Hypertension Research (2010) 33, 107–109

www.nature.com/hr

COMMENTARY

Does ACE2 contribute to the development of hypertension?

Mark C Chappell

Hypertension Research (2010) **33,** 107–109; doi:10.1038/hr.2009.207; published online 18 December 2009

It is now a decade since the discovery of the angiotensin I (Ang I)-converting enzyme homolog, ACE2 [EC 3.4.15.1]. During this period, efforts toward the characterization of this enzyme have provided greater insights into the renin–angiotensin–aldosterone system. The ongoing studies provoked more questions, particularly regarding the 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.*[1] determined the distribution, expression and activity of ACE2 within the kidney of two rat models of established hypertension and their normotensive controls. Although the reported localization of ACE2 within the kidney differs to some extent with other reports in the 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.

Both ACE and ACE2 are chloride-activated metallopeptidases that are primarily membrane bound and ubiquitously distributed in various tissues; however, 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 increase expression of the enzyme in certain circumstances.[2] Previous studies identified Ang I as the substrate for ACE2, most 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^{-1}) than that for hydrolysis of Ang I to Ang-(1–9). Indeed, ACE2 exhibits a higher catalytic efficiency in comparison with 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 *et al.*[5] 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 shows the direct utilization of Ang II by ACE2 as the precursor for Ang-(1–7) within the kidney. Induction of type 1 diabetes by streptozotocin 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 with the streptozotocin-trea-

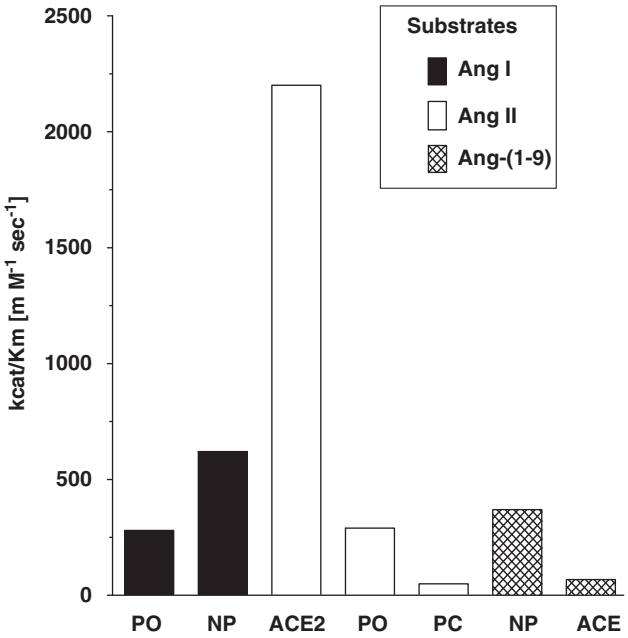

Figure 1 Comparison of the efficiency constants for Ang-(1–7)-forming enzymes. ACE2 exhibits the highest efficiency constant among the enzymes capable of forming Ang-(1–7) from the substrates Ang I, Ang II or Ang-(1–9). The efficiency constant is the ratio of the catalytic (kcat) constant to the Michaelis–Menten (Km) value for prolyl (oligo) endopeptidase (PO), neprilysin (NP), angiotensin-converting enzyme 2 (ACE2), ACE and prolyl carboxypeptidase (PC).[20,21]

Professor MC Chappell is at the Hypertension and Vascular Disease Center, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27015, USA.
E-mail: mchappel@wfubmc.edu

ted wild-type mice. In this regard, renin–angiotensin system blockade by either an ACE inhibitor or an Ang II type I (AT1) receptor antagonist is less effective in the diabetic ACE2-deficient mice or after chronic treatment with an ACE2 inhibitor than the diabetic control mice.[5,6] Moreover, Benter *et al.*[7] found that Ang-(1–7) or its nonpeptide receptor agonist, AVE0991, attenuates proteinuria and oxidative stress in hypertensive diabetic rats. 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.*[3] first reported that the ACE2 transgenic mice exhibited lower blood pressure than the wild-type mice; however, the reduced pressure likely reflects reduced cardiac output due to impaired cardiac function in older transgenic mice. 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 showed reduced protein expression of ACE2 in the kidneys of several hypertensive models, including the spontaneously hypertensive rat (SHR), the stroke-prone SHR and the Sabra salt-sensitive rat.[3] Moreover, in fetal programmed hypertension, ACE2 activity in the proximal tubules and urine is markedly reduced without changes in renal ACE.[9] Tikellis *et al.*[10] confirmed the findings by Crackower in their study of the developmental expression of ACE2 in the SHR and the normotensive Wistar Kyoto (WKY) rats. ACE2 mRNA expression and enzyme activity were significantly reduced in the SHR kidneys at 6 weeks and 12 weeks as compared with WKY rats. 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 ages as for ACE2.[10] 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 *et al.*, the authors find no change in ACE2 expression or activity in the kidneys of adult male stroke-prone SHR and transgenic (mRen2)27 rats with established hypertension, in comparison with their respective normotensive controls. Interestingly, the (mRen2)27 and Sprague–Dawley rats exhibited threefold higher renal ACE2 activity than the WKY rats or stroke-prone SHRs, emphasizing the strain differences in enzyme expression. It is not clear why the present findings in stroke-prone SHR contrast with previous studies in this strain that report a reduction in ACE2 activity.[3] The Crackower study determined ACE2 mRNA and protein expression, but not enzyme activity, in renal tissue.[3] 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-DNP.[1] However, this substrate is not completely specific for ACE2, and the addition of an ACE2 inhibitor (and possibly other peptidase inhibitors) is necessary to verify activity. In this regard, Tikellis *et al.*[10] included 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 peptides. The two most widely used ACE2 inhibitors are MLN4760, a nonpeptide 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 the 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 as a hypertensive model of an elevated tissue renin–angiotensin system. Despite the reduced expression of renal renin in the (mRen2)27, the kidney exhibits higher Ang II content.[11] We assessed renal ACE2 in hemizygous mRen2.Lewis rats originated from the backcross of the (mRen2)27 into the Lewis background.[12] The male mRen2.Lewis rats also exhibit lower renin and higher Ang II in the renal cortex, but lower ACE2 activity as compared with the Lewis control rats.[11,12] 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).

