Hypertension Highlights

A Place in Our Hearts for the Lowly Angiotensin 1-7 Peptide?

Timothy L. Reudelhuber

It is absolutely astounding that over a hundred years after the discovery of renin, important new discoveries continue to be made concerning the importance of the renin–angiotensin system (RAS) in biology and disease. In addition to its accepted role as a modulator of blood pressure and fluid volume, it is now clear that the RAS contributes to renal development (reviewed in Reference 1), and mounting evidence suggests that it modulates memory and cardiac and respiratory function. Most of the recent discoveries revolve around peptide products of the RAS that were previously thought to be simply breakdown products but that are increasingly taking center stage in physiology and pathophysiology. The RAS is classically described as a circulating enzymatic pathway of which the sole product of importance is the vasoactive peptide angiotensin II (Ang II; Figure 1). Inhibition of the RAS is now the major pharmacological target in North America for prevention of hypertension and a host of other cardiovascular complications. Although Ang II plays a key role in the biology of the RAS, it is certainly not the only biologically active peptide produced by this system, particularly within tissues (Figure 1). For example, Ang II can be converted to smaller peptide products with biological activity by the action of aminopeptidase A, which removes a single amino acid from the amino terminus of Ang II to produce angiotensin III (Ang III or Ang 2-8). Additional action of aminopeptidases can generate the hexapeptide angiotensin IV (Ang IV or Ang 3-8). Although Ang III can bind to and signal through the Ang II type 1 (AT1) and Ang II type 2 (AT2) receptors, Ang IV is a poor ligand for these receptors and has been reported to bind to a unique receptor that leads to increased renal cortical blood flow and appears to potentiate memory. Surprisingly, the Ang IV "receptor" may actually be the insulin-regulated aminopeptidase (reviewed in Reference 2).

In addition to being a substrate for Ang II production, Ang I can also be converted by neutral endopeptidase to the heptapeptide angiotensin 1-7 (Ang 1-7). Alternatively, Ang 1-7 can be produced directly from Ang II by the recently discovered carboxypeptidase angiotensin-converting enzyme (ACE2). The Ang 1-7 produced has been reported to have vasodilatory effects (recently reviewed in Reference 4) and may act through the G-coupled protein receptor mas. ACE2 can also remove a single amino acid from the carboxy terminus of Ang I to produce angiotensin 1-9 (Ang 1-9), which has no known function to date. The most surprising new discoveries in the renin–angiotensin field in the last 2 years have involved either Ang 1-7 or ACE2.

Where and When Does Ang 1-7 Act?
The physiological importance of Ang 1-7 or any of the other peptides produced by the RAS depends on 3 things: (1) the abundance, tissue distribution, affinity, and catalytic efficiency of the enzymes that produce the peptide; (2) the abundance of the substrate for the reaction; and (3) the presence and affinity of the receptor for each of the peptides. For this reason, it is perhaps more useful to think of the RAS as illustrated in Figure 2: at each junction, the peptide might either bind to a receptor or be converted to another product, depending on the above-mentioned factors. A lot can be learned by comparing the efficiency by which a given angiotensin peptide is converted by the various pathways: Rice et al recently carried out such a systematic study comparing the binding affinities and cleavage efficiencies of ACE, ACE2, and neutral endopeptidase (NEP) for Ang I, Ang II, Ang 1-7, and Ang 1–9. The first thing that is striking is that the affinity of Ang II for its receptors is about a thousand times higher than its affinity for the ACE2 protease that will convert it to Ang 1-7 (Table). What this means is that long before there would be enough Ang II to fuel the generation of the supposedly vasodilatory Ang 1-7 through ACE2, the vasoconstrictive AT1 receptor would be saturated. However, there are 2 ways around this seeming conundrum. First, if the AT1 receptor were blocked (eg, by an angiotensin receptor blocker [ARB]), Ang II would accumulate and could be converted to Ang 1-7 without stimulating the AT1 receptor. In fact, Ang 1-7 levels have been reported to increase by as much as 25-fold after either ACE inhibition or ARB treatment, presumably by using either the NEP or ACE2 pathways, respectively (see Figure 1). The second solution to this puzzle might be that the processing proteases and receptors are in different tissue compartments. Crackower et al demonstrated recently that ACE2 is particularly enriched in the coronary vasculature where it appears to play a key role in Ang 1-7 generation. Using human transplant hearts, Zisman et al showed that intracoronary infusion of [125I]-labeled Ang I led to efficient production of labeled Ang II and Ang 1-7 in the coronary circulation, both of which
decreased in parallel (by $\geq 10$-fold) when the infusions included an ACE inhibitor. These results suggest that the preferred pathway of Ang 1-7 synthesis in the coronary circulation is via Ang II and ACE2. However, Ang II receptors are abundant and functionally important on both vascular endothelial and smooth muscle cells. For these reasons, it seems likely that Ang 1-7 biology will be most important when inhibitors of the RAS are being used.

