Therapeutic Perspectives of Angiotensin-(1-7) in the Treatment of Cardiovascular Disease

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Abstract: In the last decade, new biologically active components of the renin-angiotensin system were found. Angiotensin-(1-7) (Ang-(1-7)), a metabolite of angiotensin I and angiotensin II (Ang II), is considered the most pleiotropic component of the renin-angiotensin system, acting as a counterregulatory mediator of Ang II. Ang-(1-7) exerts beneficial effects on the cardiovascular system, including reduction of blood pressure, myocardial antihypertrofic and antifibrotic actions, and reversal of renal dysfunction, among others. Recent discovery of enzymatic pathways involved in Ang-(1-7) synthesis, such as the angiotensin-converting enzyme-2 (ACE2) and the existence of a specific receptor to this heptapeptide, the Mas receptor, have increased interest in the design of therapeutic strategies aimed at increasing the biological actions of Ang-(1-7). ACE inhibitors, AT_1 receptor blockers and aldosterone antagonists enhance Ang-(1-7) levels by different mechanisms. Actually, non-peptidic Ang-(1-7) agonists and ACE2 activators are under development and could have a role in the treatment of cardiovascular diseases.

The aim of the present review is to describe the biochemical and physiological actions of Ang-(1-7), the therapeutic strategies designed to enhance Ang-(1-7) activity focussing in their possible role and limitations in the treatment of cardiovascular disease.

Keywords: Angiotensin-(1-7), angiotensin-converting enzyme-2, cardiovascular disease, Mas receptor.

INTRODUCTION

The renin-angiotensin system (RAS) is a peptidergic system with endocrine characteristics. Angiotensinogen is released from the liver and cleaved by renin to the decapeptide angiotensin (Ang) I. Ang I is then activated to the octapeptide Ang II by angiotensin converting enzyme (ACE) [1]. This view of RAS has been expanded by further findings that demonstrate the existence of different Ang receptors (AT_1, AT_2, AT_4), signal transduction pathways and alternative metabolic cascades; and the discovery of additional truncated peptides with biological activity, such as Ang-(2-8) (Ang III), Ang-(3-8) (Ang IV), and Ang-(1-7) [1]. Ang-(1-7) have gained high attraction considering its counterregulatory effects with regards to Ang II actions, and the fact that Ang-(1-7) is formed from Ang II by the action of ACE2 [1]. More recently, the discovery of a specific binding site to Ang-(1-7), named Mas receptor, confirmed the relevance of Ang-(1-7) in the regulation of cardiovascular function [2, 3]. The G-protein-coupled receptor Mas was reported to act as a physiological antagonist of the Ang II type-1 receptor, considering that this receptor can hetero-oligomerize with AT_1 receptor, inhibiting Ang II actions [4].

Taking into account the importance of Ang-(1-7) in normal physiology and pathophysiology, the aim of the present review is to comment the main mechanisms of action,

physiological functions and the pharmacological perspectives of Ang-(1-7) modulation. Several works have previously reviewed the biochemical and physiological aspects of Ang-(1-7). Therefore, the present review will be focussed on pharmacological aspects of drugs acting at the ACE2-Ang-(1-7)-Mas receptor axis, emphasizing on the possible therapeutic role and limitations of strategies aimed at increasing endogenous Ang-(1-7) levels or Mas receptor activation.

ANGIOTENSIN-(1-7) METABOLIC PATHWAYS: ROLE OF ACE-2

Synthesis of Ang-(1-7) is regulated by three known enzymes, including neprilysin 24.11 (NEP), thimet oligopeptidase 24.15 (TOP), and prolyl oligopeptidase 21.26 (POP) [5] (Fig. **1**). Additionally, angiotensin-converting enzyme-2 (ACE2), which shares 42% sequence identity to the catalytic domain of ACE [5] but is insensitive to ACE inhibitors, mediates the conversion of Ang I into Ang-(1-9), as well as the conversion of Ang II into Ang-(1-7). Therefore, ACE2 regulates in a double fashion the production of Ang-(1-7): indirectly through the cleavage of Ang I into Ang-(1-9) (which can be further metabolized into Ang-(1-7) by ACE), and directly by means of the conversion of Ang II into Ang-(1-7) (Fig. **1**). Nevertheless, direct synthesis of Ang-(1-7) seems to be most kinetically favored, considering that ACE 2 shows a higher substrate preference for Ang II than for Ang I [6].

