

# Therapeutic Implications of the Vasoprotective Axis of the Renin-Angiotensin System in Cardiovascular Diseases

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Despite the tremendous success obtained using the current pharmacotherapy, especially angiotensin (Ang)-converting enzyme (ACE) inhibitors (ACEis) and Ang II receptor blockers (ARBs), the prevalence of cardiovascular diseases (CVDs) remains high around the world. The overall death rate in the United States alone in 2005 was 278.9 per 100 000 people.<sup>1</sup> These unprecedented numbers have stimulated researchers to consider the development of new strategies and targets to control these diseases.

Since its discovery, the renin-Ang system (RAS) has been considered an important target to manage the disturbances of the cardiovascular system. In fact, RAS blockers represent the main class of drugs in the treatment of hypertension and CVDs.<sup>2</sup> Recent genomic and proteomic studies have led to significant advances in our understanding of the RAS and in experimental methods for studying regulatory mechanisms influenced by the RAS. Thus, demonstrating the existence of a counterregulatory axis within the RAS, constituted by ACE2, Ang-(1-7), and the Mas receptor, has established a new concept for this system, that is, the classic narrow cascade formed by ACE, Ang II, and Ang II type 1 receptor (AT<sub>1</sub>) has been replaced by a flexible hormonal system with many bioactive peptides, receptors, enzymes, and interactions among these components.<sup>2-4</sup> Consequently, follow-up studies have revealed new possibilities and targets to better control CVD. In this review, we discuss the following: (1) the current status of the RAS with an emphasis on evidence for the existence of the ACE2/Ang-(1-7)/Mas axis; (2) the role of this axis in the pathophysiology of the cardiovascular, renal, pulmonary, and central nervous systems; (3) the potential of this protective axis for the development of novel CVD therapeutics; and (4) future perspectives.

## Autoregulation of the RAS: ACE2/Ang-(1-7)/Mas Receptor Axis

The RAS consists of 2 distinct and counterregulatory axes (Figure). The classic ACE/AngII/AT<sub>1</sub>R axis is responsible for the vasoconstrictive, proliferative, hypertensive, and fibrotic actions of the RAS. Its hyperactivity is associated with hyper-

tension and CVDs, such as cardiac hypertrophy, heart failure, stroke, coronary artery disease, and end-stage renal disease. This axis is the primary target for the actions of the RAS blockers. The ACE2/Ang-(1-7)/Mas axis constitutes an alternative axis that represents an intrinsic mechanism to induce vasoprotective actions by counterregulating the ACE/AngII/AT<sub>1</sub>R axis, thus inducing many beneficial effects in CVDs. Ang II type 2 receptors may also contribute to this counterregulation.<sup>5</sup> The counterregulatory concept of the RAS is emerging, and studies have established a key role for its main component, ACE2.

ACE2 is a monocarboxypeptidase of which the active site domain shares ≈42% homology with the active site of ACE.<sup>6</sup> It is found in the plasma membranes of virtually all organs as an ectoenzyme and in the plasma and urine in a soluble active form.<sup>4</sup> Ang I and Ang II are the principal substrates for this enzyme. The impact of ACE2 on the metabolism of other cardiovascular relevant peptides has not been completely elucidated.<sup>6</sup>

ACE2 is one of the principal Ang-(1-7)-forming enzymes.<sup>4,6</sup> Because of the higher affinity of ACE2 for Ang II, Ang-(1-7) generation from Ang II is physiologically more relevant than from Ang I.<sup>6</sup> As a result, ACE2 plays a crucial role in maintaining the balance between both axes of the RAS, and a chronic and sustained imbalance may lead to pathophysiology of the cardiovascular, renal, pulmonary, and central nervous systems. Currently, the physiological signals that tip the balance between the 2 axes of the RAS to favor a pathological state remain unknown. It is suggested that the equilibrium between the expression/activity of the 2 ACEs is crucial.<sup>7</sup> Furthermore, ACE2 may represent an endogenous autoregulatory mechanism within the RAS, because it decreases Ang II levels in favor of Ang-(1-7) formation. The concept that ACE2 is capable of balancing the activities of both axes of the RAS is a central argument in designating this enzyme as a target for the development of novel antihypertensive and cardioprotective drugs.