Once produced, Ang 1-7 not only can bind its receptor with nanomolar affinity, but has been reported to be an inhibitor of the carboxy-terminal catalytic domain of ACE, albeit with a Michaelis constant in the micromolar range. Inhibition of this domain of ACE, however, is sufficient to reproduce the antihypertensive effects of ACE inhibitors. At pharmacological doses, Ang 1-7 may also stimulate the AT1 and AT2 receptors, and although the affinity constant is in the high micromolar range, this should be taken into consideration when interpreting any experiments where exogenous Ang 1-7 is added to culture supernatants or perfusion buffers.

Enzyme and Receptor Affinities for the Various Angiotensin Peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Protein Bound</th>
<th>Affinity</th>
<th>Catalytic Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang I</td>
<td>ACE</td>
<td>$19 \mu$mol/L</td>
<td>$3.5 \text{ S}^{-1}$</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>NEP</td>
<td>$55.1 \mu$mol/L</td>
<td>$34.1 \text{ S}^{-1}$</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>ACE2</td>
<td>$86.8 \mu$mol/L</td>
<td>$2.9 \text{ S}^{-1}$</td>
<td>6</td>
</tr>
<tr>
<td>Ang II</td>
<td>AT1R</td>
<td>$1.6 \text{ nmol/L}$</td>
<td>n/a</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>AT2R</td>
<td>$0.17 \text{ nmol/L}$</td>
<td>n/a</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>ACE2</td>
<td>$5.7 \mu$mol/L</td>
<td>$12.8 \text{ S}^{-1}$</td>
<td>6</td>
</tr>
<tr>
<td>Ang 1-7</td>
<td>mas</td>
<td>$0.8 \text{ nmol/L}$</td>
<td>n/a</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ACE</td>
<td>$7.9 \mu$mol/L</td>
<td>$2.8 \text{ S}^{-1}$</td>
<td>6</td>
</tr>
</tbody>
</table>

n/a indicates not applicable.

A Plethora of Receptor Mechanisms, a Dearth of Independent Signals

Evidence suggests that the mas oncogene is the natural receptor for Ang 1-7. First, Ang 1-7 binds with nanomolar affinity to membranes of cells transfected with the mas receptor (Reference 5 and Table). Second, tissues and cells from mice in which the gene encoding mas has been inactivated no longer respond to Ang 1-7. The mas oncogene is a 7 transmembrane G protein–coupled receptor. Originally identified because of its ability to induce tumors in nude mice, it was erroneously reported in the late 1980s as the natural receptor for Ang II. Interestingly, mas was later found to mobilize intracellular calcium in response to Ang II only when the bona fide AT1 receptor was also present in the same cell, providing the first evidence that the 2 receptors interact. More recently, Kostenis et al reported that coexpression of the AT1 and mas receptors in tissue culture cells actually leads to a decreased ability of Ang II to mobilize intracellular calcium through the AT1 receptor. By using the technique of bioluminescence energy transfer, these investigators obtained evidence for a physical interaction between the 2 receptors, and they proposed that the receptors form a heterodimer in coexpressing cells that leads to depressed activity of the AT1 partner. More strikingly, the antagonistic effect of mas on AT1 signaling was independent of the presence of Ang 1-7. This last result is hard to reconcile with...
all of the published data on the biological actions of Ang 1-7 and its signaling through the mas receptor.

