Emerging Evidence for a Functional Angiotensin-Converting Enzyme 2-Angiotensin-(1-7)-Mas Receptor Axis
More Than Regulation of Blood Pressure?

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From the initial description of renin activity over a century ago, the ongoing study of the renin-angiotensin (Ang)-aldosterone system (RAAS) continues to yield unexpected findings that redefine the functional nature of this system, as well as our concepts on the mechanisms of cardiovascular regulation. The functional arc of the RAAS can no longer be viewed solely in terms of increasing blood pressure and inducing vasoconstriction, although these still remain the dominant aspects of at least the angiotensin II receptor (AT1R) pathway. There is clearly compelling evidence that other components of the RAAS may buffer actions, because the Ang II-AT2 receptor pathway uses the identical ligand coupled to a different receptor subtype to evoke, in many cases, actions that oppose AT1 activation1 (for a more extensive review). Moreover, parallel pathways within the RAAS result in novel products distinct from Ang II that require additional “Ang”-converting enzymes (ACEs) and unique receptors for these products. Indeed, the recent discoveries of ACE2, the Ang-(1-7) [AT(1-7)] receptor, and Ang-(1-12)2 as a potentially new precursor add significant impetus to revise the RAAS as multiple systems that may amplify or oppose one another (see the Figure). In this brief review, the current evidence for the physiological relevance of these new components of the RAAS is assessed.

Kidney

Half a century after the discovery of ACE, a new homolog of the enzyme termed “ACE2” was identified. Ang I was indeed a substrate for the enzyme, but the product was the nonapeptide Ang-(1-9) and not Ang II. Subsequent studies find that the conversion of Ang II to Ang-(1-7) is the preferred pathway with a 500-fold greater efficiency than that for Ang I. Consistent with the apical expression of ACE2 in the renal epithelium, we and others find both tubular and urinary ACE2 activity that converted Ang II to Ang-(1-7) but did not process Ang I to Ang-(1-9), providing further evidence that ACE2 is likely the major catalytic path for Ang-(1-7) formation.3-5 The localization of ACE2 in the proximal tubule epithelium along with other elements of the RAAS (ACE, angiotensinogen, and Ang receptors) certainly implies a role for the enzyme in the formation of Ang-(1-7) from Ang II.4 Gurley et al4 developed additional ACE2 knockout models and, dependent on the background strain, report higher blood pressure in mice lacking this carboxypeptidase. Moreover, the relative tissue levels of renal Ang II were significantly elevated in comparison with wild-type mice, although commensurate measurements of Ang I and Ang-(1-7) were not performed. Oudit et al5 in their ACE2 knockout model found that these older mice exhibit a greater degree of glomerulosclerosis and proteinuria, which was attenuated by AT1 receptor blockade. Interestingly, the kidneys of the female ACE2 knockout mice did not reveal evidence of injury and were apparently protected from the loss of the enzyme.

Diabetic nephropathy is clearly influenced by an activated RAAS, and both ACE inhibitors and AT1 receptor antagonists are effective in attenuating the progression of injury. Renal ACE2 is reduced in the proximal tubules of the streptozotocin-induced model of type 1 diabetes, and the attenuation of renal injury by ACE inhibition is associated with increased ACE2 expression.6 In turn, chronic ACE2 inhibition in the diabetic db/db mice exacerbates the extent of albuminuria ≈3-fold.8 Although Ang content was not measured, the db/db mice exhibited increased glomerular expression of ACE and reduced ACE2 as compared with the control db/db mice. Interestingly, the localization studies revealed distinct patterns of staining for ACE2 and ACE within the glomerulus: ACE2 in podocytes and ACE in the endothelial cells.9 In this regard, Ang-(1-7) or its receptor agonist Aventis (AVE) 0991 attenuates proteinuria and improves renal vascular activity in the diabetic rat but did not reverse the urinary excretion of lysozyme, a marker of tubulointerstitial damage.10 In addition, the ratio of Ang-(1-7) to Ang II formed from Ang I was lower in glomeruli isolated from the kidneys of diabetic rats.11 Thus, a reduction in the renal expression of Ang-(1-7) in diabetes may exacerbate renal injury. These studies also suggest that the glomerulus may be a second key site within the kidney where ACE2 may influence the local expression of Ang peptides and renal function. Ang-(1-7) abrogates the Ang II-dependent activation of mitogen-activated protein (MAP) kinase in primary cultures of proximal

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Moreover, the inhibitory actions of Ang-(1-7) were blocked by the Ang-(1-7) antagonist (D-Ala7)-Ang-(1-7) consistent with the immunocytochemical evidence for the AT(1-7)-Mas receptor in the tubular epithelium.4