Ang-(1-7) has a short half-life (about 10 seconds in plasma) in rodent because of its peptidic nature [7]. However, half-life of Ang-(1-7) is approximately 29-30 min in humans [8]. ACE contributes to its metabolism, leading to the formation of the inactive metabolite Ang-(1-5) [9]. Alternative pathways are also involved in Ang-(1-7) degrada-

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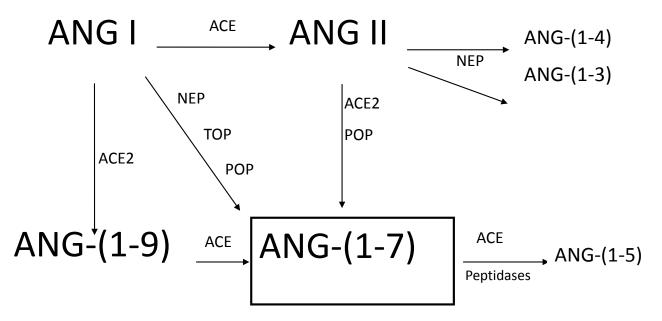
Fig. (1). Angiotensin-(1-7) synthesis and metabolism. ANG I: Angiotensin I; ANG II: Angiotensin II; ANG-(1-4): Angiotensin-(1-4); ANG-(1-3): Angiotensin-(1-3); ANG-(1-9): Angiotensin-(1-9); ANG-(1-7): Angiotensin-(1-7); ACE: Angiotensin Converting Enzyme; ACE2: Angiotensin Converting Enzyme 2; NEP: neprilysin 24.11; TOP: Thimet oligopeptidase 24.15; POP: Prolyl oligopeptidase 21.26. Adapted form ref. [3].

tion in different tissues, considering that NEP can hydrolyze the 4-tyrosine, 5-isoleucine (Tyr4-Ile5) bond of Ang-(1-7) to form Ang-(1-4) in the kidney [9].

PHARMACODYNAMIC FEATURES

The orphan G protein-coupled receptor Mas has been identified as the molecular target involved in physiological actions of Ang-(1-7) [2, 3, 10], considering that most effects of the heptapeptide can be blocked by its specific antagonist D-Ala^7-Ang-(1-7) [11]. More recently, Silva *et al.* reported pharmacological evidence for the existence of a different Ang-(1-7) receptor subtype mediating the vasodilator effect of the heptapeptide in the aorta from Sprague Dawley rats [12]. The vasodilator effect induced by the peptide is mediated by activation of Ang-(1-7) receptors sensitive to D-Pro^7-Ang-(1-7) but not to D-Ala^7-Ang-(1-7) and is dependent on endothelium derived nitric oxide (NO) [12].

Main signaling pathways involved in Ang-(1-7) responses by means of Mas receptor stimulation include PI3K-dependent Akt phosphorylation, leading to NO production by phosphorylation of NO synthase [13]; inhibition of mitogen-activated protein kinase (MAPK) phosphorylation [14]; stimulation of phospholipase A$_2$ activity and consequently release of arachidonic acid for prostanoid production [15]; and, finally, prostacyclin-mediated production of cAMP and activation of cAMP-dependent protein kinase.

