Review Article

New Cardiovascular and Pulmonary Therapeutic Strategies Based on the Angiotensin-Converting Enzyme 2/Angiotensin-(1–7)/Mas Receptor Axis

Anderson J. Ferreira, 1 Tatiane M. Murça, 1 Rodrigo A. Fraga-Silva, 2 Carlos Henrique Castro, 3 Mohan K. Raizada, 4 and Robson A. S. Santos 2

1 Department of Morphology, Institute of Biological Sciences, Federal University of Minas Gerais, 31.270-901 Belo Horizonte, MG, Brazil
2 Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, 31.270-901 Belo Horizonte, MG, Brazil
3 Department of Physiology Sciences, Federal University of Goiás, 74.001-970 Goiânia, GO, Brazil
4 Department of Physiology and Functional Genomics, College of Medicine, University of Florida, 32.610 Gainesville, FL, USA

Correspondence should be addressed to Anderson J. Ferreira, anderson@icb.ufmg.br

Received 31 August 2011; Accepted 12 October 2011

Copyright © 2012 Anderson J. Ferreira et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Angiotensin (Ang)-(1–7) is now recognized as a biologically active component of the renin-angiotensin system (RAS). The discovery of the angiotensin-converting enzyme homologue ACE2 revealed important metabolic pathways involved in the Ang-(1–7) synthesis. This enzyme can form Ang-(1–7) from Ang II or less efficiently through hydrolysis of Ang I to Ang-(1–9) with subsequent Ang-(1–7) formation. Additionally, it is well established that the G protein-coupled receptor Mas is a functional ligand site for Ang-(1–7). The axis formed by ACE2/Ang-(1–7)/Mas represents an endogenous counter regulatory pathway within the RAS whose actions are opposite to the vasoconstrictor/proliferative arm of the RAS constituted by ACE/Ang II/AT1 receptor. In this review we will discuss recent findings concerning the biological role of the ACE2/Ang-(1–7)/Mas arm in the cardiovascular and pulmonary system. Also, we will highlight the initiatives to develop potential therapeutic strategies based on this axis.

1. Introduction

The renin-angiotensin system (RAS) plays a key role in several target organs, such as heart, blood vessels, and lungs, exerting a powerful control in the maintenance of the homeostasis [1–4]. This system is activated by the conversion of the angiotensinogen to the inactive peptide angiotensin (Ang) I through the renin action [5]. Subsequently, Ang I is cleaved by the angiotensin-converting enzyme (ACE) generating Ang II [6], the main angiotensin peptide, whose actions are mediated by two G protein-coupled receptors (GPCR), AT1 and AT2 [7, 8] (Figure 1). The major physiological functions of Ang II are mediated by AT1 receptor [9, 10]. In pathological conditions, activation of this receptor induces deleterious effects, such as vasoconstriction, fibrosis, cellular growth and migration, and fluid retention [11, 12]. On the other hand, Ang II binding to the AT2 receptor generally causes opposite effects when compared with those actions mediated by the AT1 receptor [13, 14].

Recently, it has been proposed that, in addition to the ACE/Ang II/AT1 receptor axis, the RAS possesses a counter regulatory axis composed by ACE2, Ang-(1–7), and Mas receptor (Figure 1). Ang-(1–7) is a biologically active component of the RAS which binds to Mas inducing many beneficial actions, such as vasodilatation, antifibrosis, and antihypertrophic and antiproliferative effects [15–23]. This peptide is produced mainly through the action of ACE2, which has approximately 400-fold less affinity to Ang I than to Ang II [24–26]; thereby, Ang II is the major substrate for Ang-(1–7) synthesis. In fact, the conversion of Ang II to
Angiotensinogen
Asp-Arg-Val-Tyr-Ile-Pro-Phe-His-Leu-Val-Ile-

Angiotensin I
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

Renin
Prorenin

Bradykinin
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Inactive kinin
ACE

Angiotensin-(1–9)
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His

ACE2
Angiotensin-(1–5)
Asp-Arg-Val-Tyr-Ile

Tonin, Catepsin G
Catepsin A, Chymase

Angiotensin II
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

Angiotensin-(1–7)
Asp-Arg-Val-Tyr-Ile-His-Pro

Prorenin
Inactive kinin
ACE

Ang-(1–7) by ACE2 is important to regulate the RAS activity since Ang-(1–7) induces opposite effects to those elicited by Ang II [16–24]. Additionally, ACE2 can form Ang-(1–7) less efficiently through hydrolysis of Ang I to Ang-(1–9) with subsequent Ang-(1–7) formation [24].

The relevance of the RAS is highlighted by the success obtained in therapeutic strategies based on the pharmacological inhibition of this system in cardiovascular and respiratory diseases [27–32]. Blockade of the RAS with ACE inhibitors (ACEi) or AT1 receptor antagonists (ARBs) improves the outcomes of patients with hypertension, acute myocardial infarction, and chronic systolic heart failure [33–35]. Furthermore, based on the involvement of the ACE/Ang II/AT1 axis in respiratory diseases and the crucial role of the lungs in the RAS metabolism, several studies have reported the contribution of the RAS in lung pathophysiology [28, 30, 31, 36–40]. Importantly, it has been shown that administration of ACEi and ARBs causes substantial increases in plasma Ang-(1–7) levels, leading to the assumption that part of their clinical effects might be mediated by this heptapeptide [41–43]. Indeed, some effects of ACEi and ARBs can be blocked or attenuated by A-779, a Mas antagonist, confirming the role of Ang-(1–7) in the actions of these compounds [44]. The beneficial effects of Ang-(1–7), as well as its likely participation in the effects of the ACEi and ARBs, represent evidences for the potential of the ACE2/Ang-(1–7)/Mas axis as a therapeutic target.

In this review, we will focus on the recent findings related to the pathophysiology actions of the ACE2/Ang-(1–7)/Mas axis in the cardiovascular and respiratory system. Also, we will discuss the promising initiatives to develop new therapeutic strategies based on this axis to treat pathological conditions.

2. Cardiac ACE2/Ang-(1–7)/Mas Axis

The heart is one of the most important targets for the actions of the ACE2/Ang-(1–7)/Mas axis. In the heart, ACE2
is expressed in the endothelium [45], myofibroblasts [46], cardiomyocytes, and fibroblasts [47, 48]. Classical pharmacotherapeutic agents used to treat heart failure, including ACEi, ARBs, and aldosterone receptor blockers, increase ACE2 activity and/or expression, indicating its importance in the cardiac diseases establishment and progression [49–51].

Additionally, pharmacological and genetic (transgenic animals and gene transfer) approaches have evidenced the significance of ACE2 in cardiac pathologies. Despite some controversies concerning the consequences of the ACE2 deficiency, in general, evidences indicate a protective role of ACE2 in the heart [48, 52–57]. Crackower and colleagues [52] were the first to demonstrate that genetic ablation of ACE2 results in severe blood-pressure-independent systolic impairment. Also, disruption of ACE2 was able to accelerate cardiac hypertrophy and shortened the transition period to heart failure in response to pressure overload by increasing local Ang II [54]. Recently, it has been demonstrated that loss of ACE2 enhances the susceptibility to myocardial infarction, with increased mortality, infarct expansion and adverse ventricular remodeling [56]. In keeping with these genetic findings, pharmacological inhibition of ACE2 exacerbated cardiac hypertrophy and fibrosis in Ren-2 hypertensive rats [58]. On the other hand, cardiac overexpression of ACE2 prevented hypertension-induced cardiac hypertrophy and fibrosis in spontaneously hypertensive rats (SHR) and in Ang-II-infused rats [59, 60]. Indeed, transfection of Lenti–ACE2 (lentivirus containing ACE2 cDNA) or Ad–ACE2 (recombinant adenovirus carrying the murine ACE2) into the surrounding area of the infarcted myocardium was protective against pathological remodeling and cardiac systolic dysfunction in a rat model of myocardial infarction [61, 62]. This effect was associated with decreased expression of ACE and Ang II and increased expression of Ang-(1–7) [62]. Collectively, these observations reveal that ACE2 effectively plays a protective role in the cardiac structure and function.