## Biological Actions of the ACE2/Ang-(1-7)/Mas Axis Cardiovascular System

Increasing evidence indicates that the ACE2/Ang-(1-7)/Mas axis plays a critical role in cardiovascular homeostasis and

Received July 29, 2009; first decision August 26, 2009; revision accepted November 30, 2009.

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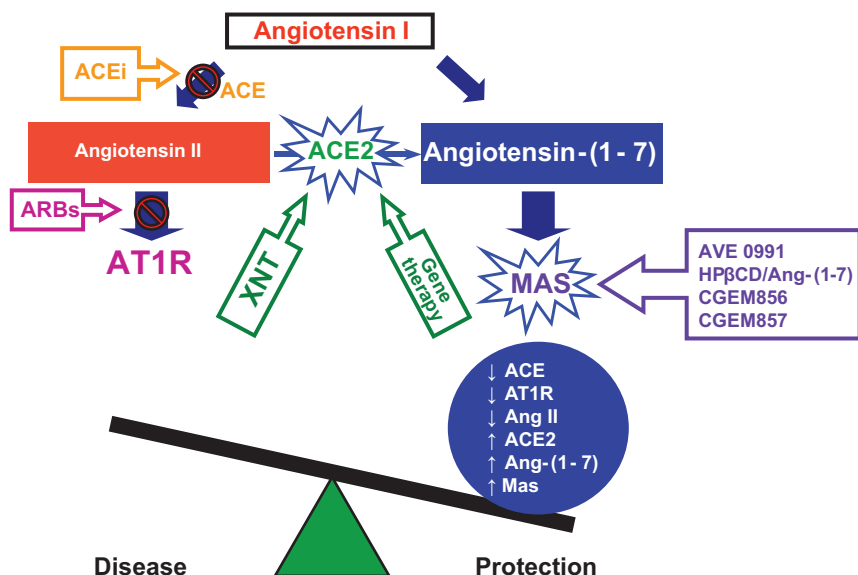
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(*Hypertension*. 2010;55:207-213.)

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*Hypertension* is available at <http://hyper.ahajournals.org>

DOI: 10.1161/HYPERTENSIONAHA.109.140145



**Figure.** Schematic diagram showing the counterregulatory axes of the RAS: ACE/Ang II/AT<sub>1</sub>R and ACE2/Ang-(1-7)/Mas receptor. The ACEs play a central role in balancing the activity of these axes. Although ACE degrades Ang I to form Ang II, ACE2 hydrolyzes Ang II to produce Ang-(1-7). AVE 0991, HPβCD/Ang-(1-7), CGEM856, and CGEM857 are Ang-(1-7) analogues and, consequently, Mas agonists. XNT is an ACE2 activator.

that alterations in its expression/function are associated with major cardiac and vascular pathophysiologies.<sup>8–10</sup> Initially, ACE2 gene disruption on the C57BL/6 background leads to a severe reduction in cardiac contractility and decreases in aortic and ventricular pressures.<sup>8</sup> In addition, transgenic mice overexpressing ACE2 in myocardial cells present cardiac rhythm disturbances.<sup>11</sup> Although subsequent studies have shown conflicting results,<sup>12</sup> it is now generally accepted that ACE2 deficiency aggravates heart failure (Table). Evidence that Mas-deficient mice present marked cardiac fibrosis and impaired cardiac function supports this view.<sup>9</sup> Furthermore, many cardiovascular actions of Ang-(1-7) are absent in these animals.<sup>9,13,14</sup> Cardiac overexpression of ACE2 in a chronic

