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Advances in the Renin Angiotensin System: Focus on Angiotensin-Converting Enzyme 2 and Angiotensin-(1–7)

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Abstract

The contribution of the renin angiotensin system to physiology and pathology is undergoing a rapid reconsideration of its mechanisms from emerging new concepts implicating angiotensin-converting enzyme 2 and angiotensin-(1–7) as new elements negatively influencing the vasoconstrictor, trophic, and pro-inflammatory actions of angiotensin II. This component of the system acts to oppose the vasoconstrictor and proliferative effects on angiotensin II through signaling mechanisms mediated by the mas receptor. In addition, a reduced expression of the vasodepressor axis composed by angiotensin-converting enzyme 2 and angiotensin-(1–7) may contribute to the expression of essential hypertension, the remodeling of heart and renal function associated with this disease, and even the physiology of pregnancy and the development of eclampsia.

I. Introduction

Research on the regulatory actions of the renin angiotensin system continues to provide a wealth of information on how cells maintain their internal homeostatic environment, regulate metabolic processes, and adapt and contribute to disease. Not that long ago, the active product of the system—angiotensin II (Ang II)—was considered the sole hormone product of an endocrine system involved in regulating blood volume and vascular tone. The demonstration of renin and angiotensinogen in multiple tissues led to a revision of concepts and the suggestion for independent regulation of tissue and circulating renin angiotensin systems, with the former acting to modulate cell growth, cell differentiation, and intercellular communication. Today, a broader and complex system is revealed by advanced genetic and molecular tools, as well as the outcome of clinical studies using medications selective for one or more of the enzymes and proteins contributing to the generation of angiotensin peptides. The further discovery that the renin angiotensin system contains both a pressor and depressor arm in exerting regulatory functions on vascular tone and cellular signaling paved the way for the generation of alternate hypothesis as to how an unbalance of their function could contribute to cardiovascular disease. In this chapter, we elaborate on these discoveries. The general tenet of the chapter focuses on providing evidence that the axis composed by angiotensin-converting enzyme 2 (ACE2), the heptapeptide angiotensin-

(1–7) [Ang-(1–7)], and the mas receptor (mas-R) function to oppose the activity of the pressor and proliferative axis composed by angiotensin-converting enzyme (ACE), Ang II, and its actions at the type 1 Ang II receptor (AT₁) (Fig. 1).

II. The Paradigm Shift

The period from approximately 1970 to 1980 may be viewed as the beginning of a shift in the concept of how the renin angiotensin system is involved in cardiovascular pathology. Renewed enthusiasm for its study resulted from the introduction of ACE inhibitors as the response to the administration of teprotide (Ondetti et al., 1977), a synthetic nonapeptide mimicking the substance contained in the venom of the *Bothrops Jararaca*, had a dramatic effect in reducing the blood pressure of hypertensive subjects (Gavras et al., 1978). Accelerated by the encouraging data regarding the antihypertensive actions of ACE inhibition, increased funding from the National Heart, Lung, and Blood Institute of the National Institutes of Health and pharmaceutical companies catapulted many laboratories to undertake newer approaches to study the physiology of Ang II, isolate its receptor, and undertake the eventual synthesis and introduction of first systemic (Brunner et al., 1976; Ondetti et al., 1977; Streeten et al., 1975a, 1975b) and then orally active Ang II receptor antagonists (Timmermans et al., 1993, 1995; Wong et al., 1990). In truth, the important work in the design, experimental, and clinical characterization of losartan, the first prototypical Ang II receptor blocker (ARB) built on the work that my mentor Irvine H. Page and his colleagues Merlin F. Bumpus, Robert Kharallai, Robert Smeby, and Mahesh Khosla from the Research Division of the Cleveland Clinic Foundation (Cleveland, OH, USA) had done in the characterization of the biological effect on amino acid substitutions in the conformational structure of the Ang II molecule. Their meticulous efforts along these lines documented the critical importance of the eighth amino acid residue, the C-terminal phenylalanine in the natural peptide, for binding and expression of the pressor and secretagogue actions of Ang II in adrenal tissue (Bumpus, 1977; Bumpus & Khosla, 1975; Bumpus et al., 1973, 1984; Khairallah et al., 1970, 1978; Khosla et al., 1972, 1973, 1974, 1976, 1977; Masaki et al., 1977; Smeby et al., 1962; Yamamoto et al., 1972).

The progress made throughout these exciting periods of discovery, nevertheless, continued to posit Ang II as the sole biologically relevant product of the biochemical cascade initiated by the proteolytic action of renin on hepatic angiotensinogen and the formation of Ang II through the cleavage of Ang I by ACE. Identification of alternate enzymes and Ang I metabolites, though demonstrated in several biological systems, was considered to have no major relevance in terms of biological function or their role in cardiovascular pathology. Although this is not the place for a discussion of these other components, it is worth mentioning the demonstration that tonin could cleave Ang I (Boucher et al., 1977; Cemassieux et al., 1976; Garcia et al., 1976, 1979; Schiffrin et al., 1981), that angiotensin-(1–9) could be produced from Ang I (Campbell et al., 1991, 1995; Donoghue et al., 2000), and that a synthetic tetradecapeptide substrate, angiotensin-(1–14) [Ang-(1–14)], was capable of forming Ang II *in vitro* (Skeggs, 1993; Skeggs et al., 1958).

III. The ACE2/Ang-(1–7)/Mas Axis

In 1998, the publication of the first description of the biological activity of the N-terminal-derived heptapeptide Ang-(1–7) would over time decisively alter the prevailing view. The initial demonstration that Ang-(1–7) stimulated the release of vasopressin from rat hypothalamic explants with a potency comparable to Ang II (Schiavone et al., 1988) suggested that more complex processes participated in the biochemical mechanisms accounting for angiotensin peptide formation and function. The heptapeptide Ang-(1–7) [Asp¹-Arg²-Val³-Tyr⁴-Ile⁵-His⁶-Pro⁷-] constituted a truncated form of Ang II since it lacked phenylalanine in the eighth position. This truncation had important consequences in terms of accepting a biological activity for this peptide because structural work on previous amino acid substitutions of the Ang II molecule indicated the criticality of maintaining the C-terminus intact for both pressor and adrenal catecholamine release (Bumpus, 1977; Khosla et al., 1973, 1974). This interpretation was in keeping with Greene et al. (1982) previous conclusion that the absence of a vascular effect of Ang-(1–7) suggested that Ang-(1–7) was an inactive fragment of Ang II.