Since the discovery of Ang-(1–7) in the late 1980s [63, 64], several studies have demonstrated important effects of this peptide in hearts. The presence of Ang-(1–7) and its receptor Mas in the heart [65, 66] and the ability of this organ to produce Ang-(1–7) [55, 67] are evidences of the role of this peptide in cardiac tissues. Functionally, Ang-(1–7) induces an antiarrhythmic effect against ischemia/reperfusion injuries in rats [17, 68] as well as prevents atrial tachycardia and fibrillation in rats and dogs [69, 70]. Treatment with Ang-(1–7) improved the coronary perfusion and cardiac function in rats after myocardial infarction [71] and after ischemia/reperfusion injury [72]. Increases in circulating Ang-(1–7) levels in transgenic rats reduced the cardiac hypertrophy [17] and fibrosis [20, 22] induced by isoproterenol administration. These effects are apparently independent of changes in blood pressure since Grobe and colleagues [18] have demonstrated that the antifibrotic and antihypertrophic actions of Ang-(1–7) are still observed in Ang-II-infused hypertensive rats. Local overexpression of Ang-(1–7) in hearts of mice and rats improved the myocardial contractility and prevented the isoproterenol- and hypertension-induced cardiac remodeling [19, 21]. Altogether, these findings support a direct effect of Ang-(1–7) in the heart.

Further evidence for the role of Ang-(1–7)/Mas in the pathophysiology of the heart came from experimental protocols utilizing mice with genetic deficiency of Mas. They revealed that the cardiac function is impaired in Mas knock-out mice likely due to the increased extracellular matrix proteins deposition in the heart [66, 73]. This profibrotic phenotype may be related to changes in matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) levels and/or activities [74, 75].

Although further elucidations regarding the signaling pathways involved in Mas activation are necessary, some mechanisms have been proposed. Overexpression of Ang-(1–7) in hearts of rats causes an improvement in the [Ca2+]i handling in cardiomyocytes and increases the expression of SERCA2a [21]. In keeping with these results, cardiomyocytes from Mas-deficient mice present slower [Ca2+]i transients accompanied by a lower Ca2+-ATPase expression in the sarcoplasmatic reticulum [66, 76]. Although acute Ang-(1–7) treatment failed to alter Ca2+-handling in ventricular myocytes of rats [76], these findings suggest an important role of the Ang-(1–7)/Mas in the long-term maintenance of the Ca2+ homeostasis in the heart.

One of the mechanisms by which Ang-(1–7) plays its effects in the heart is stimulating the nitric oxide (NO) production. Indeed, it has been demonstrated that Ang-(1–7) via Mas increases the synthesis of NO through a mechanism involving the activation of the endothelial NO synthase (eNOS). These effects were abolished by A-779 and are absent in cardiomyocytes from Mas-deficient mice [76]. Recently, Gomes et al. [77] found that the treatment of isolated cardiomyocytes of rats with Ang-(1–7) efficiently prevents the Ang-II-induced hypertrophy by modulating the calcineurin/NFAT signaling cascade. These effects were blocked by NO synthase inhibition and by guanly cyclase inhibitors, indicating that these effects are mediated by the NO/cGMP pathway.

Also, Ang-(1–7) inhibits serum-stimulated mitogen-activated protein kinase (MAPK) activation in cardiac myocytes [78] and prevents the Ang-II-mediated phosphorylation of ERK1/2 and Rho kinase in hearts in a dose-dependent manner [79]. In line with these data, activation of endogenous ACE2 significantly reduced the phosphorylation of ERK1/2 in hearts of hypertensive rats (SHRs) [48]. However, Mercure et al. [19] reported that overexpression of Ang-(1–7) in hearts of rats decreases the Ang-II-induced phosphorylation of c-Src and p38 kinase, whereas the increase in ERK1/2 phosphorylation was unaffected by the expression of the transgene, thereby suggesting a selective effect of Ang-(1–7) on intracellular signaling pathways related to cardiac remodeling.

Overall, these data reveal a key role of the ACE2/Ang-(1–7)/Mas axis in the pathophysiology of the cardiac structure and function. Activation of this axis might be an important strategy to develop a new generation of cardiovascular therapeutic agents against cardiac dysfunction and pathological remodeling of the heart.
3. Vascular ACE2/Ang-(1–7)/Mas Axis

Early studies have reported the endothelium as the major site for generation [67] and metabolism [41] of Ang-(1–7). In addition to Ang-(1–7), endothelial cells also express ACE2 and Mas [80, 81]. Thus, now it is recognized that the ACE2/Ang-(1–7)/Mas axis is present in vascular endothelial cells and modulates its function promoting vasorelaxation [82], reduction of the oxidative stress [83, 84], and antiproliferative effects [85, 86].

The vasodilatory actions of Ang-(1–7) have been reported in many studies in several vascular beds and preparations, including mouse [16, 23] and rat [15] aortic rings, canine [87] and porcine [88] coronary arteries, canine middle cerebral artery [89], porcine piglet pial arterioles [90], feline mesenteric vascular bed [91], rabbit renal afferent arterioles [92], and mesenteric microvessels of normotensive [93] and hypertensive [94] rats. Vascular Ang-(1–7) actions are still controversial in human. For example, it has been shown that Ang-(1–7) causes vasodilation in forearm circulation of normotensive subjects and patients with essential hypertension [95] while other studies were unable to report any significant effect of Ang-(1–7) in the same vascular territory in ACEi-treated patients [43].

The Mas receptor is critically involved in the vascular effects of Ang-(1–7). In fact, many of these actions are completely abolished by A-779 or partially blocked by this antagonist [3, 86, 96]. Importantly, the endothelium-dependent relaxation induced by Ang-(1–7) in mouse aortic rings is absent in vessels derived from Mas-knockout mice [16]. However, other studies have shown that Ang-(1–7) also interacts with ACE, AT1, and AT2-like receptors, suggesting the existence of additional sites of interaction for Ang-(1–7) [3, 97, 98]. Indeed, Silva et al. [99] reported evidence for the presence of a distinct subtype of Ang-(1–7) receptor sensible to D-pro7-Ang-(1–7), a second Mas antagonist, but not to A-779 in aortas of Sprague-Dawley rats.

The vascular effects of Ang-(1–7) are endothelium dependent and involve the production of vasodilator products, such as prostanoids, NO, and endothelium-derived hyperpolarizing factor (EDHF) [16, 81, 100]. Pinheiro and coworkers [101] found that Ang-(1–7) promotes an increase in NO release in Mas-transfected chinese hamster ovary (CHO) cells [101]. Furthermore, short-term infusion of Ang-(1–7) improved the endothelial function by a mechanism involving NO release in rats [102]. Mas deletion resulted in endothelial dysfunction associated with an unbalance between NO and oxidative stress [83]. Also, Mas activation by Ang-(1–7) in human endothelial cells stimulated eNOS phosphorylation/activation via the Akt-dependent pathway [81]. Other mechanisms appear to be involved in the Ang-(1–7) vascular actions. Roks et al. [103] have shown that Ang-(1–7) inhibits the vasoconstriction induced by Ang II in human internal mammary arteries, thereby suggesting that Ang-(1–7) can regulate the Ang II effects [103]. In fact, Ang-(1–7) negatively modulates the Ang II type 1 receptor-mediated activation of c-Src, and its downstream targets ERK1/2 and NAD(P)H oxidase [104]. The counterregulatory action of Ang-(1–7) on Ang II signaling has been also observed in cardiomyocytes [77], vascular smooth muscle cells [105], and fibroblasts [106]. Additionally, an interaction between Mas and bradykinin (Bk) type 2 (B2) receptors may modulate some of the Ang-(1–7) effects in blood vessels [107]. Indeed, it has been demonstrated that Ang-(1–7) potentiates the vasodilator and hypotensive effects of Bk in several vascular beds [93, 108–110].

As the major enzyme involved in Ang-(1–7) formation, ACE2 has also a crucial role in vessels. Lovren et al. [111] have demonstrated that ACE2 ameliorates the endothelial homeostasis via a mechanism involving reduction of the reactive oxygen species production [111]. Of note, this effect was attenuated by A-779 [111]. Moreover, overexpression of ACE2 in vessels of hypertensive rats resulted in reduction in the arterial blood pressure and improvement of the endothelial function associated with increased circulating Ang-(1–7) levels [112]. Overall, these data indicate that the beneficial effects of ACE2 are, at least in part, mediated by Ang-(1–7). Recently, we have demonstrated that activation of endogenous ACE2 causes a dose-dependent hypotensive effect in normotensive and hypertensive rats [113]. Also, the response to Bk administration was augmented in rats chronically treated with XNT, an ACE2 activator [113]. However, we were unable to demonstrate any significant effect of XNT on blood pressure in response to the administration of Ang II or Losartan in normotensive and hypertensive rats (Figure 2).