Ang II-infusion rat model,<sup>15</sup> in spontaneously hypertensive rats (SHRs),<sup>16</sup> or in infarcted rat hearts<sup>17</sup> protects the heart from cardiac remodeling. In keeping with these observations, data from Mercure et al<sup>18</sup> have shown that cardiac overexpression of an Ang-(1-7)-producing fusion protein protects the heart from the deleterious effects of Ang II infusion.<sup>18</sup> These findings suggest that transgenic ACE2 overexpression from birth exerts adverse effects whereas its overexpression after cardiac development completely protects the heart from pathophysiologies. It is likely that embryonic overexpression of ACE2 and/or Ang-(1-7) may induce developmental abnormalities in transgenic animals. Rentsch et al<sup>19</sup> have reported that ACE2 overexpression in the vasculature reduces blood

**Table. Summarized Phenotypic and the Main Changes in the RAS Observed in Some Animal Models of Deficiency or Overexpression of ACE2 and Mas**

Strategy	Strain	Changes in RAS	Phenotype	Reference
ACE2 overexpression in smooth muscle cells	SHRSP	↑ aortic ACE2 activity ↑ Ang-(1-7) plasma/aorta ↑ Ang-(1-7)/Ang II	Improved endothelial function	Rentsch et al <sup>19</sup>
Lentiviral ACE2 gene transfer in hearts	SHR	↑ cardiac ACE2 mRNA ↑ cardiac ACE2 activity	BP independent cardioprotection	Diez-Freire et al <sup>16</sup>
ACE2 overexpression in hearts	FVB/NTac	↑ serum ACE2 activity ↑ cardiac ACE2 expression	Cardiac rhythm disturbances and sudden death	Donoghue et al <sup>11</sup>
Adenoviral ACE2 gene transfer in hearts	SHRSP	↑ cardiac ACE2 activity ↑ Ang-(1-7) expression ↓ ACE activity	Severe cardiac fibrosis	Masson et al <sup>22</sup>
ACE2 knockout	Mixed background	↑ cardiac/plasma/renal Ang II	Impaired systolic function	Crackower et al <sup>8</sup>
ACE2 knockout	Mixed background	No basal changes	Variable	Gurley et al <sup>12</sup>
ACE2 knockout	Mixed background	No basal changes	No basal changes	Yamamoto et al <sup>61</sup>
Mas knockout	C57BL/6	↓ Ang-(1-7) binding in cardiac myocytes	Impaired heart function and cardiac fibrosis	Santos et al <sup>13</sup>
Mas knockout	C57BL/6	Not determined	↑ sympathetic tonus	Walther et al <sup>62</sup>
Mas knockout	C57BL/6	Not determined	Impaired Ang-(1-7)-mediated aortic relaxation	Peiró et al <sup>63</sup>
Mas knockout	FVB/N	Not determined	↑ BP, impaired endothelial function, and ↓ NO and eNOS	Xu et al <sup>64</sup>

SHRSP indicates stroke-prone SHR; eNOS, endothelial NO synthase.

pressure (BP) and improves endothelial function in hypertensive rats. Thus, the injurious effects viewed in ACE2 transgenic mice<sup>11</sup> may be tissue specific. Collectively, these observations suggest that the actions of ACE2, as the main Ang-(1-7)-forming enzyme, and the effects of Ang-(1-7) alone in the heart may depend on the available amount of this heptapeptide, because perfusion of isolated hearts with high concentrations of Ang-(1-7) caused cardiac rhythm disturbances,<sup>20</sup> whereas low concentrations induced an antiarrhythmic effect.<sup>21</sup> In accordance with this observation, Mason et al<sup>22</sup> have suggested that the cardiac fibrosis viewed in stroke-prone SHR overexpressing ACE2 in the myocardium, mediated by recombinant adenoassociated virus 6, is attributed to higher transduction levels achieved using this virus in comparison with lentivirus.<sup>16</sup> In fact, increased ACE2 expression mediated by lentivirus produces beneficial effects, including antifibrosis, in hearts of SHR (Table).