Heart and Vasculature

In the spontaneously hypertensive rat (SHR) heart, lentiviral expression of ACE2 has amelioratory effects on blood pressure and cardiac fibrosis, although the circulating or cardiac content of Ang II and Ang-(1-7) was not assessed.13 These findings, however, are important in demonstrating that overexpression of ACE2 can effectively alter the balance of the RAAS in this hypertensive model. Pan et al14 find that, in a porcine pacing model, cardiac ACE2 is markedly reduced, whereas ACE is increased. Tallant et al15 report that Ang-(1-7) inhibits the growth of cardiomyocytes and is associated with reduced activity of MAP kinase, responses that are opposite those for Ang II-AT1 activation. The growth inhibition and reduction in MAP kinase activity were blocked by the downregulation of the AT(1-7)-Mas receptor in the tubular epithelium.4

Recent studies from this group demonstrate that Ang-(1-7) reduces adenocarcinoma growth in vivo potentially through the reduction in the cyclooxygenase-2/proinflammatory pathway.16 Indeed, the antiproliferative actions of Ang-(1-7) may explain the clinical observation that ACE inhibitor therapy is associated with a reduced incidence of certain types of cancer. Preliminary studies also reveal that Ang-(1-7) inhibits cyclooxygenase-2 expression in cardiofibroblasts.17 Indeed, the anti-inflammatory actions of Ang-(1-7) may underlie the ability of the peptide to reverse cardiac fibrosis in the DOCA-salt rat model.18 In this case, the antifibrotic actions of Ang-(1-7) were independent of a reduction in blood pressure. The ACE2-deficient mice who develop cardiomyopathy also exhibit evidence of increased oxidative stress and inflammation, as well as MAP kinase and phosphatidylinositol 3-kinase activation.19 Ang-(1-7) reduces collagen formation, as well as the expression of transforming growth factor-β, endothelin-1, and leukemia inhibitory factor in cardiofibroblasts.20 In Ang II-dependent cardiac hypertrophy, Ang-(1-7) abolished the extent of cardiac hypertrophy and interstitial fibrosis without changes in blood pressure.21 Importantly, the coadministration of the (D-Ala7)-Ang-(1-7) reversed the cardioprotective effects of Ang-(1-7). Santos et al22 report impaired cardiac function in the Mas-deficient mouse that was associated with increased forms of collagen and fibronectin. Moreover, Ang-(1-7) or AVE 0991 provide comparable cardioprotection from induced ischemia in diabetic rats, although the extent of hyperglycemia was not attenuated.10 Similar protective actions of Ang-(1-7) and AVE 0991 were also observed in the N0-nitro-l-arginine methyl ester–treated spontaneously hypertensive rat.23 Sampaio et al24 find that Ang-(1-7) evokes phosphorylation of the serine-1177 and dephosphorylation of threonine-495 residues to activate endothelial NO synthase in human endothelial cells. Because Ang-(1-7) does not evoke an increase in intracellular calcium, these investigators implicate the AKT/phosphatidylinositol

Figure. Cascade of the processing of angiotensin peptides and their interaction with AT1 and AT(1-7) receptor systems. ACE cleaves Ang I, releasing the dipeptide His-Leu to form Ang II, and ACE2 subsequently hydrolyzes Ang II to Ang-(1-7). ACE also metabolizes Ang-(1-7) to Ang-(1-5) and the dipeptide His-Pro. Ang-(1-12) may be cleaved from angiotensinogen (Aogen) and potentially processed directly to Ang II or Ang-(1-7). Ang-(1-7) may attenuate the inflammatory and fibrotic actions of the Ang II-AT1 receptor pathway through inhibition of the MAP kinase kinase (MAPKK) pathway, the potential stimulation of cellular phosphatases, the inhibition of cyclooxygenase-2 (COX2) and other proinflammatory agents, as well as the stimulation of NO. Although not shown, the AT2 and bradykinin receptor systems may interact with these pathways as well.
3-kinase pathway in endothelial NO synthase activation. Indeed, the heptapeptide stimulated phosphorylation of AKT, of which the downstream substrate is endothelial NO synthase (Figure). Similar results on endothelial NO synthase activation and NO release were observed in the Mas–transfected but not control Chinese hamster ovary cells, further substantiating the role of the Mas receptor mediating the Ang-(1-7)—dependent activation of endothelial NO synthase. Although, these studies clearly elevate the relevance of an Ang-(1-7)-Mas pathway by demonstrating a functional pathway in human cells, the acute vasodilatory effects of the peptide have not been demonstrated in vivo.\textsuperscript{25} However, one should not discount that Ang-(1-7) lacks any effect in humans (normotensive or hypertensive), because the reliance on blood pressure or flow may hinder our view to other potential actions of the peptide.

