Chapter 10
Angiotensin-(1-7), Angiotensin-Converting Enzyme 2, and New Components of the Renin Angiotensin System

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Abstract The discovery of angiotensin-(1-7) [Ang-(1-7)] in 1988 represented the first deviation from the traditional biochemical cascade of forming bioactive angiotensin peptides. Prior to that time, the biological actions of angiotensin II (Ang II) were being investigated as it relates to cardiovascular function, including hypertension, cardiac hypertrophy and failure, as well as biological actions in the brain and kidney. We now know that Ang II elicits a whole host of actions both within and outside of the cardiovascular system. Furthermore, the discovery of Ang-(1-7) by our laboratory was also the first indication of a biologically active angiotensin peptide that further studies revealed served to counter-balance the actions of Ang II. This chapter reviews the data demonstrating the role of the vasodepressor axis of the renin angiotensin system in the regulation of cardiovascular function and the new data that shows the existence of angiotensin-(1-12) as a novel alternate substrate for the production of angiotensin peptides. The ultimate role of this discovery, as well as the continuing elucidation of mechanisms pertaining to RAS physiology, will likely be clarified in the coming years, in hopes of improving the treatment of cardiovascular disease.

Keywords Angiotensin-(1-7) · Angiotensin-(1-12) · Angiotensin II · Hypertension · Mas receptor · Blood pressure regulation

10.1 Introduction

The existence of the renin angiotensin system (RAS) as a major physiological regulator has been known since Tigerstedt and Bergman first discovered the enzyme renin over a century ago [1]. Nearly 60 years after the initial discovery of renin,

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Irvine Page from the United States and Braun Menendez from Argentina independently discovered a pressor hormone, "angiotonin" or "hypertensin," which was later agreeably called "angiotensin" (Ang). We now know this hormone to be the octapeptide pressor hormone, Ang II, which is produced from the sequential cleavage of the protein (Aogen) into Ang I by renin and Ang I into Ang II by angiotensin-converting enzyme (ACE).

This linear hydrolysis cascade was undisputed for many years until studies from our laboratory in 1988 showed that a previously considered inert metabolite of Ang II, Ang-(1-7), caused the release of vasopressin from the rat brain hypothalamus [2]. This study was the first demonstration of biological activity of a peptide within the RAS that was not Ang II-mediated. The discovery of Ang-(1-7) expanded our knowledge about the complexities of the RAS and has garnered increasing support for a potential target for the therapeutic treatment of diseases such as hypertension, heart disease, and even cancer [3, 4]. This chapter focuses on the functional role of Ang-(1-7) in the heart, as well as the important contribution that angiotensin-converting enzyme 2 (ACE2) plays in degrading Ang II into Ang-(1-7). While the evidence for a protective role for this counterbalancing arm of the RAS continues to accumulate, we also comment on the identification of a new angiotensin peptide upstream of Ang I, called angiotensin-(1-12), and how it may function in tissues as an alternate precursor for angiotensin peptide production. A diagram of the most current view of the renin angiotensin system is shown in Fig. 10.1.
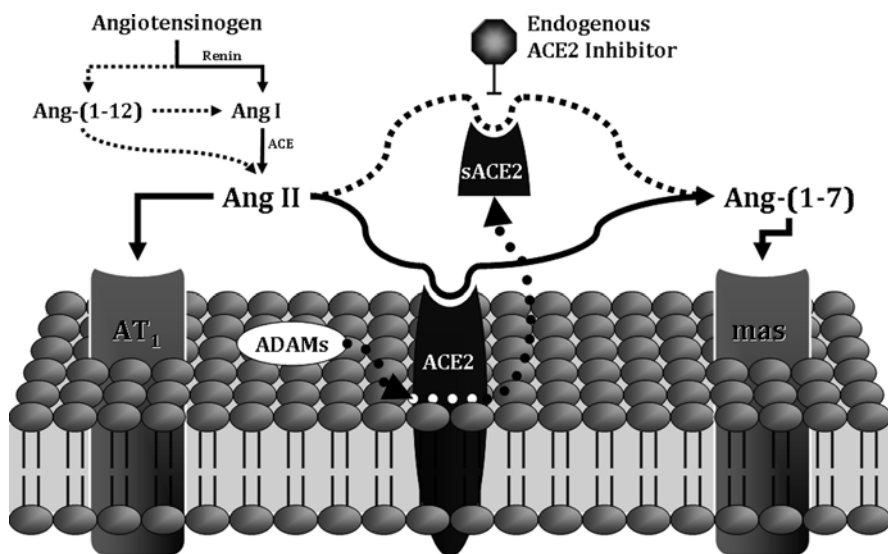