Ang-(1-7) inhibits Ang II-mediated MAPK phosphorylation, and partly suppresses Ang II-stimulated increase in the profibrotic cytokine transforming growth factor-β$_1$ (TGF-β$_1$) [16]. In addition, Giani *et al.* [17] have recently postulated that Ang-(1-7) could be a positive physiological contributor

to the actions of insulin in heart, considering that Ang-(1-7) stimulated cardiac Akt phosphorylation, meanwhile acute *in vivo* insulin-induced cardiac Akt phosphorylation was inhibited by Ang II.

Another interaction was described between Ang-(1-7) and bradykinin, which would participate in the potentiation of bradykinin response after ACE inhibitor therapy. Although the exact mechanisms involved are not completely understood, it has been proposed that ACE inhibition, non-hydrolytic interaction with ACE favoring a crosstalk between ACE and bradykinin B$_2$ receptor and receptor Mas-mediated changes in the coupling and/or signaling of bradykinin [18] could be some of the mechanisms involved.

Although most of Ang-(1-7) effects seem to be mediated by Mas receptor activation, this heptapeptide is also able to interact with AT$_1$ and AT$_2$ receptors [19-24]. In the case of AT$_1$ and AT$_2$ receptors, the low affinity of Ang-(1-7) for them makes it difficult to conceive that Ang-(1-7) acts as a physiological agonist in normal conditions. However, experimental data confirm the ability of Ang-(1-7) to interact with AT$_1$ and AT$_2$ receptors when it is administered in high pharmacological doses [25].

PATHOPHYSIOLOGICAL ACTIONS OF ANGIOTENSIN-(1-7)

Ang-(1-7) regulates cardiovascular function in healthy subjects and cardiovascular disease due to its effects on different tissues (Fig. **2**). In many tissues Ang-(1-7) and G-protein-coupled receptor Mas exert counterregulatory actions with regard to Ang II, limiting the deleterious effects of the octapeptide (for review see [3]). The fact that Ang-(1-7)
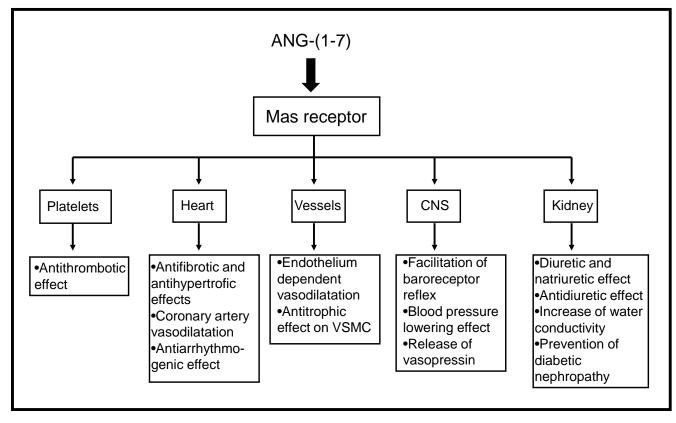
Fig. (2). Cardiovascular actions of angiotensin-(1-7). Abbreviations: CNS: central nervous system; VSMC: vascular smooth muscle cells.

cardiovascular effects are enhanced in experimental animals with cardiovascular diseases suggests a critical role for the heptapeptide in the development and maintenance of experimental hypertension and heart failure [3].

Although Ang-(1-7) cardiovascular effects have been extensively studied, the exact mechanism of action involved remains partially elusive. Ang-(1-7) exerts its cardiovascular effects mainly by means of Mas receptor activation; nevertheless, blockade of AT_1 and AT_2 receptors also prevents some actions of the heptapeptide, suggesting a crosstalk between the Mas and Ang II receptors [26]. In addition, enhancement of NO, bradykinin and prostacyclin (PGI_2) production is also involved in the mechanism of action of Ang-(1-7) effects (for review see [27]).

Ang-(1-7) exerts an antithrombotic effect due to stimulation of NO and PGI_2 release. The presence of Mas protein and specific binding for Ang-(1-7) were reported in rat and mouse platelets and the antithrombotic effect could be mediated by NO release from platelets [28]. This effect is enhanced in experimental models of cardiovascular disease, considering that the heptapeptide significantly reduced thrombus weight in 2K-1C hypertensive rats but not in normotensive animals [29]. Moreover, the antithrombotic effect of Ang-(1-7) was also blocked by an AT_1 antagonist.

