyl (oligo) endopeptidase (PO), neprilysin (NP), angiotensin converting enzyme 2 (ACE2), ACE and prolyl carboxypeptidase (PC) ²⁰.

Figure 2. The Ang II to Ang-(1-7) and ACE to ACE2 ratios are higher in the renal cortex of the hypertensive male mRen2.Lewis rats in comparison to normotensive Lewis males. Data were adapted from Pendergrass et al ¹².