There is clinical evidence to support the role of the ACE2/Ang-(1-7)/Mas axis in cardiovascular pathophysiology. Increased ACE2 expression is observed in cardiac tissue of patients with ischemic heart failure and patients with either idiopathic dilated cardiomyopathy or primary pulmonary hypertension (PH).<sup>23</sup> This increase in ACE2 is likely to be a compensatory mechanism to circumvent the chronic activation of the RAS. This view is supported by the following observations: (1) blockers of the RAS can prevent the decrease in ACE2 transcription and translation in the late phase of ventricular dysfunction in myocardial infarcted rats<sup>24</sup>; (2) expression of ACE2 and Ang-(1-7) is increased in infarcted Lewis rats after olmesartan treatment<sup>25</sup>; and (3) treatment with ACEi, ARBs, or the combined blockade of ACE and AT<sub>1</sub> receptors in rats and humans increases plasma Ang-(1-7) levels.<sup>26–28</sup> This effect is, at least in part, attributed to reduced Ang-(1-7) degradation, because this peptide is a substrate for ACE.<sup>26</sup> The cellular mechanisms and signaling pathways by which Ang-(1-7) exerts its effects have yet to be elucidated, although Dias-Peixoto et al<sup>29</sup> have demonstrated the following in cardiomyocytes of Mas-deficient mice on an FVB/N background: (1) Ang-(1-7) fails to increase NO levels; (2) there are alterations in the expression of proteins that regulate endothelial NO synthase activity; and (3) there is impaired calcium handling.<sup>29</sup> These observations suggest that Mas deficiency can lead to chronic and sustained ACE/Ang II/AT<sub>1</sub>R effects that may impair Ang-(1-7) signaling and promote cardiac ventricular dysfunction, further supporting the concept that activation of the ACE2/Ang-(1-7)/Mas axis promotes protection against CVDs (Figure).

### Renal System

ACE2, Ang-(1-7), and Mas expressions are abundant throughout renal structures and have significant pathophysiological consequences.<sup>14,30</sup> Deletion of the ACE2 gene in mice leads to the development of glomerulosclerosis with deposition of types I and III collagen and fibronectin, as well as increased albuminuria.<sup>31</sup> ACE2 expression is altered in kidneys of hypertensive rats,<sup>32</sup> diabetic animals,<sup>30</sup> and, more importantly, in kidneys of humans with renal disease.<sup>33</sup> The renal damages observed in streptozotocin-induced diabetic mice worsens with treatment with the ACE2 inhibitor

MLN4760.<sup>34</sup> Diabetic SHR chronically treated with Ang-(1-7) present an improvement in renal endothelial function and renal function and a reduction in NADPH-mediated oxidative stress.<sup>35</sup> The protective role of this axis in renal function and structure is further supported by the genetic deletion of Mas, which leads to a reduction in urine volume, sodium retention, microalbuminuria, and reduced renal blood flow.<sup>36</sup> Kidneys from these animals exhibit a reduction in glomerular tuft diameter; increased expression of collagen types III and IV and fibronectin; and increased renal AT<sub>1</sub>R.<sup>36</sup> In contrast, Shao et al<sup>37</sup> have demonstrated that chronic Ang-(1-7) treatment of streptozotocin-induced male rats accelerates diabetic renal injury. The contrasting data may be attributed to local levels of Ang-(1-7), strain variability, age, sex, dose, and/or treatment duration.

Patients with diabetic kidney disease exhibit an imbalance between the expressions of ACE and ACE2 mRNA and protein in the kidney.<sup>38,39</sup> This finding reinforces the hypothesis that the balance between the 2 ACEs is crucial to the development of hypertension, CVDs, and renal diseases (Figure). The predominance of ACE effects over those of ACE2 progresses to disease states, whereas tipping the balance toward ACE2 restores the equilibrium and promotes the attenuation or reversal of pathologies.