Other experiments support a role for Ang 1-7 in mediating the mas receptor modulation of AT1 and AT2 signaling in vivo. De Castro et al19 perfused isolated hearts from control and mas-deficient mice with subpicomolar concentrations of Ang 1-7 (well below the micromolar concentrations shown to signal through the Ang II receptors) and saw no effect on coronary vasoconstriction. However, when the AT1 receptor was blocked, the Ang 1-7 caused a vasodilation, an effect that could be blocked with an AT2 receptor antagonist and that was only seen in animals expressing the mas receptor. Conversely, when the AT1 receptor alone was blocked, the Ang 1-7 in the perfusate produced a coronary vasoconstriction, although it was not clear that this was mediated only by the AT1 receptor. These results suggest that, in the heart, circulating Ang 1-7 can act through the mas receptor to stimulate the AT1 and AT2 receptors. The net result of such an action would be that Ang 1-7 would function much like Ang II.

The mas receptor has also been proposed to have independent physiological and signaling properties that are dependent on Ang 1-7 binding. Santos et al20 reported that mice deficient for mas lost the Ang 1-7–induced natriuresis seen in control mice. Moreover, aorta from mas-deficient mice lost the ability to relax in response to Ang 1-7. These responses are different from those expected with Ang II acting through the AT1 receptor (which causes retention of sodium by the kidney and vasoconstriction of the aorta). Notably, these investigators reported that Ang 1-7 stimulated arachidonic release in mas-transfected cells and that this response was not blocked by an AT1 receptor blocker. However, although these results confirm that the endogenous mas receptor responds to Ang 1-7 stimulation and that it is capable of signaling in an AT1-independent manner, they do not entirely rule out an effect of mas interacting with the AT2 receptor. Thus, the biochemistry of the mas receptor remains somewhat controversial: it clearly is capable of interacting with Ang II receptors, may have independent signaling properties, and may not depend on the binding of Ang 1-7 for its actions in some situations. What determines the switch between these various activities and the physiological significance of these receptor modulations remain unclear.

Physiological Correlates to Some Interesting Biochemistry

With so much interesting biochemistry, what is the effect of manipulating the Ang 1-7 levels in whole animals? Although a mass of data using acute peptide administration and culture tissue or cells suggested that Ang 1-7 would be vasodilatory (recently reviewed in Reference 4), chronic manipulation of Ang 1-7 biosynthesis and signaling by gene inactivation paints a quite different picture. Because of the role of NEP and ACE2 in the generation of Ang 1-7 and the identification of mas as the receptor for this peptide (see above), one might expect that inactivation of the genes coding for these proteins would result in an increase in blood pressure. In fact, inactivation of none of these genes affects systolic blood pressure as predicted. NEP gene inactivation actually caused a drop in blood pressure,20 quite possibly because NEP is also key in the degradation of the vasodilatory bradykinin and natriuretic atrial natriuretic peptide (ANP). In contrast, mas inactivation had no apparent effect on mean systolic pressure but was reported to slightly decrease blood pressure variability in female, but not male, mice,21 perhaps as a consequence of increased sympathetic tone.22 Likewise, ACE2 inactivation had no detectable effect on blood pressure in animals up to 3 months of age.8 Surprisingly, ACE2 gene inactivation actually resulted in a decrease in systolic pressure, a phenomenon that was only seen in older male mice. The explanation for this finding came from the fact that these animals also exhibited a reduction in cardiac contractility. Interestingly, the hearts of ACE2-deficient animals had a higher Ang II content than control animals, raising the possibility that the heart defects might be because of an accumulation of Ang II resulting from the loss of ACE2 as a “clearance” enzyme. This conclusion was reinforced by the finding that breeding these animals with mice that were deficient for ACE (and, thus, unable to make Ang II) corrected the observed cardiac contractility defect.