4. Pulmonary ACE2/Ang-(1–7)/Mas Axis

In the past few years, the participation of the ACE2/Ang-(1–7)/Mas axis in the establishment and progression of pulmonary diseases has become evident. Indeed, the important role of the RAS in the lung pathophysiology and the side effects and pulmonary toxicity induced by the ACEi raised the interest to evaluate the activation of the ACE2/Ang-(1–7)/Mas axis as an alternative target to treat pulmonary pathologies. Thus, it has been reported beneficial outcomes induced by the activation of this axis in animal models of acute respiratory distress syndrome (ARDS), pulmonary hypertension (PH), fibrosis, and lung cancer [31, 37, 114–117]. These studies pointed out that the imbalance between the ACE/Ang II/AT1 and the ACE2/Ang-(1–7)/Mas axes of the RAS might be relevant in lung diseases. Taking into account that systemic hypotension is an important limitation to the use of ACEi and ARBs in pulmonary patients, therapies based on the ACE2/Ang-(1–7)/Mas axis emerge as a safe and efficient approach since studies using the ACE2 activator XNT or ACE2 gene transfer have shown that these strategies induce beneficial pulmonary outcome without changes in systemic blood pressure in rats and mice [39, 117, 118].

Imai and colleagues [37] demonstrated the role of ACE2 in ARDS pathogenesis. They found that a more severe ARDS was reached in ACE2 knockout mice, and this phenotype was reversed by double genetic deletion of the ACE2 and ACE genes or by the treatment with recombinant human ACE2 (rhACE2). Furthermore, Ang II levels were related...
to the severity of the lung injury. Of note, ACE2 is widely expressed in the pulmonary endothelium, vasculature, and pneumocytes [119, 120]. Also, rhACE2 inhibited the increase of Ang II and TNF-α levels, attenuated the arterial hypoxemia and PH, and ameliorated the distribution of the pulmonary blood flow in lipopolysaccharide-induced lung injury in piglets [121]. Therefore, these studies suggest that ACE2 is a suitable target to arrest the development of ARDS in patients at risk.

The stimulation of the ACE2/Ang-(1–7)/Mas axis has been successful used to prevent and reverse PH and fibrosis in animals. ACE2 activation using the compound XNT or induction of ACE2 overexpression by gene transfer efficiently prevented and, more importantly, reversed the increase of the right systolic ventricular pressure (RSVP), pulmonary fibrosis, imbalance of the RAS, and inflammation in animals (rats and mice) with PH induced by monocrotaline (MCT) or in rats with pulmonary fibrosis caused by bleomycin treatment [39, 117, 118]. In keeping with these findings, Ang-(1–7) gene transfer into the lungs triggered similar protective actions in MCT-treated rats [39]. In addition, Ang-(1–7) via Mas prevented the apoptosis of alveolar epithelial cells and the Jun N-terminal kinase (JNK) activation induced by bleomycin [122]. The involvement of the Ang-(1–7)/Mas in PH was further evidenced by the observation that the XNT effects are blocked by A-779 [117]. Furthermore, in both lung specimens from patients with idiopathic pulmonary fibrosis and from animals with bleomycin-induced pulmonary fibrosis were reported a reduction in mRNA, protein, and activity of ACE2 with a reciprocal increase in Ang II level [116].

A growing body of studies has focused on the relevance of the ACE2/Ang-(1–7)/Mas axis in the pulmonary cancer pathophysiology. The protein expression of ACE2 is reduced in non-small-cell lung carcinoma (NSCLC) along with an increase in Ang II levels. Moreover, overexpression of ACE2 in cultured A549 lung cancer cells and in human lung cancer xenografts inhibited the cell growth and the vascular endothelial growth factor-a (VEGFa) expression induced by Ang II [123, 124]. Gallagher and Tallant [125] evaluated the
effects of several angiotensin peptides [Ang I, Ang II, Ang-(2–8), Ang-(3–8), and Ang-(3–7)] in SK-LU-1 cancer cells.

growth, and only Ang-(1–7) showed significant attenuation of

dNA synthesis and proliferation. The antiproliferative effect of Ang-(1–7) was mediated by its receptor Mas and inhibition of the ERK1/2 pathway. Neither the blockade of AT1 nor AT2 succeeded in inhibiting the action of Ang-(1–7). In keeping with these data, the antiproliferative effect of Ang-(1–7) was observed in human A549 lung tumor xenograft growth along with a marked decrease in the vessel density in mice through a mechanism involving cyclooxygenase-2 (COX-2) [126, 127]. Of note, in a nonrandomized phase I clinical trial conducted by Petty and colleagues [38], subcutaneous injections of Ang-(1–7) were administered in 18 patients with advanced solid tumors refractory to standard therapy. Despite the mild adverse effects observed with the Ang-(1–7) treatment, generally it was well tolerated. There were no treatment-related deaths. Clinical benefits were observed in 27% of the patients. Altogether, these studies provide insights into the involvement of the ACE2/Ang-(1–7)/Mas axis in lung cancer.

5. Pharmacological Therapeutic Strategies Based on the ACE2/Ang-(1–7)/Mas Axis

Many advances have been achieved regarding the therapeutic regulation of the RAS. Current therapies based on the modulation of the RAS include the ACEi, ARBs, and renin inhibitors. In general, these drugs prevent or reverse endothelial dysfunction and atherosclerosis, reduce cardiovascular mortality and morbidity of patients with coronary artery disease, and hold antihypertensive effects [128].

Classically, the mechanisms of action of the ACEi and ARBs involve the blockade of the synthesis and actions of Ang II, respectively. However, the RAS is a complex hormonal system and, consequently, other mechanisms are likely implicated in the actions of these drugs [42, 86, 129]. They cause substantial increase in plasma levels of Ang-(1–7), leading to the assumption that their clinical effects might be partly mediated by this heptapeptide [42, 130]. Indeed, a variety of effects of the ACEi and ARBs can be abolished or attenuated by Mas antagonism, confirming the role of Ang-(1–7) in the actions of these compounds [129, 131]. The beneficial effects of Ang-(1–7) as well as its likely involvement in the effects of the ACEi and ARBs represent a strong evidence for the therapeutic potential of the activation of the ACE2/Ang-(1–7)/Mas axis (Figure 3).

5.1. Ang-(1–7) Formulations. The beneficial effects of Ang-(1–7) are well known; however, the therapeutic utilization of this peptide is limited due to its unfavorable pharmacokinetic properties. Ang-(1–7) has a short half-life (approximately 10 seconds) since it is rapidly cleaved by peptidases [132]. Furthermore, Ang-(1–7) is degraded during its passage through the gastrointestinal tract when orally administrated. Thus, new strategies are crucial to make feasible the clinical application of Ang-(1–7).

Recently, a formulation based on the Ang-(1–7) included into hydroxypropyl β-cyclodextrin [HPβCD/Ang-(1–7)] was developed by Lula and colleagues [133]. Cyclodextrins are pharmaceutical tools used for design and evaluation of drug formulations, and they enhance the drug stability and absorption across biological barriers and offer gastric protection [134]. The amphiphilic character of cyclodextrins allows the possibility of formation of supramolecular inclusion complexes stabilized by noncovalent interactions with a variety of guest molecules [133, 134]. In this regard, the formulation HPβCD/Ang-(1–7) allowed the oral administration of Ang-(1–7). Pharmacokinetic and functional studies showed that oral HPβCD/Ang-(1–7) administration significantly increases plasma Ang-(1–7) levels and promotes an antithrombotic effect that was blunted in Mas deficient mice [135]. Marques and colleagues [136] have found that chronic oral administration of HPβCD/Ang-(1–7) significantly attenuates the heart function impairment and cardiac remodeling induced by isoproterenol treatment and myocardial infarction in rats [136].

In addition, liposomal delivery systems represent an alternative method to administer Ang-(1–7) [137]. Administration of liposomes containing Ang-(1–7) in rats led to prolonged hypotensive effect for several days in contrast to the response observed when the free peptide was used [137, 138].

A strategy used to protect the Ang-(1–7) against proteolytic degradation was proposed by Kluskens and coworkers [139]. Using the ability of prokaryotes to cyclize peptides, they synthesized a cyclic Ang-(1–7) derivative [thioether-bridged Ang-(1–7)] which presented an increased stability in homogenates of different organs and plasma and enhanced the Ang-(1–7) bioavailability in rats [139]. Furthermore, cyclized Ang-(1–7) induced a relaxation in precontracted aorta rings of rats which was blocked by the Ang-(1–7) receptor antagonist D-Pro2-Ang-(1–7), providing evidence that cyclized Ang-(1–7) also interacts with Mas [139].