### Pulmonary System

The role of the ACE2/Ang-(1-7)/Mas axis in lung pathophysiology has been pursued since the identification of ACE2 in pulmonary tissues. Imai et al<sup>40</sup> have demonstrated that ACE2 deletion worsens acute lung injury induced by acid aspiration or sepsis. Replacement of ACE2 using recombinant human ACE2 improves the pulmonary damage in ACE2 knockout mice.<sup>40</sup> In addition, severe lung failure on an ACE2 knockout background was rescued by inactivation of ACE, and genetic loss of AT<sub>1a</sub> improved lung function.<sup>40</sup> Along the same line of investigation, our studies have shown that activation of endogenous ACE2, using an ACE2 activator designated “XNT” ([1{(2-[dimethylamino]ethyl)amino}-4-[hydroxymethyl]-7-[(4-methylphenyl)sulfonyl]oxy}-9H-xanthen-9-one)],<sup>41</sup> prevents the development of PH. This small molecule was also able to abolish the development of PH-associated damages, such as right ventricular and pulmonary vessel hypertrophy. These effects were completely blunted by the Ang-(1-7) antagonist A-779, indicating that the ACE2/Ang-(1-7)/Mas branch of the pulmonary RAS has important protective effects in PH.<sup>7</sup> Additional evidence that pulmonary ACE2 is important in maintaining a balance in the RAS is derived from ACE2 overexpression studies. Lenti-ACE2 particles injected into the trachea of mice were able to protect lungs from PH. In addition, this strategy is also effective in the partial reversal of PH.<sup>42</sup>

### Central Nervous System

Robust expression of ACE2 and Mas and actions of Ang-(1-7) have been reported in the brain of both animals and humans. Centrally mediated cardiovascular effects of Ang-(1-7) include baroreflex facilitation, hypotension, bradykinin (Bk) potentiation, vasopressin, Bk and NO release, and prevention of norepinephrine release.<sup>43</sup>

In addition, there are many studies establishing an altered expression of central ACE2 in pathological conditions.<sup>43–45</sup> For example, a 40% decrease in ACE2 expression has been reported in the rostral ventrolateral medulla, a cardiovascular nucleus of the brain stem, of SHR. A bilateral injection of lenti-ACE2 in this area resulted in a long-term expression of transgenic ACE2 with a concomitant decrease in BP and heart rate.<sup>45</sup> In addition to rostral ventrolateral medulla, ACE2 is shown to regulate cardiovascular functions in other brain nuclei. For instance, microinjections of MNL4760 into the nucleus tractus solitarius reduced the baroreceptor reflex sensitivity in rats.<sup>46</sup> Furthermore, overexpression of ACE2 in the subfornical organ downregulates AT<sub>1</sub> receptors and attenuates the vasopressor and drinking responses elicited by intracerebroventricular infusion of Ang II.<sup>47</sup>

More recent studies have focused on the role of the ACE2/Ang-(1-7)/Mas axis in cerebrovascular disease. Studies from our group have shown that activation of Mas with chronic or acute administration of Ang-(1-7) increases cerebral blood flow, and selective Ang-(1-7) antagonism decreases flow.<sup>48</sup> This increase in blood flow might be explained by evidence that central administration of Ang-(1-7) shortly after stroke onset increases Bk levels in the brain and upregulates Bk receptors.<sup>49</sup> In addition, NO release and endothelial NO synthase expression are increased with Ang-(1-7) treatment.<sup>50</sup> In fact, our data suggest that chronic central administration of Ang-(1-7) reduces the infarct area and provides cerebroprotection in a rodent stroke model.<sup>51</sup> Together, these observations point toward a potential mechanism of cerebroprotection mediated by Ang-(1-7) stimulation of Mas and subsequent release of Bk, leading to stimulation of Bk receptors, and release of NO, which could increase the cerebrovascular reserve during an ischemic insult.

### ACE2/Ang-(1-7)/Mas Axis Is a Potential Target for Future CVD Therapeutics

It is evident from the above discussion that significant conceptual progress has been made in the last several years that leads us to conclude that this vasoprotective axis of the RAS could serve as a new direction for improved therapeutics for CVDs. In animal models, 2 different strategies have been followed in this regard.