At the vascular system, Ang-(1-7) shows a vasodilatador action, although the mechanism of action is highly dependent on the vascular bed and animal specie (for review see [1]). Vascular effects of Ang-(1-7) were also evaluated in normotensive and hypertensive patients showing conflicting results. Sasaki *et al.* [30] found that Ang-(1-7) induced relaxation in forearm circulation of both normotensive and hypertensive subjects through a mechanism independent of NO. Other authors show a biphasic effect of Ang-(1-7) in rat aorta [31]. Whilst low concentrations of the heptapeptide induced an endothelium dependent vasorelaxation, high doses (10^{-6}-10^{-5} M) exerted vasoconstriction through an AT_1 receptor dependent mechanism [31].

Regarding the mechanism of Ang-(1-7) vasoactive effect, several reports show that the vascular effect of Ang-(1-7) is endothelium dependent [18] due to increase in NO synthesis, prostaglandins or endothelium-derived relaxing factor (for review see [1]). In addition, vascular effects of the heptapeptide could also be a consequence of a complex interaction between Mas receptor and bradykinin B_2 and angiotensin AT_2. Peiró *et al.* [32] reported that BK-mediated NO release was increased by Ang-(1-7) and blunted by D-Ala^7-Ang-(1-7) pretreatment in primary human endothelial cell cultures.

Ang-(1-7) also regulates cardiovascular function by acting at the kidneys, producing complex renal effects [1]. The peptide can regulate fluid homeostasis through different mechanisms, including stimulation of vasopressin release and acting at different nephron segments with variable effects on sodium and water excretion. Several studies have demonstrated a diuretic and natriuretic effect of Ang-(1-7) in different experimental conditions [1]. Conversely, through an action at the distal nephron, Ang-(1-7) induces opposite effects generating a Mas receptor dependent antidiuretic effect in water-loaded rats [33]. In addition, the heptapeptide potently increases water conductivity in the inner medullary collecting duct [34] through a mechanism that involves an

interaction between Mas receptor and vasopressin V_2 receptor [35].

Although Ang-(1-7) exerts opposite effects on water and sodium excretion at different segments of the nephron, the antidiuretic effect of the heptapeptide seems to predominate, considering that application of Mas antagonists stimulates renal excretion of sodium and water [1].

It is a well known fact that blood pressure is regulated by the central nervous system mainly through the control of sympathetic outflow. Ang-(1-7) is generated in central nuclei related to blood pressure regulation, such as medullary areas and the hypothalamus [36, 37]. In normotensive rats, intracerebroventricular administration of the peptide does not modify blood pressure or heart rate, although it facilitates baroreceptor reflex control of heart rate [38]. Conversely, Ang-(1-7) induces a depressor response in situations involving overactivity of Ang II. Cerebroventricular infusion of Ang-(1-7) caused a significant decrease in blood pressure and injections of a specific Ang-(1-7) monoclonal antibody increased blood pressure and heart rate in [mRen-2]27 transgenic hypertensive (Tg+) rats but not in normotensive rats [39, 40]. In previous studies from our laboratory [25, 41], we found that Ang-(1-7) blocked the pressor response to intrahypothalamic administration of Ang II in spontaneously hypertensive and sinoaortic denervated rats without affecting the hypothalamic effects of Ang II in control normotensive animals.

Central effects of the peptide are also dose-dependent. Whilst the intrahypothalamic administration of a low dose of Ang-(1-7) (5 pmol) in sinoaortic denervated rats induced a reduction of blood pressure, a higher dose (50 pmol) was without effect [25]. Moreover, the higher dose of Ang-(1-7) causes a reduction in mean arterial pressure in the presence of the AT_1 antagonist, suggesting that Ang-(1-7) at high doses does not only bind to Mas receptor but also to AT_1 receptors [25].