A. Hemodynamic and Neuro-Humoral Actions of Ang-(1–7)

Vasodilator effects of Ang-(1–7) were first observed in Sprague Dawley rats made areflexic by spinal cord transection (Benter et al., 1993). In this condition, the dose-dependent reduction in arterial pressure mediated by intravenous Ang-(1–7) infusions was abolished by administration of either the cyclooxygenase inhibitor, indomethacin, or the nonselective Ang II receptor antagonist [Sar¹-Thr⁸]Ang II (Benter et al., 1993). A more prolonged vasodepressor effect of Ang-(1–7) was then demonstrated in spontaneously hypertensive rats (SHRs), in which the antihypertensive action of Ang-(1–7) at day 7 of its chronic administration was preceded by reduction in plasma vasopressin concentration and increases in urinary prostaglandin E₂ and 6-keto prostaglandin F₁ α (6-keto-PGF₁ α) excretion (Benter et al., 1995). The possibility that Ang-(1–7) depressor actions were in part modulated by the state of the baroreceptor reflexes was further confirmed in experiments in which the heptapeptide blunted the pressor actions of systemically injected Ang II or phenylephrine (Benter et al., 1995). These earlier observations were both confirmed and extended by additional studies showing the existence of immunoreactive Ang-(1–7) within brainstem pathways involved in the central control of blood pressure (Block et al., 1988) and evidence that the heptapeptide modulated the baroreceptor reflexes at post-synaptic nerve endings in the vagal-solitarii nuclei (Campagnole-Santos et al., 1990) of the medulla oblongata (Campagnole-Santos et al., 1989, 1990). Other studies showed that the facilitatory effects of Ang-(1–7) on central baroreceptor mechanisms may involve an action on substance P pathways and the blunting of the tonic action of the heptapeptide in the brain of older rats (Sakima et al., 2005) and the (mRen-2)27 transgenic hypertensive rat (Diz & Ferrario, 1996; Diz & Pirro, 1992; Diz et al., 1997, 2008). The latter observation gained support from studies showing that immunosuppression of brain Ang-(1–7) was associated with increased blood pressure in this model of transgenic hypertension (Moriguchi et al., 1995). Over the following decades, research would demonstrate that Ang-(1–7) contributes to the cardiorenal control of blood pressure via actions that within the heart, the kidney, and

the blood vessels opposed the activity of Ang II (Ferrario, 2003, 2010; Ferrario et al., 1997, 2005).

B. Ang-(1–7) and the Heart

Ang-(1–7) suppresses the hypertrophic and profibrotic effects of Ang II in the heart (Ferreira et al., 2007b, 2010; Giani et al., 2010; Gomes et al., 2010; Grobe et al., 2006, 2007b; He et al., 2004; Iwata et al., 2005; Li et al., 2009; Mendes et al., 2005; Mercure et al., 2008; Neves et al., 1997; Santiago et al., 2010; Santos et al., 2004, 2006; Wang et al., 2005) and reduces cardiac arrhythmogenic activity (De Mello, 2004, 2009a, 2009b; De Mello et al., 2007) possibly through the release of endogenous prostaglandins (Ferreira et al., 2001).

Following the original observation of Santos et al. (1990) of the presence of increased coronary sinus levels of Ang-(1–7) after acute myocardial infarction, studies confirmed a local contribution of this hormone in the adaptation of the heart to ischemia and the progression of heart failure. Myocardial ischemia due to coronary artery ligation increases the content of Ang-(1–7) in myocytes surrounding the infarcted zone of the left ventricle (Averill et al., 2003) while administration of Ang-(1–7) attenuates the progression of cardiac fibrosis induced by chronic administration of isoproterenol in Wistar rats (Ferreira et al., 2007b) and in transgenic rats expressing an Ang-(1–7)-producing fusion protein driven by the alpha-myosin heavy chain promoter (Ferreira et al., 2010). Additional studies in the isolated heart have confirmed a cardioprotective effect of Ang-(1–7) (Ferreira et al., 2001, 2002, 2010). While the bulk of studies indicate that the counter-regulatory effect of Ang-(1–7) on cardiac function is mediated through Ang-(1–7) expression (Ferrario, 2002, 2005; Ferrario et al., 2005a; Ferreira et al., 2001, 2002, 2007a, 2007b, 2010; Grobe et al., 2006, 2007b), a study by Wang et al. (2005) suggested that circulating Ang-(1–7) accounted for the attenuation of cardiac remodeling and fibrosis induced by aortic coarctation. Since this interpretation was based on assessing the cardioprotective actions of chronically infused Ang-(1–7) on the content of cardiac Ang II, their data do not negate the possibility of a local action of Ang-(1–7) through myocyte uptake or increased autocrine expression (Wang et al., 2005).

Emerging evidence also demonstrates an action of Ang-(1–7) in regulating cardiac chronotropic activity and a consequent antiarrhythmic function. De Mello (2004) first reported that Ang-(1–7) activates the sodium pump, hyperpolarizes the heart cell, and reestablishes the impulse conduction during ischemia/reperfusion in the isolated hamster's heart. Additional studies showed that at higher concentrations [10^{-7} M], the heptapeptide induced early afterdepolarizations probably through an action on AT_1 receptors (De Mello et al., 2007). This interpretation is in keeping with previous studies showing that Ang-(1–7) binds to AT_1 receptors when administered at supraphysiological concentrations (Clark et al., 2001).

Consistent with an action of Ang-(1–7) on attenuation of cardiac remodeling are studies demonstrating that the heptapeptide has beneficial effects on the progression of heart failure. Loot et al. (2002) showed that an 8-week administration of Ang-(1–7) to Sprague Dawley rats developing heart failure due to coronary artery ligation was associated with a 40% reduction in the elevated left ventricular (LV) end-diastolic pressure, increase in the rate of

ventricular pressure incline (dp/dt) as well as restoration of endothelial function. Similar conclusions have been reported in other studies including those in which a nonpeptide analog of Ang-(1–7) (AVE-0991) was used both *in vitro* and *in vivo* (Ferreira et al., 2007a; Grobe et al., 2007b; Li et al., 2009; McMurray & Davie, 2002).