However, the inactivation of ACE2 would also be expected to reduce Ang 1-7 content in the heart, and the compound inactivation of both ACE and ACE2 could theoretically restore Ang 1-7 levels by causing an accumulation of Ang I (Figure 1). Although it is difficult with the available data to determine whether the results observed by Crackower et al8 were because of the excess cardiac Ang II or a lack of Ang 1-7, the chronic overproduction of Ang II in the heart by other approaches has not lead to similar findings. Our own group targeted expression of an Ang II–releasing fusion protein to cardiomyocytes resulting in a 30- to 50-fold chronic increase in Ang II content in the heart.23 Ang II could be also be detected in the perfusate of Langendorf-mounted hearts, demonstrating that some of the peptide was released into the coronary circulation. The resulting animals exhibited increased interstitial fibrosis, and, although we did not directly test cardiac function, 12-week-old male mice were normotensive, suggesting that they did not suffer from the cardiac insufficiency seen by Crackower et al.8 Indeed, after our original publication, we tested mice ≤1.5 years of age and found them to be asymptomatic (Jorge P. van Kats and Timothy Reudelhuber, unpublished data, 2002). Thus, an excess of cardiac Ang II does not seem to be sufficient to generate cardiac insufficiency. Could some of the observed effects be because of a chronic cardiac deficiency in Ang 1-7?

In an effort to directly test for the cardioprotective effect of Ang 1-7, Santos et al24 made transgenic rats who expressed a fusion protein capable of releasing Ang 1-7 from the cells in which it is expressed. Although they used a viral promoter in an effort to get widespread expression of the fusion protein in the rats, the transgene was primarily expressed in the testes. Nevertheless, male rats had an ≈2-fold increase in circulating venous and arterial Ang 1-7 over a lifetime. Once again, the transgenic rats had no detectable difference in blood pressure in spite of the reported vasodilatory role of Ang 1-7. However, the rats had a slightly increased heart rate and were less susceptible to induction of cardiac hypertrophy by isoproterenol injection. Because the hearts also showed a reduction in the
duration of reperfusion arrhythmias when mounted in Langendorf preparations, the authors suggested that chronic exposure to Ang I-7 had resulted in a “reprogramming” of the heart that was maintained even when the heart were removed from the Ang I-7–enriched in vivo setting. These results are the first to suggest that Ang I-7 can be cardioprotective, and they raise the possibility that the nefarious effects of ACE2 inactivation might be, in part, mediated by an Ang I-7 deficiency.

If ACE2 deficiency causes cardiac insufficiency, could increased ACE2 expression in the heart actually provide additional protection? Surprisingly, targeting ACE2 overexpression to the cardiomyocytes results in heart block, ventricular tachycardia, and sudden death in transgenic mice. The idea that expression of the transgenic ACE2 was responsible was supported by the correlation between the severity of the symptoms and the level of transgene expression. A downregulation of connexin40 and connexin43 in transgenic cardiomyocytes might well explain the electrophysiological defects and suggests that ACE2 overexpression has a direct effect on the cardiomyocyte. How can these results be reconciled with the ACE2 inactivation experiments of Crackower et al? Perhaps the site of expression of ACE2 is important. In the normal animals, ACE2 is expressed primarily in the coronary vasculature, whereas expression of the ACE2 transgene used by Donoghue et al was targeted to the cardiomyocyte. Perhaps ACE2 in the myocardium carries out a different function than that in the lumen of the vasculature. Indeed, ACE2 has a number of peptide substrates in addition to Ang II that might lead to the production of noxious regulators of connexin expression.