In addition, Ang-(1-7) may also affect central regulation of blood pressure by changing vasopressin release. It was demonstrated that Ang-(1-7) stimulates vasopressin release with the same potency that Ang II does [42].

Several lines of evidence support a cardioprotective role of Ang-(1-7). Ang-(1-7) exerts antiarrhythmogenic, antifibrotic, antihypertrofic effects and vasodilatation in heart vasculature [1]. In addition, Ang-(1-7) endogenous levels are increased in cardiovascular diseases, including heart failure and acute myocardial infarction [1].

Coronary artery vasodilatation induced by Ang-(1-7) is dependent of bradykinin receptors and NO release [43, 44]. In other experiments, it was also demonstrated that the vasodilatory effect of the heptapeptide in perfused mouse hearts involved an interaction of Mas with AT_1 and AT_2 receptors [45]. However, higher concentrations of the peptide could exert opposite effects possibly by means of AT_1 receptors activation [46].

Ang II decreases conduction velocity and enhances the dispersion of action potential duration in the failing heart inducing cardiac arrhythmias [47]. Considering the counter-regulatory actions of Ang-(1-7), an antiarrhythmogenic effect of Ang-(1-7) has been postulated. Ferreira *et al*. [48]

have demonstrated that Ang-(1-7) perfusion reduced the incidence and duration of reperfusion arrhythmias after local ischemia induced by coronary ligation. The antiarrhythmic effect of low concentrations of Ang-(1-7) is mediated by Mas receptor activation and release of endogenous prostaglandins. The heptapeptide also induces hyperpolarization of heart cells and enhances conduction velocity in cardiomyophatic hamsters [49]. However, the authors also found that high concentrations of Ang-(1-7) induce proarrhythmic effects by increasing duration of the action potential and consequently generating early-after depolarizations [49]. Therefore, excessive generation of the heptapeptide in the heart could be harmful by means of increasing the incidence of proarrhythmia.

Ang-(1-7) also exerts cardioprotective effects through inhibition of cardiac hypertrophy and remodeling induced by different adverse stimuli. Different authors found that an increase in cardiac Ang-(1-7) levels prevents cardiac hypertrophy and fibrosis in chronically Ang II infused rats [50], spontaneously hypertensive rats [51] and deoxycorticosterone acetate (DOCA)-salt rat model [52]. Moreover, overproduction of Ang-(1-7) in a transgenic model in rats induces resistance to isoproterenol-induced cardiac hypertrophy [53]. In addition, these transgenic rats showed a reduced duration of reperfusion arrhytmias and an improved post ischemic function in isolated Langerdorff heart preparation [53].

It is also important to mention that antiremodeling effects of the heptapeptide are induced by activation of cardiac Mas receptor and are independent of its effects on blood pressure or Ang II receptors in the cardiac tissue [54]. Co-infusion of AngII and Ang-(1-7) in Sprague Dawley rats results in significant attenuation of myocyte hypertrophy and interstitial fibrosis, without significant effects on blood pressure [54].

Ang-(1-7) also prevents cardiac fibrosis, considering that the heptapeptide reduces collagen synthesis and decreases mRNA expression of growth factors in adult rat cardiac fibroblasts [55]. In addition, pretreatment with Ang-(1-7) inhibits Ang II profibrotic actions [55]. Effects of Ang-(1-7) on cardiac fibrosis are mediated by means of Mas receptor activation, considering that application of A-779 but not AT_1 or AT_2 reduced the affinity of the heptapeptide to adult rat cardiac fibroblasts [55]. On the other hand, the work of Mercure *et al*. [56] sustains that cardiac Ang-(1-7) selectively modulates some of the downstream signaling effectors of cardiac remodeling and the heptapeptide can reduce hypertension-induced cardiac remodeling through a direct effect on the heart.