C. Ang-(1–7) Vascular Actions

Weak vasodilator actions of Ang-(1–7) given systemically contrasts with the more pronounced effects of the peptide when administered to isolated blood vessels (Almeida et al., 2000; Brosnihan et al., 1996, 1998; Fernandes et al., 2001; Gorelik et al., 1998; Kozlovski et al., 2007; Machado et al., 2002; Meng & Busija, 1993; Neves et al., 2004; Oliveira et al., 1999; Osei et al., 1993; Sampaio et al., 2007; Soares de et al., 2004; Tirapelli et al., 2006), a finding that underscores a paracrine rather than a systemic effect of this angiotensin peptide. There is agreement that Ang(1–7) vasodilator responses are in part related to release of bradykinin and endothelium-derived nitric oxide as well as a direct effect of Ang-(1–7) on the mas-R (Almeida et al., 2000; Brosnihan et al., 1996, 1998; Davie & McMurray, 1999; Fernandes et al., 2001; Gorelik et al., 1998; Heitsch et al., 2001; Kozlovski et al., 2007; Lemos et al., 2005; Li et al., 1997a; Li et al., 1997b; Marangoni et al., 2006; Oliveira et al., 1999, 2002, 2003; Sampaio et al., 2007; Soares de et al., 2004; Tirapelli et al., 2006). In the precontracted mesenteric bed of SHR, Ognibene et al. (2009) reported that Ang II induces a type 2 Ang II (AT_2) receptor-mediated vasodilator effect via activation of Ang-(1–7) and bradykinin receptors. In addition, there is evidence that vasodilator prostaglandins may contribute as well in the effects of Ang-(1–7) on vascular tone (Oliveira et al., 1999, 2003).

Consistent with direct actions of Ang-(1–7) on vascular function are reports that Ang-(1–7) opposes the proatherogenic effects of Ang II (Dantas & Sandberg, 2005; Fraga-Silva et al., 2008; Heringer-Walther et al., 2009; Igase et al., 2005, 2008; Langeveld et al., 2005, 2008; Nie et al., 2009; Nishimura et al., 1999; Strawn et al., 1999, 2000, 2004) and acts as an anti-inflammatory factor in part via the inhibition of oxidative stress and the generation of radical oxygen species (Esteban et al., 2009; Gava et al., 2009; Heitsch et al., 2001; Polizio et al., 2007; Rajendran et al., 2005, 2007; Santos et al., 2010). Additional actions of Ang-(1–7) in the regulation of insulin resistance and the evolution of diabetes underscore a role for this peptide in antagonizing the actions of Ang II (Benter et al., 2007, 2008; Dharmani et al., 2007; Ebermann et al., 2008; Oliveira et al., 2002; Rastelli et al., 2007; Santos et al., 2010; Yousif et al., 2007).

D. Renal Actions of Ang-(1–7)

The functional importance of Ang II action on the kidneys in the long-term regulation of blood pressure has been shown through its critical involvement in maintaining body volume and electrolyte homeostasis. However, pathological activation of Ang II not only modifies renal hemodynamics and water and sodium balance but also imposes significant alteration in cell growth and proliferation resulting in hypertension and progression of renal disease. Ang-(1–7) counteracts the effects of Ang II on nephron function by mechanisms that are often not associated with changes in renal hemodynamics (Ferrario & Varagic, 2010).

As shown in the systemic circulation, the renal production of Ang-(1–7) from Ang I is influenced by prolyl oligopeptidase, neprilysin, and thimet oligopeptidase (Ferrario & Varagic, 2010). Activities of all three enzymes are documented in the kidney (Berti et al., 2009; Ebihara et al., 2003; Edwards et al., 1999; Kato et al., 1980; Li et al., 1996; Pendergrass et al., 2008; Schulz et al., 1991; Shaltout et al., 2007). Velez et al. (2009) recently showed that Ang-(1–7) production from Ang I in fresh isolated glomeruli depends heavily on neprilysin activity. Interestingly, renal cortical neprilysin activity and protein expression were higher in female congenic (mRen-2)²⁷ hypertensive rats when compared to their male counterparts (Pendergrass et al., 2008). Since both Ang-(1–7) and Ang II can be metabolized into Ang(1–4) by neprilysin (Ferrario et al., 2002a), the observed lower renal Ang II but not Ang-(1–7) may be accounted for by higher neprilysin activity and, at least in part, explain the gender difference in blood pressure and proteinuria in this hypertensive strain.

The alternative pathway of Ang-(1–7) generation in the kidney includes degradation of Ang II by ACE2 which is highly expressed in the kidney (Chappell, 2010; Shaltout et al., 2009). Several reports showed that ACE and ACE2 colocalize on the apical surface of the proximal tubules while ACE2 is expressed in podocytes and parietal epithelial cells of the Bowman's capsule within the glomerulus (Li et al., 2005; Wysocki et al., 2006; Ye et al., 2006). Similarly, in renal arterioles, ACE2 expression was demonstrated in vascular smooth muscle cells while expression of ACE was confined in the vascular endothelium (Soler et al., 2009). While these reports clearly support the concept of the functionally active Ang-(1–7) machinery, they also underline the necessity for further studies aiming to functionally dissect the specific localization of the participating enzyme and corresponding substrates in structurally diverse nephron segments.

Ang-(1–7) exerts its renal action by acting on the G-protein-coupled receptor mas (Santos et al., 2003). The mas-R expression in afferent arterioles, glomerular mesangial cells, and apical surface of the tubular epithelium (Alenina et al., 2008; Su et al., 2006; Zhang et al., 2010) are consistent with a critical role of Ang-(1–7) in the regulation of renal hemodynamics, and tubuloglomerular structure and function. In isolated blood vessels including the renal afferent arterioles, Ang-(1–7) induced robust vasodilation (Ren et al., 2002) supporting the concept that Ang-(1–7) acts in an autocrine/paracrine fashion rather than exerting classical hormonal effects. Additionally, Ang II-induced renal vasoconstriction in isolated kidney was abolished by mas-R-mediated Ang-(1–7) effects in both Wistar Kyoto (WKY) and SHR (Dharmani et al., 2007). Chronic increases in circulating Ang-(1–7) in transgenic rats expressing an Ang-(1–7)-producing fusion protein did not affect blood pressure but profoundly decreased vascular resistance in many tissues including the kidneys (Botelho-Santos et al., 2007). Similarly, the increases in regional blood flow, including the renal vascular bed, in response to Ang-(1–7) infusion was partially abolished by the specific mas-R antagonist [D-Ala⁷]-Ang-(1–7) (A-779) (Sampaio et al., 2003).