Fig. 10.1 Current view of the renin angiotensin system. Abbreviations: ADAMs, tumor necrosis factor-α convertases, such as ADAM17; sACE2, secreted ACE2

10.2 Angiotensin-(1-7): Gaining Favor in the 21st Century

The discovery of Ang-(1-7) in the late 1980s did not lend itself to ready acceptance [2, 5–7]. However, studies investigating the role of Ang-(1-7) are on the rise, and a whole new array of data has been emerging on this bioactive peptide since the turn of the 21st century. Most of the biological effects of Ang-(1-7) that are discussed below have been attributed to the mas receptor, which was identified as a functional receptor for Ang-(1-7) [8].

10.2.1 Angiotensin-(1-7) and the Regulation of Cardiac Dynamics

The presence of Ang-(1-7) in the heart and the ability of the heart to produce Ang-(1-7) were not known for some time. Ang-(1-7) was identified in cardiomyocytes of the heart, but not cardiac fibroblasts, and Averill et al. [9] further showed that its expression was augmented after coronary artery ligation. Several studies have shown that Ang-(1-7) can be synthesized by the heart, and we showed a direct conversion of Ang II into Ang-(1-7) in isolated hearts from normal and hypertensive rats [10]. Early studies investigating a direct role for Ang-(1-7) actions in the heart showed that it was protective against ischemia-induced cardiac dysfunction [11–13], which may be due in part to the activation of the sodium pump [14]. Additional studies in the cardiomyopathy hamster showed that the anti-arrhythmic effects of Ang-(1-7) are mediated through hyperpolarization of the heart cell [15]. Further evidence that Ang-(1-7) is a direct positive effector in the heart stems from data showing its anti-fibrotic and anti-hypertrophic actions [16–20]. Because Ang-(1-7) is a peptide and thus has a short half-life, several studies have investigated a more stable analog of Ang-(1-7), called AVE0991. The administration of this Ang-(1-7) analog is associated with improvement of cardiac function in diabetic rats [21], improved baroreceptor sensitivity [22], and potentiation of the vasodilator actions of bradykinin [23].

The mas receptor mediates the signaling mechanisms produced by Ang-(1-7) [8]. We further showed that transfection of cultured myocytes with an antisense oligonucleotide to the mas receptor blocked the Ang-(1-7)-mediated inhibition of serum-stimulated mitogen-activated protein kinase (MAPK) activation, whereas a sense oligonucleotide was ineffective [19]. In keeping with these findings, chronic mas deficiency leads to impaired Ca^{2+} handling in cardiomyocytes in culture [24].

10.2.2 Salt and the ACE2/Ang-(1-7)/mas Axis

A clear relationship between salt intake and prevalence of hypertension has been shown in abundant epidemiological and interventional studies [25–28], giving support for the current recommendation for sodium intake of 2,400 mg per day by the American Heart Association. On the other hand, despite numerous studies suggesting that interruption of ACE2/Ang-(1-7)/mas receptor axis may lead to hypertension and cardiac dysfunction [29–31], little is known about its response to

altered sodium intake with respect to blood pressure changes or target organ damage. Our laboratory was among the first one to report the importance of a tonic depressor activity of Ang-(1-7) to the maintenance of blood pressure in the spontaneously hypertensive rats, with endogenous RAS activation induced by chronic salt depletion [32]. Furthermore, in the face of unchanged plasma Ang-(1-7), an enhanced vascular sensitivity to endogenous Ang-(1-7) in salt-restricted state suggests significant amplification in Ang-(1-7) receptor–signaling interaction. Subsequent studies also revealed that, under the condition of increased renal Ang II due to salt depletion [33] or 2K1C Goldblatt hypertension [34], endogenous Ang-(1-7) counterbalanced the effects of Ang II to maintain a glomerular filtration rate and renal plasma flow [34]. Thus, could it be possible that insufficient synthesis or activity of ACE2/Ang-(1-7)/mas may be a critically important mechanism in salt-sensitive hypertension?