Ang-(1-7) induces antitrophic effects on vascular smooth muscle cells of normotensive rats, possibly by means of increased release of prostaglandins [57], enhancing thereby production of cAMP and activation of cAMP-dependent protein kinase and by attenuation of MAPK activation [58]. Moreover, vascular ACE2 overexpression in spontaneously hypertensive stroke-prone rats reduces hypertension probably by locally degrading Ang II and improved endothelial function [59].

On the other hand, it was demonstrated that Ang-(1-7) reduces in a dose dependent manner the incorporation of [^{3}H]-thymidine, a biomarker of cellular proliferation, in vascular smooth muscle cells obtained from aorta induced by

different stimuli, including fetal bovine serum, platelet-derived growth factor (PDGF) and Ang II [60]. In addition, chronic administration of Ang-(1-7) diminishes neointimal formation after vascular injury without affecting medial area of the injured or the contralateral uninjured artery, and these effects occurred in the absence of changes in blood pressure [61]. Regarding the receptor involved in the antiproliferative actions of Ang-(1-7), reduced thymidine incorporation induced by Ang-(1-7) was not affected by pretreatment with AT_1 or AT_2 antagonists, but was prevented by application of [D-Ala^7]-Ang-(1-7), a Mas receptor blocker [57].

THERAPEUTIC PERSPECTIVES OF ANGIOTENSIN-(1-7)

As mentioned above, Ang-(1-7), at physiological or pharmacological concentrations, regulates cardiovascular function acting on several tissues. Ang-(1-7) also exerts antiproliferative actions that could be of interest in several cardiovascular diseases including heart failure and atherosclerosis. The potential therapeutic benefit of Ang-(1-7) is also supported by the fact that endogenous Ang-(1-7) levels or the effect of the heptapeptide are modified in pathological conditions. Moreover, Rodgers *et al.* [62] reported a Phase I/II dose escalation study of Ang-(1-7) administered before and after chemotherapy in patients with newly diagnosed breast cancer.

Preclinical studies have demonstrated the involvement of Ang-(1-7) in blood pressure regulation in different models of hypertension [25, 63-65]. Compromise of Ang-(1-7) in clinical hypertension was also suggested by the fact that urinary excretion of Ang-(1-7) is reduced in essential hypertensive patients with regard to normotensive subjects [66]. Moreover, urinary concentrations of Ang-(1-7) correlated inversely with systolic and diastolic blood pressure.

Beneficial effects of Ang-(1-7) in hypertension are not only related to blood pressure lowering effect, but also to reduction in target organ damage. Grobe *et al.* [52] have found that chronic infusion of Ang-(1-7) prevented collagen deposition in the DOCA-salt model of hypertension without affecting blood pressure and cardiac hypertrophy. The heptapeptide is also able to prevent cardiac remodeling induced by Ang II. Ang-(1-7) improves renal, cardiac, and vascular function in spontaneously hypertensive rats treated with NG-nitro-L-arginine methyl ester (L-NAME) and these effects were similar to many of the beneficial effects of RAS blockade with an ACE inhibitor [64].

Ang-(1-7) also seems to play a protective role during acute myocardial infarction, considering that chronic infusion of the heptapeptide reduces arrhythmias [48] and attenuates development of heart failure after myocardial infarction in rats [67].

Indirect evidence that also supports a role for Ang-(1-7) in cardiovascular pathologies is the fact that ACE2-deficient mice develop cardiomyopathy associated with increased oxidative stress and inflammation, as well as MAPK and PI3K activation [68]. Moreover, Burrell *et al.* [69] have demonstrated the existence of an increase in ACE2 after myocardial infarction, suggesting that the enzyme plays an important role in the negative modulation of the RAS after cardiac injury.

Clinical studies also indirectly show a protective role of Ang-(1-7) in heart failure. Activity of NEP and ACE2 were increased in failing human heart ventricles, suggesting that enhanced Ang-(1-7) cardiac levels could exert a cardioprotective effect in heart failure [70].