After the first report that Ang-(1–7) increased glomerular filtration rate and water and sodium excretion in isolated kidney (DelliPizzi et al., 1994), studies from our laboratory showed that the heptapeptide facilitated diuresis and natriuresis by inhibiting tubular Na⁺/K⁺-ATPase at doses that did not alter renal blood flow (Handa et al., 1996). At the time when

the mas-R-mediated effects of Ang (1–7) were not yet known, Heller et al. (2000) reported that the increases in water and sodium excretion produced by intrarenal Ang-(1–7) infusion in dogs were mediated through a non-AT₁/AT₂ receptor. Subsequent studies revealed that Ang-(1–7) abolished the stimulatory effect of Ang II on the Na⁺/K⁺-ATPase activity in proximal tubule through the mas-R (Lara et al., 2002). These effects of Ang-(1–7) in the kidneys are linked to the release of cyclooxygenase products coupled with downregulation of AT₁ receptors (Clark et al., 2001; 2003), activation of phospholipase A (Andreatta-van et al., 1993), and increased phosphatidylcholine (Gironacci et al., 2002). There is some discrepancy regarding the receptors involved in mediating the actions of Ang-(1–7) in the kidney. In a distal tubule epithelial-derived cell line from Madin–Darby canine kidney (MDCK), Ang-(1–7) inhibitory action on Na⁺/K⁺-ATPase involved the activation of a protein kinase C signaling pathway via AT₁ receptors (Lara et al., 2005). By contrast, inhibitory effects of both Ang II and Ang-(1–7) on Na⁺/K⁺-ATPase of inner cortex from pig kidney were completely reversed by AT₂ but not by either AT₁ or mas-R antagonists (De Souza et al., 2004). The same group has also reported an antidiuretic action of Ang-(1–7) in water-loaded rats which is abrogated by blockade of AT₁ receptors or genetic deletion of the mas-R (Baracho et al., 1998; Santos et al., 1996, 2003; Simoes e Silva et al., 1998). Consistently, A-779, a selective mas-R antagonist, blocked the antidiuretic effects of Ang-(1–7) mimetic AVE 0991 in water-loaded mice (Pinheiro et al., 2004). Although the foregoing studies demonstrate a modulatory role of Ang-(1–7) in the regulation of both proximal and distal tubular function, continuing research efforts are warranted to further clarify the role of Ang-(1–7) signaling mechanisms in water and electrolyte transport along the different parts of the nephron. In addition, little regard has been taken in evaluating whether species differences influenced the nature of the receptors involved in Ang-(1–7) renal function. As suggested by Ferrario & Varagic (2010), the interaction between the angiotensins and other autocoid and their receptors in terms of allosteric regulation or heterodimerization cannot be ruled out and should be considered when specific effects of different components of the renin angiotensin system are evaluated.

As in other tissues, Ang-(1–7) exerts antigrowth activity in proximal tubular cells inhibiting Ang II-induced phosphorylation of mitogen-activated protein kinases (MAPK) [p38, extracellular signal-related kinase, (ERK1/2), and *c*-Jun N-terminal kinase (JNK)] and Ang II stimulation of transforming growth factor β_1 (TGF- β_1) (Su et al., 2006). Consistently, in genetically modified mice lacking the mas-R, tubulointerstitial and glomerular fibrosis as well as renal dysfunction were associated with upregulation of AT₁ receptors and TGF- β_1 mRNA. Interestingly, a MAPK phosphatase may mediate Ang-(1–7) antiproliferative actions in vascular smooth muscle cells and cardiac fibroblasts (Tallant et al., 2006 and Tallant and Gallagher, unpublished observations).

E. Renoprotective Effects of Ang-(1–7)

A large body of experimental and clinical evidence supports the concept that the Ang-(1–7) axis counterbalances the renal effects of Ang II. Similar to the effects in the heart and aorta (Igase et al., 2005, 2008; Ishiyama et al., 2004), ACE2 gene expression in kidney arterioles increased in response to blockade of AT₁ receptors (Soler et al., 2009). In accord with these studies, a differential regulation of ACE and ACE2 by Ang II has been observed in

hypertensive renal disease (Koka et al., 2008). Upregulation of renal ACE mRNA accompanied by activation of ERK1/2 and p38 MAPK was associated with downregulation of renal ACE2 mRNA in hypertensive patients (Koka et al., 2008). Moreover, inhibition of endogenous ACE2 accelerated hypertension and decreased renal hemodynamics in two-kidney one-clip (2K1C) hypertensive rats by further increasing kidney content of Ang II while averting the protective increase in Ang-(1–7) (Burgelova et al., 2009). A protective role of Ang-(1–7) axis has also been suggested in other forms of nonhypertensive renal disease such as in renal failure (Simoes e Silva et al., 2006; Velkoska et al., 2010), nonhypertensive glomerulopathy (Lely et al., 2004), chemically induced glomerulonephritis (Zhang et al., 2010), renal fibrosis resulting from mas-R genetic deletion (Pinheiro et al., 2009), and diabetic nephropathy (Benter et al., 2007, 2008; Konoshita et al., 2006; Mizuiri et al., 2008; Oliveira et al., 2002; Reich et al., 2008; Soler et al., 2008; Tikellis et al., 2003, 2008; Wang et al., 2008a, 2009; Zhang et al., 2008; Wysocki et al., 2008, 2010).

The existence of Ang-(1–7) as a functional nephron component is buttressed by prior observations of reduced urinary content of Ang-(1–7) in untreated hypertensive subjects (Ferrario et al., 1998) or hypertensive rats (Yamada et al., 1999), and a significant increase in urinary excretion rates of Ang-(1–7) in response to chronic administration of captopril (Luque et al., 1996) or the dual peptidase inhibitor omapatrilat (Ferrario et al., 2002a, 2002b). These prior studies are reinforced by the observation of a strong positive correlation between proteinuria and urinary ACE2 expression in diabetic patients (Wang et al., 2008a).