Indeed, it has been shown that in female Dahl salt-sensitive rats fed high-salt diet, chronic Ang-(1-7) supplementation reduced increase in blood pressure and improved aortic and renal blood flow by increasing prostacyclin and prostaglandinE_2 release. It was believed that an increase in plasma levels of nitric oxide following Ang-(1-7) infusion was responsible for this observed vasodilatory effect [35]. It has also been shown that acute vasodilation by Ang-(1-7) was augmented in rats fed high-sodium versus low-salt diet due to an increase in vasodilatory and a decrease in vasoconstrictor prostanoids [36]. But the antagonistic and nitric oxide-independent effect of Ang-(1-7) on Ang II-induced vasoconstriction in aortic rings from the rats fed high-sodium diet was abolished in rats fed low-sodium diet [37]. Thus, further studies are warranted to define precisely a fine-tuning mechanism of Ang-(1-7) in the regulation of blood pressure and flow as well as vascular reactivity in different status of sodium intake. In this context, it is important to note that in salt-sensitive hypertensive patients, omapatrilat, a dual ACE and neprilysin inhibitor, effectively reduced blood pressure and increased urinary excretion of Ang-(1-7) [38]. This study clearly pointed out that besides the inhibition of Ang II production and degradation of atrial natriuretic peptide and bradykinin, an Ang-(1-7) of renal origin may contribute to the hypotensive effect of omapatrilat in the patients whose blood pressure is sensitive to sodium intake.

Finally, having in mind the anti-hypertrophic and anti-fibrotic effects of Ang-(1-7), it is intriguing to hypothesize that salt-induced left ventricular remodeling and renal injury observed in different forms of experimental and human hypertension [39–43] may be, at least in part, governed by alteration of the ACE2/Ang-(1-7)/ mas axis. In fact, in Dahl salt-sensitive rats fed high-salt diet, cardiac enlargement and fibrosis were associated with an increased cardiac angiotensinogen but reduced cardiac ACE2 mRNA. Treatment with AT_1 receptor antagonist, but not mineralocorticoid receptor blocker, reversed the effect of salt on ACE2 gene expression [44]. Importantly, both therapies ameliorated salt-induced cardiac remodeling along with a reduction in angiotensinogen and ACE mRNA. Therefore, it seems that the effects of salt-intake variation or RAS blockade may ultimately depend on their net effects on the two opposing arms of the RAS. Furthermore, low sodium intake in Wistar rats reduced renal ACE, but not ACE2 mRNA and

activity; this effect was not amplified during ACE inhibition [45]. Neither plasma Ang II nor Ang-(1-7) were affected by low sodium intake, but ACE inhibition increased plasma Ang-(1-7) shifting the balance between the two opposing peptides toward Ang-(1-7) more effectively during a low sodium intake. Moreover, blood pressure was the lowest in the group treated with ACE inhibitor and low-salt intake. The findings from these studies corroborate well with previous conclusion that anti-hypertensive and cardio-renal protective effects of RAS blockade stemmed, at least in part, from Ang-(1-7) pathway activation [46–48] and that these effects may be more pronounced if followed by dietary sodium restriction [49]. Further studies are clearly necessary to explore whether the beneficial effects of dietary sodium alteration and/or pharmacological intervention indeed depend on preferable ACE/ACE2 and ultimately Ang II/Ang-(1-7) balance in the target organs.

10.3 ACE2: A Critical Enzyme Regulator in the Heart

The discovery of the biological effector peptide, Ang-(1-7) in 1988 represented the first expansion of the classical RAS cascade in that it was the only other known peptide member of the RAS to elicit some physiological function. However, the formation of Ang-(1-7) remained elusive for several years. Welches and colleagues [50] first showed that Ang-(1-7) could be formed from the traditional RAS precursor peptide, Ang I, by endopeptidases including prolyl oligopeptidase (POP, E.C. 21.26), neprilysin (NEP, E.C. 24.11), and thimet oligopeptidase (TOP, E.C. 24.15). While it was known that prolyl oligopeptidase could cleave the Pro^7-Phe^8 bond of Ang II, the studies were not supported by convincing in vivo evidence. Moreover, studies by Yang et al. [51] found that prolyl carboxypeptidase (PCP, E.C. 16.2), a lysosomal enzyme with an acidic pH optimum, could cleave Ang II into Ang-(1-7). The hydrolysis appeared to be an intracellular cleavage, and the observation that the acidic pH optimum of PCP of 5.0 provided some doubt as to the physiological role for this enzyme in producing Ang-(1-7).