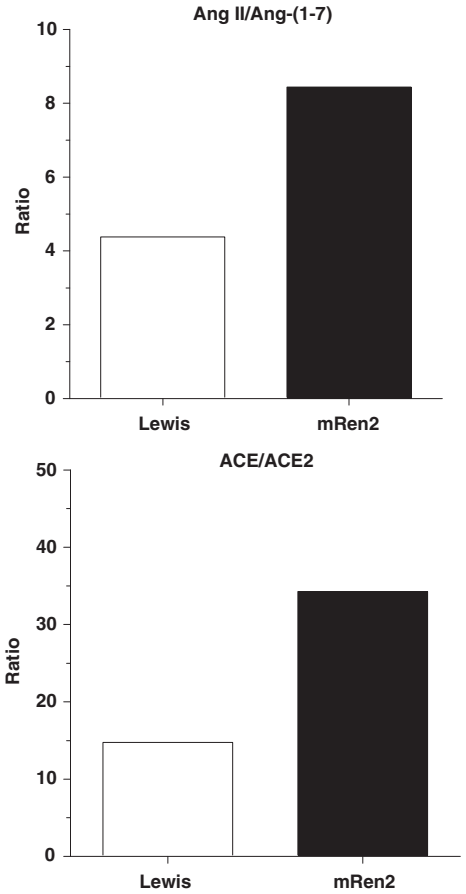

Figure 2 The Ang II to Ang-(1–7) and ACE to ACE2 ratios are higher in the renal cortex of the hypertensive male mRen2.Lewis rats (mRen2) in comparison with normotensive Lewis males. Data were adapted from Pendergrass *et al.*,[12] used with permission by the American Journal of Physiology—Heart and Circulatory Physiology.

Moreover, chronic renin–angiotensin system 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 cortex.[12] However, Ang II-dependent activation of AT1 receptor reduces the expression of ACE2;[13] thus, the issue remains as to whether lower ACE2 activity increases Ang II or whether Ang II downregulates ACE2 in the kidneys of the mRen2.Lewis rats.

Finally, the Kamilic study reports that the localization of ACE2 in the rat kidney is predominantly localized to the glomerulus with high expression in the visceral epithelial cells and with moderate expression in both endothelial and medial aspects of the renal vasculature, but no evidence for ACE2 in the proximal tubules.[1] The authors used the Millennium ACE2 antibody, in which staining was blocked by the immunogenic peptide, as well as showed 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 show 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.*[17] found that ACE2 expressed in the MDCK tubular cells preferentially localizes or traffics to the apical aspect of the cell membrane, whereas ACE is found on both basolateral and apical areas consistent with *in situ* studies. 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 species.[18] 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 that 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 renin–angiotensin–aldosterone system, 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 such as 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 renin–angiotensin–aldosterone system.[19]

ACKNOWLEDGEMENTS

This study was supported in part by grants from the National Institute of Health grants (HL-56973, HL-51952).

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. *Hypertens Res* 2009 (e-pub ahead of print 20 November 2009; doi:10.1038/hr.2009.191).

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**: 2605–2610.

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**: 822–828.

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**: 2218–2225.

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**: 1018–1025.

6 Wysocki J, Ye M, Soler MJ, Gurley SB, Xiao HD, Bernstein KE, Coffman TM, Chen S, Batlle D. ACE and ACE2 activity in diabetic mice. *Diabetes* 2006; **55**: 2132–2139.

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.

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**: 718–726.

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**: 404–408.

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.

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**: 1778–1785.

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.

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.

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.

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**: 1610–1616.

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.

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**: 39353–39362.

18 Gwathmey TM, Pendergrass KD, Reid SD, Rose JM, Diz DI, Chappell MC. Angiotensin-(1-7)-ACE2 pathway attenuates reactive oxygen species formation to angiotensin II within the cell nucleus. *Hypertension* (e-pub ahead of print 30 November 2009; doi:10.1161/HYPERTENSIONAHA.109.141622).

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**: 596–599.

20 Welches WR, Brosnihan KB, Ferrario CM. A comparison of the properties, and enzymatic activity of three angiotensin processing enzymes: angiotensin converting enzyme, prolyl endopeptidase and neutral endopeptidase 24.11. *Life Sci* 1993; **52**: 1461–1480.

21 Rice GI, Thomas DA, Grant PJ, Turner AJ, Hooper NM. Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. *Biochem J* 2004; **383**: 45–51.