F. Ang-(1–7) and the Mas Receptor

Prior studies (Brosnihan, 1998; Brosnihan et al., 1996, 1998; Diz & Pirro, 1992; Iyer et al., 1998; Jaiswal et al., 1992) showing that Ang-(1–7) actions were not abrogated by blockade of either AT₁ or AT₂ receptors culminated with the identification of the mas-R as the protein responsible for its physiological effects (Santos et al., 2003). Engagement of the mas-R as a site for Ang-(1–7) binding and actions has been confirmed to mediate attenuation of [³H]leucine incorporation in neonatal cardiac myocytes while transfection of cultured myocytes with an antisense oligonucleotide to the mas-R blocked the Ang-(1–7)-mediated inhibition of serum-stimulated MAPK activation (Tallant et al., 2005). Findings involving the application of A-779 to evaluate Ang-(1–7) actions appear to confirm the involvement of the mas-R (Santos et al., 1994). Using this antagonist, studies have reported that A-779 prevents the inhibitory actions of Ang-(1–7) on nerve stimulation-mediated release of norepinephrine and neuropeptide Y (Byku et al., 2010), myocardial contractility (Castro-Chaves et al., 2009), and stimulation of cardiac Akt phosphorylation and Ang II-stimulated ERK1/2 and Rho kinase phosphorylation (Giani et al., 2008). The use of mice with genetic deletion of the mas-R has affirmed conclusions derived from pharmacological blockade of Ang-(1–7) (Santos et al., 2003, 2006). Further evidence for a role of the mas-R in mediating Ang-(1–7) actions has been derived from the observation of hypertension in mas-deficient mice backcrossed to the inbred mouse strain FVB/N genetic background (Alenina et al., 2008), the disappearance of Ang-(1–7) reduction in coronary perfusion pressure in mas knockout mice (Castro et al., 2005), and the suppression of Ang-(1–7)-mediated release of nitric oxide from cardiomyocytes isolated from mas-deficient mice (Dias-Peixoto et al., 2008) or Chinese hamster ovary (CHO)-mas cells (Sampaio et al., 2007). Transfection of

COS or Human Embryonic Kidney 293 (HEK293) cells with an assortment of mas-related gene (Mrg) receptors shows that of the six studied receptors, MrgD and MRG initiated significant arachidonic acid release after stimulation with Ang-(1–7), a finding that implicates other Mrg's in mediating the actions of Ang-(1–7) (Gembardt et al., 2008).

IV. Angiotensin-Converting Enzyme 2 as a Component of the Ang-(1–7)/Mas Axis

The pace of research on the counter lever role of Ang-(1–7) on Ang II was expanded with the identification of an ACE homologue, ACE2, that cleaves Ang II into Ang-(1–7) (Donoghue et al., 2000; Tipnis et al., 2000). ACE2 cloned from a human lymphoma (Tipnis et al., 2000) and the heart of a heart failure patient (Donoghue et al., 2000) was found to be a close homologue of the ACE cDNA library. Despite a close similarity between ACE and ACE2, the two enzymes function in a completely different way. ACE functions as a dipeptidyl carboxypeptidase, whereas ACE2 functions as a mono carboxypeptidase. In addition, ACE2 activity is not inhibitable by the administration of ACE inhibitors.

A. ACE2 Enzyme Kinetic and Protein Structure

Shortly, after the discovery of ACE2, Vickers et al. (2002) showed that ACE2 cleaved Ang II into Ang-(1–7) with high affinity (catalytic efficiency of ACE2 for Ang II, $k_{\text{cat}}/K_m = 1.9 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$). The ACE2 catalytic efficiency is 400-fold higher for Ang II as compared to Ang I. In addition, ACE2 competes with ACE for Ang I, forming Ang-(1–9) with a lower turnover number ($k_{\text{cat}} = 0.035 \text{ s}^{-1}$). ACE2 also cleaves apelin-13 and dynorphin A 1–13 substrate with high catalytic efficiency (K_m value is $<10 \text{ }\mu\text{M}$, and k_{cat}/K_m value is $>1 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$). In addition, ACE2 cleaves des-Arg⁹-bradykinin peptides, β -casomorphin, and neurotensin 1–8 with substantial catalytic efficiency ($k_{\text{cat}}/K_m = 1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) (Vickers et al., 2002). The optimum proteolytic activity of ACE2 is pH 6.5 and this enzyme is almost inactive at pH 5.0. However, ACE2 maintains substantial catalytic activity under basic condition (pH 7.0–9.0). ACE2 proteolytic activity is greatly enhanced by high chloride or fluoride ions while changes in bromide ion concentrations have no effect. Vickers et al. (2002) also noted that ACE2 catalytic activity is optimal in the presence of 1.0 M NaCl and is stable for more than 6 h at room temperature in assay buffer. Additional studies by Rice et al. (2004) confirmed the high catalytic efficiency of ACE2 for Ang II ($k_{\text{cat}}/K_m = 2.2 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$).

Detailed information about ACE2 protein crystal structure and the schematic view of active site was reported by Guy et al. (2003), Natesh et al. (2003), and Towler et al. (2004). Unlike ACE which has two catalytic sites, ACE2 has one catalytic domain. ACE2 shares 42% sequence identity in the catalytic regions and 61% sequence similarity with ACE (Tipnis et al., 2000). In contrast to ACE, ACE2 contains a single His-Glu-Xaa-Xaa-His (HEXXH) zinc active-site motif. The gene encoding human ACE has been localized to the chromosome 17q23 and is encoded by a 21 kb gene that consists of 26 exons and 25 introns (Rigat et al., 1990). The human ACE2 gene, on the other hand, is located on the X chromosome (Tipnis et al., 2000). Analysis of the DNA sequence of mouse ACE2 shows 83% identity with human ACE2 and the gene is also mapped to chromosome X 70.5 cM (Komatsu et al., 2002).

B. Tissue Distribution of ACE2

ACE2 is widely distributed in many tissues with higher levels in the heart, kidney, and testis (Batlle et al., 2006, 2008; Ferrario & Varagic, 2010; Ferrario et al., 2005a, 2005b; Gembardt et al., 2005; Hamming et al., 2004, 2008; Ohtsuki et al., 2010; Oudit et al., 2006). Our studies showed that ACE2-dependent Ang-(1–7) formation from Ang II was significantly higher from the heart of hypertensive rats as compared to normal rat heart (Trask et al., 2007). ACE2 is a cell surface protein distributed evenly to detergent-soluble regions of the plasma membrane in CHO cells. However, in polarized MDCK cells under steady-state conditions, ACE2 is localized predominantly to the apical surface (~92%) where it is proteolytically cleaved within its ectodomain to release a soluble form (Warner et al., 2005). ACE2 protein was also detected in membrane preparation from whole testis and Leydig cells from adult rats (Douglas et al., 2004). In this study, they also reported the cloning and sequencing of ACE2 from a human testis and noted that the cDNA sequence was identical to that reported for ACE2 from cardiac tissue.