It was not until 2000, when two independent research groups described a homolog of ACE, called angiotensin-converting enzyme 2 (ACE2) [52, 53], that a viable Ang-(1-7)-forming enzyme from Ang II was discovered. Shortly after its discovery, Vickers et al. [54] showed that ACE2 could cleave Ang II into Ang-(1-7) with high affinity. ACE2 also cleaved apelin, des-Arg^9-bradykinin, and the opioid peptide dynorphin A 1-13 with similar affinities, but its involvement in modulating these peptides in vivo remains to be clarified. Subsequent studies revealed the generation of Ang-(1-7) in human failing heart tissue, which was dependent on Ang II [55], suggesting that ACE2 was required for the cleavage of Ang II into Ang-(1-7). Studies from our laboratory represented the first direct in vivo evidence for ACE2's participation in hydrolyzing Ang II into Ang-(1-7) in hearts isolated from both normal and hypertensive rats [10]. We further showed that the hypertrophied hearts from hypertensive rats were almost completely reliant on ACE2 for the

production of Ang-(1-7) from Ang II, whereas ACE2 in the normal heart was of less importance.

ACE2 is widely expressed in many tissues in humans [56] and rodents [56, 57], including the heart. In addition, work from this laboratory first demonstrated that cardiac expression of ACE2 mRNA was regulated by the actions of Ang II via an AT_1 receptor pathway [58]. A more recent study showed that the negative actions of Ang II on cardiac ACE2 mRNA could be mimicked by the addition of endothelin-1 and that both effects could be blocked by inhibitors of mitogen-activated protein (MAP) kinase kinase 1, suggesting that Ang II or endothelin-1 activate ERK1/ERK2 to reduce ACE2 [59].

The importance of ACE2 in the regulation of cardiac function was determined when Crackower and colleagues [29] demonstrated that genetic inactivation of ACE2 in mice resulted in severe blood-pressure-independent systolic impairments in cardiac function, which was associated with significant accumulation of circulating and cardiac Ang II. The concomitant genetic inactivation of ACE completely rescued the ACE2-null cardiac phenotype, further implicating elevated Ang II in cardiac dysfunction observed in the ACE2-null mice. These studies were the first to support the in vivo importance of ACE2 in regulating cardiac function and Ang II metabolism. In addition to these findings, two additional ACE2-null mice strains were generated by separate groups [60, 61]. One strain exhibited cardiac dysfunction only in response to pressure overload, which was also associated with increased cardiac Ang II [61]. The impairments in cardiac dysfunction were abrogated with the co-administration of the AT_1 receptor antagonist, candesartan. In contrast, Gurley et al. [60] reported that genetic inactivation of ACE2 in 129/SvEv, C57BL/6, or mixed mouse backgrounds did not induce any functional impairments in the heart, suggesting that the importance of cardiac ACE2 may be dependent on the genetic background of the animal model [62]. In this context, Mercure et al. [63] reported that an eightfold increase in Ang(1-7) in the heart of transgenic animals was associated with less ventricular hypertrophy and fibrosis than their nontransgenic littermates in response to a hypertensive challenge.

A view from an opposite approach to determine the physiological importance of ACE2 further favors a cardioprotective role for the enzyme. Indeed, the overexpression of ACE2 protects the heart from Ang II-induced cardiac hypertrophy and myocardial fibrosis in rats [64]. Moreover, elevations in cardiac ACE2 exhibited a partial rescue of the cardiac functional deficits induced by coronary artery ligation in rats [65]. The cardioprotective persona given to ACE2 is further illustrated by its regulation in pathological conditions. Three very important independent studies showed that cardiac ACE2 was upregulated in both humans and rodent models of heart failure [66–68]. Moreover, we first showed that secreted ACE2 (sACE2) was elevated in the cardiac effluent of hypertrophied hearts, suggesting that the enzyme was attempting to protect the heart from progressing toward overt failure as is known in the Ren-2 transgenic rats [10]. These data were very recently supported by human studies that measured sACE2 activity in human plasma, and the authors showed that the sACE2 was indeed markedly increased in patients diagnosed with heart failure [69]. Intriguingly, Lambert et al. [70] recently demonstrated that the tumor necrosis

factor convertase, ADAM17, participated in the shedding of ACE2 from the membrane, and other studies have reported that ADAM17 is upregulated in heart failure [71].