5.2. Synthetic Mas Receptor Agonists. AVE 0991 was the first nonpeptide synthetic compound developed with the intention of stimulating the Mas receptor. This compound mimics the Ang-(1–7) effects in several organs such as vessels [140, 141], kidney [101], and heart [142, 143]. Similar to Ang-(1–7), AVE 0991 induced a vasodilation effect which was absent in aortic rings of Mas-deficient mice [140]. Moreover, its effects in aortic rings were blocked by the two Ang-(1–7) receptor antagonists A-779 and D-Pro2-Ang-(1–7) [140]. AVE 0991 potentiated the acetylcholine-induced vasodilation in conscious normotensive rats, and this effect was abolished by A-779 and L-NAME [102]. Similarly, it was able to increase the hypotensive effect of Bk in normotensive rats, and A-779 also blocked this effect [107]. Ferreira et al. [142, 143] reported that AVE 0991 protects the heart against cardiac dysfunction and remodeling caused by isoproterenol treatment or by myocardial infarction in rats [142, 143]. In Mas-transfected cells, AVE 0991 induced NO release which was blunted by A-779 and not by AT1 or AT2 antagonists [101]. All these data support the concept that AVE 0991 is
an Ang-(1–7) mimetic and that its actions are mediated by the interaction with Mas.

Using a computational discovery platform for predicting novel naturally occurring peptides that may activate GPCR, two novel peptides, designated as CGEN-856 and CGEN-857, with amino acid sequence unrelated to angiotensin peptides, were found to display high specificity for Mas [23]. These peptides elicited Ca^{2+} influx in CHO cells overexpressing Mas without any activity in AT_1 or AT_2 receptors [144]. CGEN-856S, a derivative of the CGEN-856 peptide, produced beneficial cardiovascular effects similar to those caused by Ang-(1–7) [23]. This compound competes with Ang-(1–7) for the same bind site in Mas-transfected cells. Furthermore, similar to Ang-(1–7), CGEN-856S produced a vasodilation effect which was absent in Mas-deficient mice, indicating that this compound also acts via Mas [23]. This was confirmed by the inhibition of the CGEN-856S effects by the Mas antagonist A-779. Importantly, Savergnini et al. [23] showed that CGEN-856S promotes antiarrhythmogenic effects and produces a small dose-dependent decrease in arterial pressure of conscious SHR [23].

5.3. ACE2 Activators. A new approach addressing the therapeutic potential of the activation of the ACE2/Ang-(1–7)/Mas axis was proposed by Hernández Prada et al. [113]. Based on the crystal structure of ACE2 and using a virtual screening strategy, it was identified small molecules that may interact with this enzyme leading to changes in its conformation and, consequently, enhancing its activity [113]. Thus, the ACE2 activator, namely XNT, was identified and its administration in SHR decreased blood pressure, induced an improvement in cardiac function, and reversed the myocardial and perivascular fibrosis observed in these animals [48, 113]. The beneficial effects of XNT were also
observed in rats with PH induced by MCT [117]. Furthermore, this compound attenuated the thrombus formation and reduced the platelet attachment to vessels in hypertensive rats [145].

It appears that the pharmacological activation of ACE2 promotes its beneficial effects due to an increased Ang-(1–7) production with concomitant degradation of Ang II. In fact, coadministration of A-779 abolished the protective effects of XNT on PH [117]. In addition, the antifibrotic effect of XNT observed in hearts of SHR was associated with increases in cardiac Ang-(1–7) expression [48]. However, it is also pertinent to point out that off-target effects of XNT on these beneficial outcomes cannot be ruled out at the present time.

6. Conclusions

The complexity of the RAS is far beyond what we could suspect few years ago. There is growing evidence that changes in the novel components of the RAS [Ang-(1–7), ACE2, and Mas] may take part of the establishment and progression of cardiovascular and respiratory diseases. Importantly, these new components of the RAS, due to their counter regulatory actions, are candidates to serve as a concept to develop new cardiovascular and respiratory drugs.

References

[1] J. E. Hall, A. C. Gutyon, and H. L. Mizelle, “Role of the renin-angiotensin system in control of sodium excretion and arterial pressure,” Acta Physiologica Scandinavica, Supplement, vol. 139, no. 591, pp. 48–62, 1990.
[2] A. C. Gutyon, “Kidneys and fluids in pressure regulation: small volume but large pressure changes,” Hypertension, vol. 19, no. 1, pp. 12–18, 1992.
[3] R. A. S. Santos, M. J. Campagnole-Santos, and S. P. Andrade, “Angiotensin-(1–7): an update,” Regulatory Peptides, vol. 91, no. 1–3, pp. 45–62, 2000.
[4] R. P. Marshall, “The pulmonary renin-angiotensin system,” Current Pharmaceutical Design, vol. 9, no. 9, pp. 715–722, 2003.
[5] C. M. Ferrario and W. B. Straw, “Role of the renin-angiotensin-aldosterone system and proinflammatory mediators in cardiovascular disease,” The American Journal of Cardiology, vol. 98, no. 1, pp. 121–128, 2006.
[6] T. Kokubu, E. Ueda, T. Joh, and K. Nishimura, “Purification and properties of angiotensin I-converting enzyme in human lung and its role on the metabolism of vasoactive peptides in pulmonary circulation,” Advances in Experimental Medicine and Biology B, vol. 120, pp. 467–475, 1979.
[7] T. Inagami, “A memorial to Robert Tegerstedt: the centennial of renin discovery,” Hypertension, vol. 32, no. 6, pp. 953–957, 1998.
[8] R. M. Touyz and C. Berry, “Recent advances in angiotensin II signaling,” Brazilian Journal of Medical and Biological Research, vol. 35, no. 9, pp. 1001–1015, 2002.
[9] T. Matsusaka and I. Ichikawa, “Biological functions of angiotensin and its receptors,” Annual Review of Physiology, vol. 59, pp. 395–412, 1997.
[10] A. M. Allen, J. Zhuo, and F. A. O. Mendelsohn, “Localization and function of angiotensin AT1 receptors,” American Journal of Hypertension, vol. 13, no. 1, pp. 315–385, 2000.
[11] S. Kim and H. Iwao, “Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases,” Pharmacological Reviews, vol. 52, no. 1, pp. 11–34, 2000.
[12] P. K. Mehta and K. K. Griendling, “Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system,” American Journal of Physiology—Cell Physiology, vol. 292, no. 1, pp. C82–C97, 2007.
[13] M. Horiuichi, W. Hayashida, T. Kambe, T. Yamada, and V. J. Dzau, “Angiotensin type 2 receptor dephosphorylates Bel-2 by activating mitogen-activated protein kinase phosphatase-1 and induces apoptosis,” Journal of Biological Chemistry, vol. 272, no. 30, pp. 19022–19026, 1997.
[14] R. M. Touyz, D. Endemann, G. He, J. S. Li, and E. L. Schiffrin, “Role of AT2 receptors in angiotensin II-stimulated contraction of small mesenteric arteries in young SHR,” Hypertension, vol. 33, no. 1, pp. 366–372, 1999.
[15] Y. le Tran and C. Forster, “Angiotensin-(1–7) and the rat aorta: modulation by the endothelium,” Journal of Cardiovascular Pharmacology, vol. 30, no. 5, pp. 676–682, 1997.
[16] R. A. S. Santos, A. C. Simoes e Silva, C. Maric et al., “Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas,” Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 14, pp. 8258–8263, 2003.
[17] R. A. S. Santos, A. J. Ferreira, A. P. Nadu et al., “Expression of an angiotensin-(1–7)-producing fusion protein produces cardioprotective effects in rats,” Physiological Genomics, vol. 17, pp. 292–299, 2004.
[18] I. L. Grobe, A. P. Mecca, M. Lingis et al., “Prevention of angiotensin II-induced cardiac remodeling by angiotensin-(1–7),” American Journal of Physiology—Heart and Circulatory Physiology, vol. 292, no. 2, pp. H736–H742, 2007.
[19] C. Mercure, A. Yogi, G. E. Callera et al., “Angiotensin(1–7) blunts hypertensive cardiac remodeling by a direct effect on the heart,” Circulation Research, vol. 103, no. 11, pp. 1319–1326, 2008.
[20] A. P. Nadu, A. J. Ferreira, T. L. Reudelhuber, M. Bader, and R. A. S. Santos, “Reduced isoproterenol-induced renin-angiotensin changes and extracellular matrix deposition in hearts of TGR(A1–7)3292 rats,” Journal of the American Society of Hypertension, vol. 2, no. 5, pp. 341–348, 2008.
[21] A. J. Ferreira, C. H. Castro, S. Guatimosim et al., “Attenuation of isoproterenol-induced cardiac fibrosis in transgenic rats harboring an angiotensin-(1–7)-producing fusion protein in the heart,” Therapeutic Advances in Cardiovascular Disease, vol. 4, no. 2, pp. 83–96, 2010.
[22] N. M. Santiago, P. S. Guimarães, R. A. Sirvente et al., “Lifetime overproduction of circulating angiotensin-(1–7) attenuates deoxycorticosterone acetate-salt hypertension-induced cardiac dysfunction and remodeling,” Hypertension, vol. 55, no. 4, pp. 889–896, 2010.
[23] S. Q. Savergnini, M. Beiman, R. Q. Lautner et al., “Vascular relaxation, antihypertensive effect, and cardioprotection of a novel peptide agonist of the Mas receptor,” Hypertension, vol. 56, no. 1, pp. 112–120, 2010.
[24] C. Vickers, P. Hales, V. Kaushik et al., “Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase,” Journal of Biological Chemistry, vol. 277, no. 17, pp. 14838–14843, 2002.
[25] S. R. Tipnis, N. M. Hooper, R. Hyde, E. Karran, G. Christie, and A. J. Turner, “A human homolog of angiotensin-converting enzyme: cloning and functional expression as a captopril-insensitive carboxypeptidase,” Journal of Biological Chemistry, vol. 275, no. 43, pp. 33238–33243, 2000.
[26] M. Donoghue, F. Hsieh, E. Baronas et al., “A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9,” Circulation Research, vol. 87, no. 3, pp. E1–E9, 2000.