### Brain

The brain is also a key area for cardiovascular regulation containing multiple RAAS components, if not a complete system. Doobay et al\textsuperscript{26} find ACE2 mRNA and protein expression in the mouse brain, specifically in cardiovascular relevant areas, such as the subfornical organ, area postrema, paraventricular nucleus, and ventrolateral medulla. ACE2 expression was exclusively relegated to neurons; however, the staining was predominantly cytoplasmic, which is surprising given that the enzyme is primarily a membrane-anchored protein with the catalytic domain facing the extracellular surface. The processing pathways for the brain RAAS remain equivocal, but perhaps the cytosolic location of ACE2 may indicate a unique mechanism for the neuronal expression and/or regulation of intracellular Ang II and Ang-(1-7). Yamazato et al\textsuperscript{27} report that ACE2 expression in the rostral area of the ventrolateral medulla was lower in the SHR versus normotensive controls, which is consistent with reduced renal content in this hypertensive model. Most interestingly, the overexpression of ACE2 in this area reduced both blood pressure and heart rate in the SHR. Additional studies are required to determine the exact mechanisms for the blood pressure–lowering actions of ACE2; however, Ang-(1-7) is known to elicit cardiovascular actions in this area. Sakima et al\textsuperscript{28} find that the reduced control of heart rate in older rats is associated with the loss of an Ang-(1-7) effect in the nucleus tractus solitarius. Preliminary findings by Diz et al\textsuperscript{29} reveal that ACE2 inhibition within the nucleus tractus solitarius impairs the baroreflex to a similar extent as the Ang-(1-7) antagonist and clearly support a functional role for ACE2 in this region.

### Conclusion

Evidence that ACE2 directly forms Ang-(1-7) and for a distinct Ang-(1-7) receptor has stimulated resurgence in experimental studies resulting in a wider re-examination of Ang-(1-7). Additional interest, apart from the functional aspects, lies in elucidating the complex regulation of these components within the RAAS. Ang II directly downregulates ACE2 through activation of the AT\textsubscript{1} receptor\textsuperscript{30} consistent with studies in the intact animal that ACE or AT\textsubscript{1} blockade increases ACE2 expression.\textsuperscript{4} Although Ang-(1-7) alone did not influence the basal expression of ACE2 in the cell experiments, the peptide attenuated the inhibitory effects of Ang II on ACE2 via a receptor-dependent mechanism. Current evidence suggests that aldosterone downregulates ACE2,\textsuperscript{31,32} whereas the mineralocorticoid is known to increase the expression of ACE, AT\textsubscript{1} receptors, and intrarenal Ang II.\textsuperscript{33} The downregulation of ACE2 leading to higher Ang II may potentially be compensated by AT\textsubscript{1} receptor binding of Ang II. In turn, either ACE inhibition or AT\textsubscript{1} blockade may promote ACE2-dependent formation of Ang-(1-7) and its receptor-associated pathways. Indeed, the relationship between the AT\textsubscript{1} and Ang-(1-7) cellular pathways is an area requiring further study. Finally, the demonstration of endogenous Ang-(1-12) in the rat may portend renin-independent pathways that culminate in the formation of biologically active peptides.\textsuperscript{2} The peptide bond Tyr\textsuperscript{12}-Tyr\textsuperscript{13} hydrolyzed to yield Ang-(1-12) from rat angiotensinogen is structurally distinct from the Leu\textsuperscript{9}-Leu\textsuperscript{10} bond recognized by rat renin to form Ang I. Moreover, these bonds are also distinct for human angiotensinogen. Although the Ang-(1-12) peptide may not exhibit any functional action, the processing to this intermediate peptide may convey an additional level of regulation within the RAAS (Figure). Since this research on Ang-(1-7) originated by my colleagues at the Cleveland Clinic began 18 years ago, one could scarcely envisage the expansion of the RAAS that has occurred. There is little doubt that studies in the next few years will resolve the relevance of an endogenous Ang-(1-7)-ACE2-Mas pathway, particularly its role in tissue injury, inflammation, and cellular growth, and perhaps reveal whether this pathway is of therapeutic value. In this regard, the orally active agonist AVE 0991 or the development of related compounds may provide additional cardiovascular benefits.

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### Disclosures

None.

### References


16. Menon J, Sota-Pantoja DR, Callahan MF, Chappell ACE2 and Ang-(1-7) 599


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