Collectively, the data on the physiological importance of cardiac ACE2 are clear: it exerts a cardioprotective role from the early stages of cardiac hypertrophy through overt heart failure, although its efforts may be insufficient to overcome the progression of heart disease. However, further studies are required to determine the importance of the discovery of an endogenous ACE2 inhibitor [72], as well as the emerging data that ACE2 is a functional receptor for the SARS coronavirus [73], as these may provide alternative therapeutic targets for the treatment of cardiovascular disease.

10.4 Angiotensin-(1-12)

Over the years, questions have been raised regarding the capability of cardiac and vascular tissue to synthesize Ang II [74–78]. The heart remains a critical example. Although a large body of evidence suggests the existence of local tissue RAS in the regulation of cardiac function and remodeling, most studies revealed low levels of gene expression for both cardiac renin and Aogen [79]. Neither the identification of renin in cardiac mast cells [80] excludes an uptake mechanism from the blood compartment nor does the finding of renin activation by binding of prorenin to the prorenin/renin receptor [81–83] can be construed as evidence for local production of cellular renin. Likewise, Aogen gene expression in cardiac tissue has been reported at very low expression levels while the question of how much of the Aogen mRNA is due to its presence in the endothelial cells of intracoronary vessels and cardiac fibroblast has not been answered.

Our current view of the RAS as a complex system entailing several levels of regulation and processing is now further expanded with the identification of proangiotensin-12 [angiotensin-(1-12), Ang-(1-12)] as an upstream propeptide to Ang I [84]. These investigators first isolated this novel Aogen-derived peptide from the rat small intestine. Consisting of 12 amino acids, this peptide was termed proangiotensin-12 based on its possible role as an Ang II precursor. Ang-(1-12) constricted aortic strips and, when infused intravenously, raised blood pressure in rats. The vasoconstrictor responses to Ang-(1-12) were abolished by either captopril or the AT1 blocker CV-11974. Current studies from this laboratory now demonstrate the existence of Ang-(1-12) in both the heart and the kidneys of spontaneously hypertensive rats (SHR), primarily restricted to cardiac myocytes and renal tubular cells [85]. In addition, the cardiac content of Ang-(1-12) was significantly augmented in the heart of SHR compared to Wistar-Kyoto (WKY) controls [85]. Moreover, an insight into the processing of Ang-(1-12) into Ang I, Ang II, and Ang-(1-7) was accomplished by studying the effects of exogenously administered Ang-(1-12) in isolated hearts from both normotensive and hypertensive rat strains [86]. In these studies, we showed processing of Ang-(1-12) into Ang I, Ang II, and Ang-(1-7). Moreover, in the group of WKY

and SHR investigated in this study, the addition of a specific renin inhibitor to the preparation in no manner altered the production of angiotensins from Ang-(1-12). These data showed that Ang-(1-12) is processed into the active angiotensin peptides by a non-renin mechanism. While further work will be required to ascertain the biological role of Ang-(1-12), these data expand on our knowledge of the mechanisms by which the RAS regulates the expression of angiotensins in tissues [87–90].

10.5 Conclusions

The discovery of the counter-balancing ACE2/Ang-(1-7)/mas arm of the RAS has expanded knowledge of the intrinsic mechanisms by which the system regulates homeostasis and tissue perfusion in both physiology and pathology [91]. Rapid advances in this field now suggest alternate approaches to suppress the pathological actions of Ang II by enhancing the counter-regulatory actions of Ang-(1-7), augmenting the activity of ACE2, or both. Moreover, the discovery of Ang-(1-12) in multiple tissues including the heart may provide additional mechanistic insights that could lead to the better treatment and management of hypertension and heart failure.

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