[27] C. M. Ferrario, “The renin-angiotensin system: importance in physiology and pathology,” Journal of Cardiovascular Pharmacology, vol. 15, supplement 3, pp. S1–S5, 1990.

[28] R. I. Cargill and B. J. Lipworth, “Lisinopril attenuates acute hypoxic pulmonary vasoconstriction in humans,” Chest, vol. 109, no. 2, pp. 424–429, 1996.

[29] M. G. Nichols, A. M. Richards, and M. Agarwal, “The importance of the renin-angiotensin system in cardiovascular disease,” Journal of Human Hypertension, vol. 12, no. 5, pp. 295–299, 1998.

[30] R. P. Marshall, R. J. McAnulty, and G. J. Laurent, “Angiotensin II is mitogenic for human lung fibroblasts via activation of the type I receptor,” American Journal of Respiratory and Critical Care Medicine, vol. 161, no. 6, pp. 1999–2004, 2000.

[31] S. E. Orfanos, A. Armaganidis, C. Glynos et al., “Pulmonary capillary endothelium-bound angiotensin-converting enzyme activity in acute lung injury,” Circulation, vol. 102, no. 16, pp. 2011–2018, 2000.

[32] I. Fleming, K. Kohlstedt, and R. Busse, “The tissue renin-angiotensin system and intracellular signalling,” Current Opinion in Nephrology and Hypertension, vol. 15, no. 1, pp. 8–13, 2006.

[33] E. L. Schiffrin, “Vascular and cardiac benefits of angiotensin receptor blockers,” The American Journal of Medicine, vol. 115, no. 5, pp. 409–418, 2002.

[34] T. K. W. Ma, K. K. H. Kam, B. P. Yan, and Y. Y. Lam, “Renin-angiotensin-aldosterone system blockade for cardiovascular diseases: current status,” British Journal of Pharmacology, vol. 160, no. 6, pp. 1273–1292, 2010.

[35] K. Vijayaraghavan and P. Deedwania, “Renin-angiotensin-aldosterone blockade for cardiovascular disease prevention,” Cardiology Clinics, vol. 29, no. 1, pp. 137–156, 2011.

[36] F. Fournier, C. Chopin, B. Wallaert et al., “Compared evolution of plasma fibronectin and angiotensin-converting enzyme levels in septic ARDS,” Chest, vol. 87, no. 2, pp. 191–195, 1985.

[37] Y. Imai, K. Kuba, S. Rao et al., “Angiotensin-converting enzyme 2 protects from severe acute lung failure,” Nature, vol. 436, no. 7047, pp. 112–116, 2005.

[38] W. J. Petty, A. A. Miller, T. P. McCoy, P. E. Gallagher, E. A. Tallant, and F. M. Torti, “Phase I and pharmacokinetic study of angiotensin-(1–7), an endogenous antiangiogenic hormone,” Clinical Cancer Research, vol. 15, no. 23, pp. 7398–7404, 2009.

[39] V. Shenoy, A. J. Ferreira, R. A. Fraga-Silva et al., “The angiotensin-converting enzyme 2/angiogenesis-(1–7)/Mas axis confers cardiopulmonary protection against lung fibrosis and pulmonary hypertension,” American Journal of Respiratory and Critical Care Medicine, vol. 182, no. 8, pp. 1065–1072, 2010.

[40] B. D. Uhal, X. Li, A. Xue, X. Gao, and A. Abdul-Hafez, “Regulation of alveolar epithelial cell survival by the ACE2/angiotensin 1–7/Mas axis,” American Journal of Physiology—Lung Cellular and Molecular Physiology, vol. 301, no. 3, pp. L269–L274, 2011.

[41] M. C. Chappell, N. T. Pirro, A. Sykes, and C. M. Ferrario, “Metabolism of angiotensin-(1–7) by angiotensin-converting enzyme,” Hypertension, vol. 31, no. 1, pp. 362–367, 1998.

[42] S. N. Iyer, C. M. Ferrario, and M. C. Chappell, “Angiotensin-(1–7) contributes to the antihypertensive effects of blockade of the renin-angiotensin system,” Hypertension, vol. 31, no. 1, pp. 356–361, 1998.

[43] A. P. Davie and J. J. V. McMurray, “Effect of angiotensin-(1–7) and bradykinin in patients with heart failure treated with an ACE inhibitor,” Hypertension, vol. 34, no. 3, pp. 457–460, 1999.

[44] R. R. Britto, R. A. S. Santos, C. R. Fagundes-Moura, M. C. Khosla, and M. J. Campagnole-Santos, “Role of angiotensin-(1–7) in the modulation of the baroreflex in renovascular hypertensive rats,” Hypertension, vol. 30, no. 3, pp. 549–556, 1997.

[45] G. Y. Oudit, M. A. Crackower, P. H. Backx, and J. M. Penninger, “The role of ACE2 in cardiovascular physiology,” Trends in Cardiovascular Medicine, vol. 13, no. 3, pp. 93–101, 2003.

[46] J. L. Guy, D. W. Lambert, A. J. Turner, and K. E. Porter, “Functional angiotensin-converting enzyme 2 is expressed in human cardiac myofibroblasts,” Experimental Physiology, vol. 93, no. 5, pp. 579–588, 2008.

[47] P. E. Gallagher, C. M. Ferrario, and E. A. Tallant, “Regulation of ACE2 in cardiac myocytes and fibroblasts,” American Journal of Physiology—Heart and Circulatory Physiology, vol. 295, no. 6, pp. H2373–H2379, 2008.

[48] A. J. Ferreira, V. Shenoy, Y. Qi et al., “Angiotensin-converting enzyme 2 activation protects against hypertension-induced cardiac fibrosis involving extracellular signal-regulated kinases,” Experimental Physiology, vol. 96, no. 3, pp. 287–294, 2011.

[49] C. M. Ferrario, J. Jessup, M. C. Chappell et al., “Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2,” Circulation, vol. 111, no. 20, pp. 2605–2610, 2005.

[50] S. Keidar, A. Gamliel-Lazarovich, M. Kaplan et al., “Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients,” Circulation Research, vol. 97, no. 9, pp. 946–953, 2005.

[51] Kaisiang Ji, M. Minakawa, K. Fukui, Y. Suzuki, and I. Fukuda, “Olmesartan improves left ventricular function in pressure-overload hypertrophied rat heart by blocking angiotensin II receptor with synergic effects of upregulation of angiotensin converting enzyme 2,” Therapeutic Advances in Cardiovascular Disease, vol. 3, no. 2, pp. 103–111, 2009.

[52] M. A. Crackower, R. Rao, G. Y. Oudit et al., “Angiotensin-converting enzyme 2 is an essential regulator of heart function,” Nature, vol. 417, no. 6891, pp. 822–828, 2002.

[53] S. B. Gurley, A. Allred, T. H. Le et al., “Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice,” Journal of Clinical Investigation, vol. 116, no. 8, pp. 2218–2225, 2006.

[54] K. Yamamoto, M. Ohishi, T. Katsuya et al., “Deletion of angiotensin-converting enzyme 2 accelerates pressure overload-induced cardiac dysfunction by increasing local angiotensin II,” Hypertension, vol. 47, no. 4, pp. 718–726, 2006.