C. Role of ACE2 in Cardiovascular Function

Crackower et al. (2002) demonstration that deletion of the ACE2 gene in mice resulted in major defects in cardiac contractility and upregulation of hypoxia-inducible genes awakened the interest of investigators to consider ACE2 and Ang-(1–7) as components of the renin angiotensin system involved in cardiovascular function. Studies in transgenic Ren-2 hypertensive rats from our group revealed an ACE2-dependent cardiac generation of Ang-(1–7) from Ang II as well as increased collagen deposition following chronic ACE2 inhibition (Trask et al., 2007, 2010). Other studies involving genetic knockout mice models also showed the *in vivo* importance of ACE2 in cardiac hypertrophy and fibrosis (Gurley & Coffman, 2008; Huentelman et al., 2005; Yamamoto et al., 2006). A recent study from our laboratory showed that a 28-day intravenous infusion of the specific ACE2 inhibitor, MLN-4760, resulted in increased cardiac Ang II levels, worsening of cardiac hypertrophy, and augmented content of cardiac interstitial collagen fraction in (mRen-2)²⁷ transgenic rats (Trask et al., 2010).

Der Sarkissian et al. (2008) showed that cardiac overexpression of ACE2 exerts protective influence on the heart during myocardial infarction by preserving cardiac function, LV wall motion, and contractility, and by attenuating LV wall thinning. Earlier, a study from Huentelman et al. (2005) found that ACE2 overexpression induced by intracardiac injection of a lentiviral vector encoding the mouse ACE2 attenuated the development of Ang II-induced cardiac hypertrophy and fibrosis. Additional studies agree with the conclusion that ACE2 counteracts cardiac remodeling induced by myocardial injury, renal hypertension, and heart failure, as well as retarding the progression of atherosclerosis and vascular injury (Burgelova et al., 2009; Burrell et al., 2005; Crackower et al., 2002; Diez-Freire et al., 2006; Dong et al., 2008; Epelman et al., 2008; Ferrario, 2005; Ferrario et al., 2005a; Goulter et al., 2004; Grobe et al., 2007a, 2007b; Hamming et al., 2008; Heeneman et al., 2007; Hu et al., 2007; Igase et al., 2005, 2008; Ji et al., 2008; Kaiqiang et al., 2009; Kassiri et al., 2009; Lovren et al., 2008; Ohtsuki et al., 2010; Pan et al., 2007; Sluimer et al., 2008; Trask et al., 2010; Varagic et al., 2010; Velkoska et al., 2010; Yamamoto et al., 2006; Zulli et al., 2006).

These findings demonstrate that ACE2 plays a central role in balancing the pressor and proliferative activity of the ACE/Ang II/AT₁ receptor axis.

The role of ACE2 in the development of human heart failure is not fully understood. Ohtsuki et al. (2010) found that the upregulation of the ACE2 gene in the myocardium of the LV of patients with severe heart failure correlated with the degree of LV dilatation. These data are in keeping with the observation that the hypertensive effects of infused Ang II in mice can be prevented by coinfusion of human recombinant ACE2 (hrACE2) enzyme in association with increased formation of Ang-(1–7) from Ang II (Wysocki et al., 2010). Since blunting effect of hrACE2 on blood pressure was unaffected by the specific Ang-(1–7) receptor blocker A-779, the mechanism of hrACE2 action may result from the combination of increased systemic ACE2 activity and reduction in plasma Ang II. Further validation of the results obtained in these experiments (Wysocki et al., 2010) may lead to the design of therapeutic approaches geared to increasing ACE2 activity in states of Ang II overactivity. Since the loss of ACE2 facilitates adverse postmyocardial infarction ventricular remodeling by potentiation of Ang II effects on AT₁ receptors, supplementation of ACE2 may be a potential therapy for ischemic heart disease (Kassiri et al., 2009). The studies reported by Wysocki et al. (2010) and Kassiri et al. (2009) provide strong evidence for a direct role of ACE2 in modulation of adverse cardiac remodeling and tissue expression of Ang-(1–7).

Several studies suggest that Ang II peptide play a significant role in regulation of ACE2 expression, but molecular mechanisms of ACE2 regulation in cardiovascular system are poorly understood. Signaling pathways studies show that the downregulation of ACE2 by Ang II or endothelin-1 was blocked by mitogen-activated protein extracellular kinase (MEK) inhibitors to reduce ERK activities, suggesting that both agonists downregulate ACE2 through activation of the MAPK (ERK1 and/or ERK2) pathways (Koka et al., 2008). The influence that Ang II exerts on the expression and activity of ACE2 requires further study since a recent report in human cardiac fibroblast showed that both Ang II and Ang-(1–7) upregulate ACE2 expression via AT₁ and mas-R-mediated actions, respectively (Lin et al., 2010). This finding is not consistent with observations of increased ACE2 mRNA and activity induced by AT₁ receptor blockade *in vivo* (Agata et al., 2006; Ferrario et al., 2005a, 2005b; Igase et al., 2005, 2008; Ishiyama et al., 2004; Jessup et al., 2006) and *in vitro* (Gallagher & Tallant, 2004; Gallagher et al., 2006, 2008a, 2008b), and the demonstration that Ang-(1–7) activates a MAPK phosphatase to counteract the inhibitory effect of Ang II on ACE2 (Gallagher et al., 2008a).

The clinical translation for a role of ACE2 in human disease is buttressed by the report of Calo et al. (2010) who showed increased ACE2 expression and Ang-(1–7) levels in mononuclear cells obtained from Bartter's/Gitelman's patients. In addition, ACE2-mediated Ang II degrading activity is significantly increased in prehypertensive patients (Keidar et al., 2007). Completion of the Human Genome Project has opened a new window to investigate genetic means for the treatment of hypertension through overexpression of ACE2 gene (Ferreira & Raizada, 2008; Katovich et al., 2005). Polymorphisms of the ACE2 gene have been associated with essential hypertension (Fan et al., 2007; Huang et al., 2006; Liu et al., 2005; Mo et al., 2010; Niu et al., 2007; Yi et al., 2006), LV hypertrophy, coronary artery

disease, and myocardial infarction (Lieb et al., 2006; Palmer et al., 2008; Sotoodehnia et al., 2009; Wang et al., 2008b; Yan et al., 2008; Yang et al., 2006).