Recently, the ACE2 enzyme was also identified as the severe acute respiratory syndrome (SARS) coronavirus receptor. Infection with the SARS virus leads to severe pneumonia and frequently to respiratory failure. Kuba et al showed that SARS surface Spike protein binds to ACE2 and causes its downregulation. Lavaging the lungs of mice with an acid solution resulted in lung injury that is worsened in mice pretreated with an intraperitoneal injection of SARS coronavirus. The detrimental action of the Spike protein correlated with an increase in lung Ang II. Importantly, the increase in lung injury mediated by the Spike protein could be prevented by treating the mice with the ARB losartan. Imai et al extended these studies to investigate other forms of acute respiratory distress syndrome triggered by sepsis and found very similar results: lung injury (as measured by pulmonary edema, leukocyte infiltration, and increased lung elastance) was worsened in ACE2 knockout mice, whereas combined ACE and ACE2 knockout reduced the degree of lung injury to that seen in control animals. In addition, they showed that the severity of lung injury was decreased in Ang II AT1 knockout mice and increased in AT2 knockout mice as compared with control animals. Furthermore, they obtained similar results when they pretreated the animals with either an AT1- or an AT2–specific receptor antagonist. Interestingly, these last experiments were carried out in mice with an intact ACE2 gene, which apparently was not able to clear enough of the accumulated Ang II to prevent the worsening of the injury in the AT2 knockout animals, raising the possibility that it is limiting, at least in lung injury. Nevertheless, these results confirm that ACE2 protects the lung from injury and strongly suggest that the AT1 receptor does as well.

**What Does It All Mean?**

Although it only relatively recently arrived on the scene, the ACE2 enzyme has breathed new life into an ancient physiological system. In spite of its demonstrated role in cardiac function and protection of the lungs, the means by which it provides these cardiopulmonary benefits is still largely correlative. In spite of the fact that ACE2 mediates the cleavage of a number of peptides other than Ang II, the evidence strongly suggests that it mediates its effects on cardiac and pulmonary function through the RAS. First, its inactivation leads to tissue accumulation of Ang II, suggesting that it serves as a natural clearance pathway for Ang II. Second, the effects of ACE2 inactivation in cardiac and pulmonary function can be reversed by concomitant inactivation of ACE. Finally, ARBs provide some measure of protection in the same models of lung injury used to demonstrate the role of ACE2. However all of these conditions (ACE2 deletion, ACE inactivation, and ARB usage) also modulate the levels of Ang 1-7, the role of which has not, to date, been thoroughly investigated in the biology of ACE2. If, indeed, the mas oncogene is the bona fide Ang 1-7 receptor, it should be possible to use existing mas knockout animals to directly test the role of Ang 1-7 in these experimental models, and these experiments are likely under way. It is safe to say, however, that there is no clear evidence that Ang 1-7 lowers blood pressure in the mouse models studied so far. Plovsving et al also found no response of blood pressure, renal function, or aldosterone release when Ang 1-7 was infused in humans at doses equivalent to those at which Ang II affects all of these parameters. Thus, from what we know about the biochemistry of the RAS and in considering the available data, Ang 1-7 is a peptide hormone that seems more likely to manifest local actions in tissues, perhaps most often in the context of RAS inhibition.

If ACE inhibition and ARBs increase Ang 1-7, and if it has potentially beneficial effects, why should we study its biology? Major clinical trials have suggested that treatment of patients with either ACE inhibitors or ARBs might have advantages beyond blood pressure control in preventing cardiovascular morbidity and mortality. However, whether these effects are truly independent of blood pressure, whether ACE inhibitors or ARBs are more effective, and how they mediate their cardiovascular protection remains a topic of some debate. The recent data showing a role for ACE2 in cardiopulmonary pathophysiology suggests that Ang 1-7 and its biologically relevant signaling pathways merit another look in this regard.

**References**


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Hypertension. 2006;47:811-815; originally published online March 6, 2006;
doi: 10.1161/01.HYP.0000209020.69734.73

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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