[55] A. J. Trask, D. B. Averill, D. Ganten, M. C. Chappell, and C. M. Ferrario, “Primary role of angiotensin-converting enzyme-2 in cardiac production of angiotensin-(1–7) in transgenic Ren-2 hypertensive rats,” American Journal of Physiology—Heart and Circulatory Physiology, vol. 292, no. 6, pp. H3019–H3024, 2007.
[56] Z. Kassiri, J. Zhong, D. Guo et al., “Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction,” Circulation: Heart Failure, vol. 2, no. 5, pp. 446–455, 2009.

[57] J. Zhong, D. Guo, C. B. Chen et al., “Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2,” Hypertension, vol. 57, pp. 314–322, 2011.

[58] A. J. Trask, L. Groban, B. M. Westwood et al., “Inhibition of angiotensin-converting enzyme 2 exacerbates cardiac hypertrophy and fibrosis in ren-2 hypertensive rats,” American Journal of Hypertension, vol. 23, no. 6, pp. 687–693, 2010.

[59] M. J. Huentelman, J. L. Grobe, J. Vazquez et al., “Protection from angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats,” Experimental Physiology, vol. 90, no. 5, pp. 783–790, 2005.

[60] C. Diez-Freire, J. Vazquez, M. F. Correa de Adjounian et al., “ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR,” Physiological Genomics, vol. 27, no. 1, pp. 12–19, 2006.

[61] S. Der Sarkissian, J. L. Grobe, L. Yuan et al., “Cardiac overexpression of angiotensin converting enzyme 2 protects the heart from ischemia-induced pathophysiology,” Hypertension, vol. 51, no. 3, pp. 712–718, 2008.

[62] Y. X. Zhao, H. Q. Yin, Q. T. Yu et al., “ACE2 overexpression ameliorates left ventricular remodeling and dysfunction in a rat model of myocardial infarction,” Human Gene Therapy, vol. 21, no. 11, pp. 1545–1554, 2010.

[63] R. A. S. Santos, K. B. Brosnihan, M. C. Chappell et al., “Converting enzyme activity and angiotensin metabolism in the dog brainstem,” Hypertension, vol. 11, no. 2, pp. 1153–1157, 1988.

[64] M. T. Schiavone, R. A. S. Santos, K. B. Brosnihan, M. C. Khosla, and C. M. Ferrario, “Release of vasopressin from the rat hypothalamo-neurohypophyseal system by angiotensin-(1–7) heptapeptide,” Proceedings of the National Academy of Sciences of the United States of America, vol. 85, no. 11, pp. 4095–4098, 1988.

[65] D. B. Averill, Y. Ishiyama, M. C. Chappell, and C. M. Ferrario, “Cardiac angiotensin-(1–7) in ischemic cardiomyopathy,” Circulation, vol. 108, no. 17, pp. 2141–2146, 2003.

[66] R. A. S. Santos, C. H. Castro, E. Gava et al., “Impairment of in vitro and in vivo heart function in angiotensin-(1–7) receptor Mas knockout mice,” Hypertension, vol. 47, no. 5, pp. 996–1002, 2006.

[67] R. A. S. Santos, K. B. Brosnihan, D. W. Jacobsen, P. E. DiCorleto, and C. M. Ferrario, “Production of angiotensin-(1–7) by human vascular endothelium,” Hypertension, vol. 19, no. 2, pp. 1156–1161, 1992.

[68] A. J. Ferreira, R. A. Santos, and A. P. Almeida, “Angiotensin-(1–7): cardioprotective effect in myocardial ischemia/reperfusion,” Hypertension, vol. 38, no. 3, pp. 665–668, 2001.

[69] A. J. Ferreira, P. L. Moraes, G. Foureaux, A. B. Andrade, R. A. Santos, and A. P. Almeida, “‘The angiotensin-(1–7)/Mas receptor axis is expressed in sinoatrial node cells of rats,” Journal of Histochemistry and Cytochemistry, vol. 59, no. 8, pp. 761–768, 2011.

[70] E. Liu, Z. Xu, J. Li, S. Yang, W. Yang, and G. Li, “Enalaprilat, irbesartan, and angiotensin-(1–7) prevent atrial tachycardia-induced ionic remodeling,” International Journal of Cardiology, vol. 146, no. 3, pp. 364–370, 2011.

[71] A. E. Loot, A. J. M. Roos, R. H. Henning et al., “Angiotensin-(1–7) attenuates the development of heart failure after myocardial infarction in rats,” Circulation, vol. 105, no. 13, pp. 1548–1550, 2002.

[72] A. J. Ferreira, R. A. S. Santos, and A. P. Almeida, “Angiotensin-(1–7) improves the post-ischemic function in isolated perfused rat hearts,” Brazilian Journal of Medical and Biological Research, vol. 35, no. 9, pp. 1083–1090, 2002.

[73] C. H. Castro, R. A. S. Santos, A. J. Ferreira, M. Bader, N. Alenina, and A. P. Almeida, “Effects of genetic deletion of angiotensin-(1–7) receptor Mas on cardiac function during ischemia/reperfusion in the isolated perfused mouse heart,” Life Sciences, vol. 80, no. 3, pp. 264–268, 2006.

[74] C. H. Pan, C. H. Wen, and C. S. Lin, “Interplay of angiotensin II and angiotensin-(1–7) in the regulation of matrix metalloproteinases of human cardiocytes,” Experimental Physiology, vol. 93, no. 5, pp. 599–612, 2008.

[75] Z. Pei, R. Meng, G. Li et al., “Angiotensin-(1–7) ameliorates myocardial remodeling and interstitial fibrosis in spontaneous hypertension: role of MMPs/TIMPs,” Toxicology Letters, vol. 199, no. 2, pp. 173–181, 2010.

[76] M. F. Dias-Peixoto, R. A. S. Santos, E. R. M. Gomes et al., “Molecular mechanisms involved in the angiotensin-(1–7)/Mas signaling pathway in cardiomyocytes,” Hypertension, vol. 52, no. 3, pp. 542–548, 2008.

[77] E. R. M. Gomes, A. A. Lara, P. W. M. Almeida et al., “Angiotensin-(1–7) prevents cardiomyocyte pathological remodeling through a nitric oxide/guanosine 3′,5′-cyclic monophosphate-dependent pathway,” Hypertension, vol. 55, no. 1, pp. 153–160, 2010.

[78] E. A. Tallant, C. M. Ferrario, and P. E. Gallagher, “Angiotensin-(1–7) inhibits growth of cardiac myocytes through activation of the Mas receptor,” American Journal of Physiology—Heart and Circulatory Physiology, vol. 289, no. 4, pp. H1560–H1566, 2005.

[79] J. F. Giani, M. M. Gironacci, M. C. Muñoz, D. Turyn, and F. P. Dominici, “Angiotensin-(1–7) has a dual role on growth-promoting signalling pathways in rat heart in vivo by stimulating STAT3 and STAT5a/b phosphorylation and inhibiting angiotensin II-stimulated ERK1/2 and Rho kinase activity,” Experimental Physiology, vol. 93, no. 5, pp. 570–578, 2008.

[80] L. M. Burrell, C. I. Johnston, C. Tikellis, and M. E. Cooper, “ACE2, a new regulator of the renin-angiotensin system,” Trends in Endocrinology and Metabolism, vol. 15, no. 4, pp. 166–169, 2004.

[81] W. O. Sampaio, R. A. S. Santos, R. Faria-Silva, L. T. Da Mata Machado, E. L. Schiffrin, and R. M. Touyz, “Angiotensin-(1–7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways,” Hypertension, vol. 49, no. 1, pp. 185–192, 2007.

[82] R. A. S. Santos, A. J. Ferreira, S. V. B. Pinheiro, W. O. Sampaio, R. Touyz, and M. J. Campagnole-Santos, “Angiotensin-(1–7) and its receptor as a potential target for new cardiovascular drugs,” Expert Opinion on Investigational Drugs, vol. 14, no. 8, pp. 1019–1031, 2005.

[83] P. Xu, A. C. Costa-Goncalves, M. Todiras et al., “Endothelial dysfunction and elevated blood pressure in Mas gene-deleted mice,” Hypertension, vol. 51, no. 2, pp. 574–580, 2008.

[84] L. A. Rabelo, N. Alenina, and M. Bader, “ACE2-angiotensin(1–7)-Mas axis and oxidative stress in cardiovascular disease,” Hypertension Research, vol. 34, no. 2, pp. 154–160, 2011.

[85] E. A. Tallant and M. A. Clark, “Molecular mechanisms of inhibition of vascular growth by angiotensin-(1–7),” Hypertension, vol. 42, no. 4, pp. 574–579, 2003.
[86] A. J. Ferreira, R. A. S. Santos, C. N. Bradford et al., “Therapeutic implications of the vasoprotective axis of the renin-angiotensin system in cardiovascular diseases,” Hypertension, vol. 55, no. 2, pp. 207–213, 2010.