D. Role of ACE2 in Renal Axis

ACE2, expressed at high levels in the kidney, has been demonstrated as renoprotective in mouse model of chronic kidney disease (Dilauro et al., 2010). In 5/6 nephrectomized mice, administration of an ACE2 inhibitor worsened proteinuria in association with increased renal content of Ang II. While AT₁ receptor blockade reversed proteinuria, a similar effect could not be obtained by Ang-(1–7) administration (Dilauro et al., 2010). These data suggest that some of the effects induced by ACE2 inhibition may not be expressed to increased renal expression of Ang-(1–7). Dilauro et al.'s (2010) interpretation of these findings agree with the report that administration of hrACE2 blunts the hemodynamic effects of acute increases in Ang II by a mechanism that is independent of circulating Ang-(1–7) (Wysocki et al., 2010). Likewise, the role of ACE2 in the development of diabetic nephropathy remains unclear. A recent study on a streptozotocin-induced diabetic ACE2-knockout mice (ACE2-KO) model shows more severe progression of albuminuria, as well as a severe time-dependent increase in glomerular/tubulointerstitial damage in diabetic ACE2-KO mice compared to wild-type mice (C57BL/6) (Shiota et al., 2010). The renal-protective effect of ACE2 might involve more than just suppressing Ang II-mediated AT₁ receptor signaling because Senador et al. (2010) found that short-term AT₁ blockade treatment in C57BL/6 mice (fed with a 60% fructose diet for 8 weeks in combination with losartan treatment with 30 mg/kg/day on week 9) reversed the pressor effect in a high fructose diet group, demonstrating that there are prominent interactions between a dietary regimen that produces glucose intolerance and an antihypertensive drug that antagonizes Ang II signaling. These findings suggest that the mechanism of change may be via renal Ang II rather than the ACE2/Ang-(1–7) pathway because the fructose losartan combination resulted in lowered renal Ang II content without changes in Ang-(1–7) level (Senador et al., 2010).

Protection against oxidative stress and cell damage through the ACE2/Ang-(1–7) pathway has also been reported within the nucleus in the kidneys. Studies showed that the ACE2 inhibitor (MLN-4760) reduced the exacerbation of Ang II-dependent formation of reactive oxygen species in renal nuclei isolated from older sheep (Gwathmey et al., 2010). These changes were associated with abolition of Ang-(1–7) formation from Ang II (Gwathmey et al., 2010). A protective role of hrACE2 to slow the progression of kidney injury has also been reported in diabetic Akita mice [Ins2 (WT/C96Y)] (Oudit et al., 2010). Treatment with hrACE2 increases the Ang-(1–7) levels, lowers Ang II levels, and reduces NADPH oxidase activity in diabetic Akita mice [Ins2(WT/C96Y)] as compared to control C57BL/6J mice [(WT/WT)] (Oudit et al., 2010). Furthermore, an *in vitro* study also reported that hrACE2 attenuated both high glucose and Ang II-induced oxidative stress and NADPH oxidase activity which suggest that the protective effect of hrACE2 is due to reduction in Ang II and increases in Ang-(1–7) signaling. The changes in renin angiotensin system components appear to be tissue specific and possibly related to enhancement of fatty acid synthesis, epididymal adipose tissue mass, and hypertension. Coelho et al. (2010) found that the tissue level Aogen, ACE, AT₁, and AT₂ genes as well as protein expression were unaltered in sucrose-fed rats while Ang II, Ang-(1–7), and ACE activity increased in their kidneys.

Although the expression of ACE2 in the diabetic kidney has been well studied (Mizuiru et al., 2008; Moon et al., 2008; Tikellis et al., 2003, 2008; Wysocki et al., 2006), little is known about the ACE2 expression in non-diabetic renal disease. Recently, a study conducted in acute kidney injury induced by subtotal nephrectomy showed a marked reduction in cortical and medullary ACE2 activity compared with control rats (Velkoska et al., 2010). ACE inhibition with ramipril produced beneficial effects in association with increased renal ACE2 activity (Velkoska et al., 2010). Renal biopsies obtained from both control and renal diseased patients showed that ACE2 is present in human kidney (Lely et al., 2004). In control kidneys, the immunohistochemical staining for ACE2 was localized in tubular and glomerular epithelium. However, in diseased patients (all primary and secondary renal diseases, and renal transplants) neo-expression of ACE2 was found to be restricted to the glomerular and peritubular capillary endothelium (Lely et al., 2004).

V. The Ang-(1–7)/ACE2/Mas-R Axis in the Regulation of Pregnancy

Normal pregnancy is a physiological condition characterized by decreased total peripheral resistance, decreased or normal blood pressure (August et al., 1990), and an increased renin angiotensin system activity (Nasjletti & Masson, 1972; Oelkers, 1996). Within the first few weeks of pregnancy, a rise in plasma Ang II levels is accompanied by increases in angiotensinogen, plasma renin activity (PRA), urinary and plasma aldosterone, and downregulation of AT₁ receptors (Baker et al., 1990). Pregnant women possess decreased vascular responsiveness to Ang II (Gilbert et al., 2008; Rosenfeld, 2001; Shah, 2005) which is associated with downregulation of the AT₁ receptor (Baker et al., 1990, 1992). New data suggest that increased estrogen and Ang-(1–7) levels compensate for the increased renin angiotensin system in pregnancy (Li et al., 1997). When nulliparous third trimester normal pregnant patients were compared to nonpregnant subjects, plasma Ang-(1–7) levels were significantly elevated in normal pregnant women as compared to nonpregnant women (Merrill et al., 2002). According to Merrill et al. (2002), plasma Ang-(1–7) and Ang II levels were 34 and 50% higher, respectively, in normal pregnant women as compared to nonpregnant subjects. Nogueira et al. (2007) confirmed the increase in plasma Ang I and Ang-(1–7) levels in a small sample of women with pregnancy and further reported a blunting of the Ang-(1–7) increase in pregnant women with gestational diabetes. Involvement of Ang-(1–7) in the evolution of normal pregnancy has been demonstrated by Valdes et al. (2001), who described a progressive increase in Ang-(1–7) urinary excretion throughout the gestational period to levels that were 10-fold higher than those reported during the menstrual cycle. In agreement with these observations, 24 h urinary excretion of Ang I, Ang II, and Ang-(1–7) was increased by 93, 44, and 60%, respectively, as compared to virgin animals in the diestrus phase of the estrus cycle of pregnant rats (Neves et al., 2003). The increase in urinary Ang-(1–7) levels may reflect local synthesis of the heptapeptide as kidney Ang-(1–7) and ACE2 immunostaining were enhanced in the inner cortex/outer medulla proximal and distal tubules throughout pregnancy in Sprague Dawley rats (Joyner et al., 2007). These results suggested that Ang-(1–7) through its associated enzyme ACE2 may function in blood pressure and/or hydro mineral balance during pregnancy. Additional studies involving blockade of Ang-(1–7) concluded that the diuresis seen during late gestation in normal pregnancy can be mechanistically regulated by Ang-(1–7) through increased water intake,