#### Pharmacotherapy

Development of a small molecule ACE2 activator, XNT, is currently being pursued to translate the vasoprotective concept into an effective cardiovascular therapy. XNT has been shown to decrease BP and to cause improvements in cardiac function and reversal of myocardial and perivascular fibrosis.<sup>41</sup> Similar beneficial effects of XNT are observed in PH induced by monocrotaline treatment in rats.<sup>7</sup>

Mas agonists, such as AVE0991 and hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD/Ang-(1-7)), are also under investigation. AVE0991 was the first orally active Ang-(1-7) analogue capable of mimicking Ang-(1-7) actions.<sup>52</sup> The protective effects of AVE0991 are qualitatively comparable to those of Ang-(1-7) in the endothelium,<sup>52</sup> blood vessels,<sup>53</sup> kidneys,<sup>14</sup> and heart.<sup>54</sup> Recently, Lula et al<sup>55</sup> have proposed that complexing Ang-(1-7) with HP $\beta$ CD renders Ang-(1-7) to a more

stable complex, thus allowing a formulation for oral administration of Ang-(1-7) with an increased half-life.<sup>55</sup> Initial testing has revealed that HP $\beta$ CD/Ang-(1-7) reduces the deleterious effects induced by myocardial infarction on cardiac function and reduces infarct size. Furthermore, this compound attenuates the isoproterenol effects on cardiac function and decreases the cardiac hypertrophy and cardiac damage induced by isoproterenol without any change in the systemic BP.<sup>56</sup> Independently, Kluskens et al<sup>57</sup> have reported a stable cyclized derivative of Ang-(1-7) for which the vasodilator activity is 2-fold higher when compared with the normal Ang-(1-7).<sup>57</sup> Finally, 2 more Mas agonists have been discovered (CGEM856 and CGEM857), and initial data demonstrate that these peptides produce endothelium-dependent vasorelaxation, cardioprotection, and antihypertensive effects in SHR.<sup>58</sup>

#### Gene Transfer Therapy

A target gene therapy strategy holds significant potential to translate the available fundamental research of ACE2 into therapeutics. In fact, initial animal experiments have been extremely encouraging. For example, viral-mediated ACE2 overexpression in the heart has been shown to protect the animals from myocardial injuries<sup>15</sup>; to decrease high BP; and attenuate perivascular cardiac fibrosis.<sup>16</sup> This strategy also preserves cardiac function, as well as left ventricular wall motion and contractility, and attenuates left ventricular wall thinning induced by myocardial infarction.<sup>17</sup> The efficacy of ACE2 gene transfer is not limited to the heart as illustrated by ACE2 overexpression studies in the brain.<sup>45,47</sup> ACE2 overexpression in the rostral ventrolateral medulla causes significant decreases in BP and HR.<sup>45</sup> In addition, its overexpression in the lungs protects mice from PH.<sup>42</sup> Similar beneficial effects are observed with Ang-(1-7) fusion protein.<sup>59</sup>

Taken together, this exciting and rapidly evolving field of investigation is likely to provide a better understanding of the role of both RAS axes in CVD pathophysiology and will position us to develop new strategies for cardiovascular therapeutics. Compared with ACEis and ARBs, we believe that targeting ACE2 has the following therapeutic advantages described here. First, it degrades Ang II to generate Ang-(1-7). Thus, targeting ACE2 would not only produce the vasoprotective/antiproliferative peptide Ang-(1-7) but would also influence the vasoconstrictive/proliferative effects of the ACE/Ang II/AT<sub>1</sub>R axis. Second, it is a multifunctional enzyme with many biologically active substrates. The effects of ACE2 on substrates other than Ang I and Ang II still need to be determined. They could hold an unidentified relevance for the treatment of CVD. Third, unlike ARB/ACEi therapy, ACE2 is an endogenous regulator of the RAS. Fourth, it is a part of the vasodilatory/antiproliferative axis of the RAS that seems to be effective in the control of fibrosis and structural remodeling without changes in systemic BP, which could prove to be extremely beneficial for PH. Fifth, although treatment with ACEis or ARBs indirectly increases ACE2 expression, direct activation of this enzyme could result in a better outcome in CVDs because ACE2 is not the main target for ACEis and ARBs.