[87] K. B. Brosnihan, P. Li, and C. M. Ferrario, “Angiotensin-(1–7) dilates canine coronary arteries through kinins and nitric oxide,” Hypertension, vol. 27, no. 3, pp. 523–528, 1996.

[88] I. Porsti, A. T. Bara, R. Busse, and M. Hecker, “Release of nitric oxide by angiotensin-(1–7) from porcine coronary endothelium: implications for a novel angiotensin receptor,” British Journal of Pharmacology, vol. 111, no. 3, pp. 652–664, 1994.

[89] K. Feterik, L. Smith, and Z. S. Katusic, “Angiotensin-(1–7) causes endothelium-dependent relaxation in canine middle cerebral artery,” Brain Research, vol. 873, no. 1, pp. 75–82, 2000.

[90] W. Meng and D. W. Busija, “Comparative effects of angiotensin-(1–7) and angiotensin II on piglet pial arterioles,” Stroke, vol. 24, no. 12, pp. 2041–2045, 1993.

[91] S. Y. Osei, R. S. Ahima, R. K. Minkes, J. P. Weaver, M. C. Khosla, and P. J. Kadowitz, “Differential responses to angiotensin-(1–7) in the feline mesenteric and hindquarters vascular beds,” European Journal of Pharmacology, vol. 234, no. 1, pp. 35–42, 1993.

[92] Y. Ren, J. L. Garvin, and O. A. Carretero, “Vasodilator action of angiotensin-(1–7) on isolated rabbit afferent arterioles,” Hypertension, vol. 39, no. 3, pp. 799–802, 2002.

[93] M. A. Oliveira, Z. B. Fortes, R. A. S. Santos, M. C. Kosla, and M. H. C. de Carvalho, “Synergistic effect of angiotensin-(1–7) on bradykinin arteriolar dilation in vivo,” Peptides, vol. 20, no. 10, pp. 1195–1201, 1999.

[94] L. Fernandes, Z. B. Fortes, D. Nigro, R. C. A. Tostes, R. A. S. Santos, and M. H. Catelli de Carvalho, “Potentiation of bradykinin by angiotensin-(1–7) on arterioles of spontaneously hypertensive rats studied in vivo,” Hypertension, vol. 37, no. 2, pp. 703–709, 2001.

[95] S. Sasaki, Y. Higashi, K. Nakagawa, H. Matsuura, G. Kajiyama, and T. Oshima, “Effects of angiotensin-(1–7) on forearm circulation in normotensive subjects and patients with essential hypertension,” Hypertension, vol. 38, no. 1, pp. 90–94, 2001.

[96] M. A. Bayorth, D. Eatman, M. Walton, R. R. Socci, M. Thierry-Palmer, and N. Emmett, “1A-779 attenuates angiotensin-(1–7) depressor response in salt-induced hypertensive rats,” Peptides, vol. 23, no. 1, pp. 57–64, 2002.

[97] P. Li, M. C. Chappell, C. M. Ferrario, and K. B. Brosnihan, “Angiotensin-(1–7) augments bradykinin-induced vasodilation by competing with ACE and releasing nitric oxide,” Hypertension, vol. 29, no. 1, pp. 394–400, 1997.

[98] P. E. Walters, T. A. Gaspari, and R. E. Widdop, “Angiotensin-(1–7) acts as a vasodilator agent via angiotensin II type 2 receptors in conscious rats,” Hypertension, vol. 45, no. 5, pp. 960–965, 2005.

[99] D. M. R. Silva, H. R. Vianna, S. F. Cortes, M. J. Campagnole-Santos, R. A. S. Santos, and V. S. Lemos, “Evidence for a new angiotensin-(1–7) receptor subtype in the aorta of Sprague-Dawley rats,” Peptides, vol. 28, no. 3, pp. 702–707, 2007.

[100] M. M. Muthalif, I. F. Benter, M. R. Uddin, J. L. Harper, and K. U. Malik, “Signal transduction mechanisms involved in angiotensin-(1–7)-stimulated arachidonic acid release and prostanooid synthesis in rabbit aortic smooth muscle cells,” Journal of Pharmacology and Experimental Therapeutics, vol. 284, no. 1, pp. 388–398, 1998.

[101] S. V. B. Pinheiro, A. C. Simões e Silva, W. O. Sampaio et al., “Nonpeptide AVE 0991 is an angiotensin-(1–7) receptor Mas agonist in the mouse kidney,” Hypertension, vol. 44, no. 4, pp. 490–496, 2004.

[102] R. Faria-Silva, F. V. Duarte, and R. A. Santos, “Short-term angiotensin-(1–7) receptor Mas stimulation improves endothelial function in normotensive rats,” Hypertension, vol. 46, no. 4, pp. 948–952, 2005.

[103] A. J. M. Roks, P. P. Van Geel, Y. M. Pinto et al., “Angiotensin-(1–7) is a modulator of the human renin-angiotensin system,” Hypertension, vol. 34, no. 2, pp. 296–301, 1999.

[104] J. Zhong, Z. M. Zhu, and Y. J. Yang, “Inhibition of PKC and ERK1/2 in cultured rat vascular smooth muscle cells by angiotensin-(1–7),” Acta Physiologica Sinica, vol. 53, no. 5, pp. 361–363, 2001.

[105] N. Hayashi, K. Yamamoto, M. Ohishi et al., “The counter-regulating role of ACE2 and ACE2-mediated angiotensin 1–7 signaling against angiotensin II stimulation in vascular cells,” Hypertension Research, vol. 33, no. 11, pp. 1182–1185, 2010.

[106] J. Zhang, N. A. Noble, W. A. Border, and Y. Huang, “Infusion of angiotensin-(1–7) reduces glomerulosclerosis through counteracting angiotensin II in experimental glomerulonephritis,” American Journal of Physiology—Renal Physiology, vol. 298, no. 3, pp. F579–F588, 2010.

[107] M. B. L. Carvalho, F. V. Duarte, R. Faria-Silva et al., “Evidence for Mas-mediated bradykinin potentiation by the angiotensin-(1–7) nonpeptide mimic AVE 0991 in normotensive rats,” Hypertension, vol. 50, no. 4, pp. 762–767, 2007.

[108] S. Ueda, S. Masumori-Maemoto, A. Wada, M. Ishii, K. B. Brosnihan, and S. Umemura, “Angiotensin-(1–7) potentiates bradykinin-induced vasodilation in man,” Journal of Hypertension, vol. 19, no. 11, pp. 2001–2009, 2001.

[109] C. V. Lima, R. D. Paula, F. L. Resende, M. C. Khosla, and R. A. S. Santos, “Potentiation of the hypotensive effect of bradykinin by short-term infusion of angiotensin-(1–7) in normotensive and hypertensive rats,” Hypertension, vol. 30, no. 3, pp. 542–548, 1997.

[110] A. P. Almeida, B. C. Frábregas, M. M. Madureira, R. J. S. Santos, M. J. Campagnole-Santos, and R. A. S. Santos, “Angiotensin-(1–7) potentiates the coronary vasodilatory effect of bradykinin in the isolated rat heart,” Brazilian Journal of Medical and Biological Research, vol. 33, no. 6, pp. 709–713, 2000.

[111] F. Lovren, Y. Pan, A. Quan et al., “Angiotensin converting enzyme-2 confers endothelial protection and attenuates atherosclerosis,” American Journal of Physiology—Heart and Circulatory Physiology, vol. 295, no. 4, pp. H1377–H1384, 2008.

[112] B. Rentzsch, M. Todiras, R. Iliescu et al., “Transgenic angiotensin-converting enzyme 2 overexpression in vessels of SHRSP rats reduces blood pressure and improves endothelial function,” Hypertension, vol. 52, no. 5, pp. 967–973, 2008.

[113] J. A. Hernández Prada, A. J. Ferreira, M. J. Katovich et al., “Structure-based identification of small-molecule angiotensin-converting enzyme 2 activators as novel antihypertensive agents,” Hypertension, vol. 51, no. 5, pp. 1312–1317, 2008.

[114] H. Ikram, A. H. Maslowski, and M. G. Nicholls, “Haemodynamic and hormonal effects of captopril in primary pulmonary hypertension,” British Heart Journal, vol. 48, no. 6, pp. 541–545, 1982.
[115] M. Ghazi-Khansari, A. Mohammadi-Karakan, M. Sotoudeh, P. Mokhtary, E. Pour-Esmaeli, and S. Maghsoud, “Antifibrotic effect of captopril and enalapril on paraquat-induced lung fibrosis in rats,” Journal of Applied Toxicology, vol. 27, no. 4, pp. 342–349, 2007.