decreased plasma arginine vasopressin (AVP), and downregulation of kidney aquaporin 1 (Joyner et al., 2008). Recent studies have shown wide distribution and generally colocalization of Ang-(1–7) and ACE2 throughout the human and rat uteroplacental unit during gestation (Joyner et al., 2008; Valdes et al., 2006). In the pregnant human uterus, Ang-(1–7) and ACE2 were found in the invading trophoblasts and in trophoblasts cells lining the uterine spiral arteries (Anton et al., 2009). While the uterine concentration of Ang I and Ang-(1–7) and ACE2 mRNA did not change with pregnancy, the concentration of Ang II was lower in the human uterus during pregnancy as compared to nonpregnant subjects (Anton et al., 2009). The relative gene expression of AT₁ receptor, AT₂ receptor, and mas-R was decreased in the uterus during normal pregnancy as compared to the uterus of nonpregnant subjects (Anton et al., 2009).

Temporal–spatial studies in the rat uterus during early and late gestation suggest that Ang-(1–7) and ACE2 may play an important role in implantation (Neves et al., 2008). During early pregnancy, Ang-(1–7) and ACE2 immunostaining was present in the implantation and interimplantation sites (decidua, luminal, and glandular epithelium, embryo, and ectoplacental cone) (Neves et al., 2003), whereas during late gestation, Ang-(1–7) and ACE2 were found on epithelial cells of the yolk sac and amnion.

The main sites of human placental Ang-(1–7) expression were in the syncytiotrophoblasts, cytotrophoblasts, blood vessel endothelium, and vascular smooth muscle of the primary and secondary villi (Valdes et al., 2006). Ang-(1–7) was also expressed in the maternal stroma in extravillous cytotrophoblasts, intravascular cytotrophoblasts, and decidual cells. ACE2 was expressed mainly in the syncytiotrophoblasts, cytotrophoblasts, villous blood vessel endothelium, and vascular smooth muscle cells of the primary villi while in the maternal stroma, ACE2 was expressed in the invading and intravascular trophoblast and the decidual cells (Valdes et al., 2006). The coincident location of Ang-(1–7) and ACE2 suggests an autocrine function of Ang-(1–7). Additionally, Ang-(1–7) may integrate with other vasodilators including nitric oxide, bradykinin, and prostaglandins to produce an autocrine/paracrine role in endometrial receptivity; trophoblast invasion; regulation of fetal, placental, and uterine perfusion; and the prevention of platelet aggregation in the intervillous space. Ang-(1–7) in the placenta may regulate vascular growth since Ang-(1–7) was shown to have antiangiogenic effects on human umbilical vein endothelial cells through a unique AT_{1–7} receptor that was sensitive to losartan (Anton et al., 2007).

The regulatory role of the Ang-(1–7)/ACE2/mas-R axis in blood pressure regulation in the pregnant state is further strengthened by reports of reduced plasma Ang-(1–7) levels in preeclamptic women (Velloso et al., 2007) and nulliparous preeclamptic third trimester patients matched for parity, race, and gestational age as compared to third trimester normal pregnant patients (Merrill et al., 2002). Additionally, Merrill et al. (2002) observed that there was a negative correlation between plasma Ang-(1–7) and both systolic and diastolic blood pressures, suggesting a potential contribution of reduced Ang-(1–7) on elevated blood pressure in preeclampsia.

VI. Conclusion

As reviewed elsewhere (Ferrario, 2010; Ferrario & Varagic, 2010), even from this limited overview of the intricate internal mechanisms regulating the pathways determining the production of angiotensin peptides, it is obvious that the renin angiotensin system is embodied with a great capacity to use alternate mechanisms in bypassing blockade of primary pathways. In unraveling the complexity of the biochemical physiology of the system, it is also apparent that formation of angiotensin peptides within the cellular environment may not follow what has been characterized in the circulation or the extracellular compartment. The intertwined relationship between the ACE/Ang II/AT₁ receptor axis and its counterlever ACE2/Ang-(1–7)/mas-R axis is now buttressed on a firm literature and its clinical impact is beginning to be fully appreciated in terms of the possibility that many cases of essential hypertension, particularly those associated with low renin, may be explained by a genetic or acquired deficiency of ACE2 or Ang-(1–7) activity. Research in this field is extending knowledge of disease processes outside the cardiovascular system as altered function of the ACE2/Ang-(1–7)/mas-R axis is being implicated in the pathology of cancer (Altundag et al., 2006; Menon et al., 2007; Rodgers et al., 2002, 2003, 2006; Soto-Pantoja et al., 2009; Zhou et al., 2009), liver disease (Alfany-Fernandez et al., 2009; Carvalho et al., 2007; Herath et al., 2007, 2009; Lubel et al., 2008, 2009; Pereira et al., 2007), blood disorders (Ellefson et al., 2004; Heringer-Walther et al., 2009; Kucharewicz et al., 2002), and autoimmune diseases (Pignone et al., 2007).

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Abbreviations

ACE	angiotensin-converting enzyme
ACE2	angiotensin-converting enzyme 2
ACE2-KO	ACE2-knockout mice
Ang II	angiotensin II
AT₁	type 1 angiotensin II receptor (R)
AT₁₋₇	angiotensin-(1–7) receptor
AT₂	type 2 angiotensin II receptor
CHO	Chinese hamster ovary
hrACE2	human recombinant ACE2
LV	left ventricular
MDCK	Madin–Darby canine kidney

Mrg	mas-related gene
rACE2	recombinant ACE2
SHR	spontaneously hypertensive rat
TGF-β_1	transforming growth factor β_1
mas-R	mas receptor

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FIGURE 1.
Schematic diagram of the key biochemical steps involved in the formation of angiotensin peptides.