## Future Directions

The above discussion supports the concept that regulated increases in the activity of the vasoprotective axis of the RAS can produce beneficial outcomes in CVDs. Both pharmacotherapy and gene therapy strategies have shown promise in animal experiments. Moreover, it has been reported that treatment with ACEis or ARBs increases plasma Ang-(1-7) levels in humans<sup>28</sup> and that increased soluble ACE2 activity in humans correlates with worsened cardiac performance. Therefore, it has prognostic value as a predictor of adverse events.<sup>60</sup> However, many issues and potential pitfalls must be addressed to take this leap. Some of these issues are as follows. First, the mechanism of an interdependent regulation of both axes of the RAS needs to be elucidated. Colocalization of ACE and ACE2 may provide some indication if the expression of 1 enzyme could directly regulate the other enzyme's activity. The possibility that the coregulation could be at the level of signaling by the AT1R and Mas should be considered. Second, we must understand the mechanisms of action of ACE2/Ang-(1-7) at the tissue/cellular levels. Is the target for ACE2/Ang-(1-7)/Mas endothelial cells or do they act in a paracrine/autocrine manner involving multiple vascular cells to control the RAS? Third, ACE2 is a ubiquitous enzyme as well as the receptor for the severe acute respiratory syndrome virus. We must determine the consequences of a long-term activation of ACE2. The effects of ACE2 activators on the immune competence of animals and their vulnerability to severe acute respiratory syndrome infection must be tested before these molecules are ready for preclinical trials. Fourth, can genetically modified endothelial progenitor cells overexpressing ACE2 or Ang-(1-7) be a better approach for targeting disease-induced endothelial damage? This combinatorial approach is exciting and offers a faster track for testing the concept in patients. Fifth, the RAS has been shown to play a role in stroke. Because ARBs produce limited beneficial outcomes, it would be interesting to determine whether activation of the ACE2/Ang-(1-7)/Mas axis provides an improved protection and reversal from ischemia-induced neural damage. Sixth, is the benefit of ACE2 intervention organ specific? We must establish a consensus about the influences of sex, strain, organ, dose, timing, and methodology on ACE2 effects. Seventh, gene therapy strategy holds great promise because it could be targeted to the diseased organ, but it is not without inherent pitfalls. For example, an ideal vector that can produce high levels of transduction without immune or other adverse effects is not currently available but is within reach in near future. One must develop a transduction system that can be turned on/off on demand in the event of adverse effects of a long-term transgene expression.

## Perspectives

It is becoming evident that normal cardiovascular homeostasis is a result of a balance between the activities of the vasoconstrictive, hypertrophic, proliferative, and fibrotic (ACE/AngII/AT<sub>1</sub>R) and the vasoprotective (ACE2/Ang-(1-7)/Mas) axes of the RAS. Any chronic imbalance leads to major cardiovascular pathophysiology. Both animal and human studies provide strong conceptual support for this view. They also demonstrate that pharmacological or genetic intervention in restoring this balance

by increasing the activity of the ACE2/Ang-(1-7)/Mas axis produces impressive outcomes against cardiovascular and pulmonary diseases. Thus, it is reasonable to suggest that the vasoprotective axis offers a novel target for the development of improved and more successful therapy for cardiopulmonary diseases.

## Sources of Funding

This work was supported by the National Institutes of Health grants HL56921 and HL33610.

## Disclosures

R.A.S.S. is a consultant of Compugen (Tel Aviv, Israel).

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KEY WORDS: ACE2 ■ angiotensin-(1-7)/Mas receptor ■ hypertension ■ cardiopulmonary disease prevention/control ■ cardiovascular disease

## Therapeutic Implications of the Vasoprotective Axis of the Renin-Angiotensin System in Cardiovascular Diseases

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*Hypertension*. 2010;55:207-213; originally published online December 28, 2009;  
doi: 10.1161/HYPERTENSIONAHA.109.140145

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231  
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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