[116] X. Li, M. Molina-Molina, A. Abdul-Hafez, V. Uhal, A. Xaubet, and B. D. Uhal, “Angiotensin converting enzyme-2 is protective but downregulated in human and experimental lung fibrosis,” American Journal of Physiology—Lung Cellular and Molecular Physiology, vol. 295, no. 1, pp. L178–L185, 2008.

[117] A. J. Ferreira, V. Shenoy, Y. Yamazato et al., “Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension,” American Journal of Respiratory and Critical Care Medicine, vol. 179, no. 11, pp. 1048–1054, 2009.

[118] Y. Yamazato, A. J. Ferreira, K.-H. Hong et al., “Prevention of pulmonary hypertension by angiotensin-converting enzyme 2 gene transfer,” Hypertension, vol. 54, no. 2, pp. 365–371, 2009.

[119] I. Hamming, W. Timens, M. L.C. Bulthuis, A. T. Lely, G. J. Navis, and H. van Goor, “Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis,” Journal of Pathology, vol. 203, no. 2, pp. 631–637, 2004.

[120] L. Baginski, G. Tachon, F. Falson, J. S. Patton, U. Bakowsky, and C. Ehrhardt, “Reverse Transcription Polymerase Chain Reaction (RT-PCR) analysis of proteolytic enzymes in cultures of human respiratory epithelial cells,” Journal of Aerosol Medicine and Pulmonary Drug Delivery, vol. 24, no. 2, pp. 89–101, 2011.

[121] B. Treml, N. Neu, A. Kleinsasser et al., “Recombinant angiotensin-converting enzyme 2 improves pulmonary blood flow and oxygenation in lipopolysaccharide-induced lung injury in piglets,” Critical Care Medicine, vol. 38, no. 2, pp. 596–601, 2010.

[122] B. D. Uhal, X. Li, A. Xue, X. Gao, and A. Abdul-Hafez, “Regulation of alveolar epithelial cell survival by the ACE-2/angiotensin 1–7/ Mas axis,” American Journal of Physiology—Lung Cellular and Molecular Physiology, vol. 301, no. 3, pp. L269–L274, 2011.

[123] Y. Feng, H. Wan, J. Liu et al., “The angiotensin-converting enzyme 2 in tumor growth and tumor-associated angiogenesis in non-small cell lung cancer,” Oncology Reports, vol. 23, no. 4, pp. 941–948, 2010.

[124] Y. Feng, L. Ni, H. Wan et al., “Overexpression of ACE2 produces antitumor effects via inhibition of angiogenesis and tumor cell invasion in vivo and in vitro,” Oncology Reports, vol. 26, no. 5, pp. 1157–1164, 2011.

[125] P. E. Gallagher and E. A. Tallant, “Inhibition of human lung cancer cell growth by angiotensin-(1–7),” Carcinogenesis, vol. 25, no. 11, pp. 2045–2052, 2004.

[126] J. Menon, D. R. Soto-Pantoja, M. E. Callahan et al., “Angiotensin-(1–7) inhibits growth of human lung adenocarcinoma xenografts in nude mice through a reduction in cyclooxygenase-2,” Cancer Research, vol. 67, no. 6, pp. 2809–2815, 2007.

[127] D. R. Soto-Pantoja, J. Menon, P. E. Gallagher, and E. A. Tallant, “Angiotensin-(1–7) inhibits tumor angiogenesis in human lung cancer xenografts with a reduction in vascular endothelial growth factor,” Molecular Cancer Therapeutics, vol. 8, no. 6, pp. 1676–1683, 2009.

[128] T. Unger, “The role of the renin-angiotensin system in the development of cardiovascular disease,” The American Journal of Cardiology, vol. 89, no. 2, pp. 3A–9A, 2002.

[129] I. Kucharewicz, R. Pawlak, T. Matys, D. Pawlak, and W. Buczko, “Antithrombotic effect of captopril and losartan is mediated by angiotensin-(1–7),” Hypertension, vol. 40, no. 5, pp. 774–779, 2002.

[130] S. N. Iyer, M. C. Chappell, D. B. Averill, D. I. Diz, and C. M. Ferrario, “Vasodepressor actions of angiotensin-(1–7) unmasked during combined treatment with lisinopril and losartan,” Hypertension, vol. 31, no. 2, pp. 699–705, 1998.

[131] J. P. Collister and M. D. Hendel, “The role of Ang (1–7) in mediating the chronic hypotensive effects of losartan in normal rats,” Journal of the Renin-Angiotensin-Aldosterone System, vol. 4, no. 3, pp. 176–179, 2003.

[132] K. Yamada, S. N. Iyer, M. C. Chappell, D. Ganten, and C. M. Ferrario, “Converting enzyme determines plasma clearance of angiotensin-(1–7),” Hypertension, vol. 32, no. 3, pp. 496–502, 1998.

[133] I. Lula, ˆA. L. Denadai, J. M. Resende et al., “Study of angiotensin-(1–7) vasoactive peptide and its β-cyclodextrin inclusion complexes: complete sequence-specific NMR assignments and structural studies,” Peptides, vol. 28, no. 11, pp. 2199–2210, 2007.

[134] K. Uekama, “Design and evaluation of cyclodextrin-based drug formulation,” Chemical and Pharmaceutical Bulletin, vol. 52, no. 8, pp. 900–915, 2004.

[135] R. A. Fraga-Silva, F. P. Costa-Fraga, N. Alenina et al., “An orally active formulation of angiotensin-(1–7) produces an antithrombotic effect,” Clinics, vol. 66, no. 5, pp. 837–841, 2011.

[136] F. D. Marques, A. J. Ferreira, R. Sinisterra et al., “An oral formulation of angiotensin-(1–7) produces cardioprotective effects in infarcted and isoproterenol-treated rats,” Hypertension, vol. 57, no. 3, pp. 477–483, 2011.

[137] N. M. Silva-Barcellos, S. Caligiorne, R. A. S. Santos, and F. Frézard, “Site-specific microinjection of liposomes into the brain for local infusion of a short-lived peptide,” Journal of Controlled Release, vol. 95, no. 2, pp. 301–307, 2004.

[138] N. M. Silva-Barcellos, F. Frézard, S. Caligiorne, and R. A. S. Santos, “Long-lasting cardiovascular effects of liposome-entrapped angiotensin-(1–7) at the rostral ventrolateral medulla,” Hypertension, vol. 38, no. 6, pp. 1266–1271, 2001.

[139] L. D. Kluskens, S. A. Nelemans, R. Rink et al., “Angiotensin-(1–7) with thioether bridge: an angiotensin-converting enzyme-resistant, potent angiotensin-(1–7) analog,” Journal of Pharmacology and Experimental Therapeutics, vol. 328, no. 3, pp. 849–855, 2009.

[140] V. S. Lemos, D. M.R. Silva, T. Walther, N. Alenina, M. Bader, and R. A. S. Santos, “The endothelium-dependent vasodilator effect of the nonpeptide Ang(1–7) mimic AVE 0991 is abolished in the aorta of Mas-knockout mice,” Journal of Cardiovascular Pharmacology, vol. 46, no. 3, pp. 274–279, 2005.

[141] G. Wiemer, L. W. Dobrucki, F. R. Louka, T. Malinski, and H. Heitsch, “AVE 0991, a nonpeptide mimic of the effects of angiotensin-(1–7) on the endothelium,” Hypertension, vol. 40, no. 6, pp. 847–852, 2002.

[142] A. J. Ferreira, B. A. Jacoby, C. A. A. Araújo et al., “The nonpeptide angiotensin-(1–7) receptor Mas agonist AVE-0991 attenuates heart failure induced by myocardial infarction,” American Journal of Physiology—Heart and Circulatory Physiology, vol. 292, no. 2, pp. H1113–H1119, 2007.
[143] A. J. Ferreira, T. L. Oliveira, M. C. M. Castro et al., “Iso-
proterenol-induced impairment of heart function and re-
modeling are attenuated by the nonpeptide angiotensin-(1–
7) analogue AVE 0991,” *Life Sciences*, vol. 81, no. 11, pp. 916–
923, 2007.

[144] R. Shemesh, A. Toporik, Z. Levine et al., “Discovery and
validation of novel peptide agonists for G-protein-coupled
receptors,” *Journal of Biological Chemistry*, vol. 283, no. 50,
pp. 34643–34649, 2008.

[145] R. A. Fraga-Silva, B. S. Sorg, M. Wankhede et al., “ACE2
activation promotes antithrombotic activity,” *Molecular
Medicine*, vol. 16, no. 5-6, pp. 210–215, 2010.