Ang-(1-7) may also have a therapeutic role in the treatment of diabetic complications. Benter *et al.* [71] have demonstrated that Ang-(1-7) prevents cardiovascular dysfunction in streptozotocin-treated rats, an experimental model of diabetes, suggesting that activation of Ang-(1-7) signal transduction could be an important therapeutic strategy to reduce cardiovascular events in diabetic patients. Diabetic nephropathy could also be reduced by activation of Mas receptor. For instance, Ang-(1-7) or its receptor agonist AVE 0991 attenuates proteinuria and improves renal vascular activity in the diabetic rat [71].

More indirect evidences demonstrated that renal ACE2 is reduced in the proximal tubules of the streptozotocin-induced model of type-1 diabetes, and the attenuation of renal injury by ACE inhibition is associated with increased ACE2 expression [72]. Moreover, chronic ACE2 inhibition in the diabetic db/db mice exacerbates the extent of albuminuria in a 3-fold manner [73].

Enhancement of ACE2-Ang-(1-7)-Mas axis seems to be also attractive to overcome insulin resistance, considering that Mas knockout (Mas-/-) mice exhibit many common features of metabolic syndrome [10]. Moreover, recently we have demonstrated that chronic Ang-(1-7) infusion ameliorates insulin resistance in fructose fed rats, an experimental model of metabolic syndrome, through a mechanism that could involve modulation of insulin signaling [74].

On the other hand, other effects were also reported for possible therapeutic perspectives. A role of Ang-(1-7) was also demonstrated in the prevention of certain types of cancer [75], treatment of chemotherapy-induced cytopenias [76] and dermal repair [77]. Moreover, a role of ACE2 in severe acute respiratory syndrome coronavirus (SARS-CoV) induced lung injury in mice. ACE2 could be SARS-CoV receptor *in vivo* and injection of SARS-CoV spike into mice worsens acute lung failure which can be attenuated by blocking the renin-angiotensin pathway [78, 79].

THERAPEUTIC STRATEGIES TO ENHANCE ENDOGENOUS ANG-(1-7) LEVELS OR MAS RECEPTOR ACTIVATION

Taking into account the beneficial effects of Ang-(1-7) and the involvement of the peptide in the pathophysiological state of several diseases, enhancement of the ACE2-Ang-(1-7)-Mas axis could be a novel therapeutic strategy in the treatment of cardiovascular diseases. It is known that ACE2 activity, in an Ang-(1-7)-dependent fashion, improves homeosthasis through a mechanism that may involve attenuation of NADPHox-induced reactive oxygen species production. Therefore, enhancement of ACE2 activity may be an interesting approach to limit aberrant vascular responses and atherothrombosis [80].

From a pharmacological perspective, Mas receptor activation could be enhanced either by strategies aimed at increasing endogenous levels of Ang-(1-7) or by the systemic administration of Mas receptor agonists (Fig. **3**).
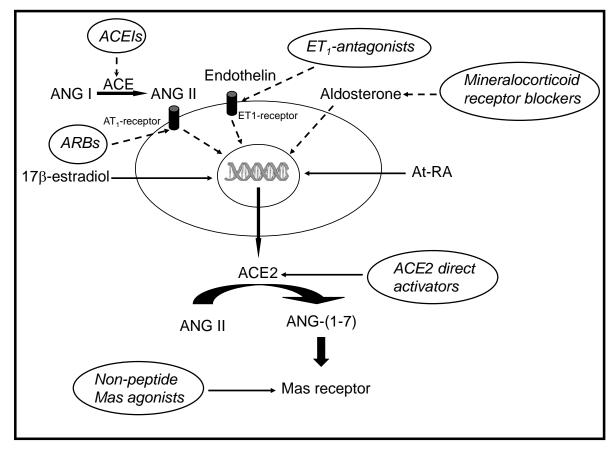
Fig. (3). Pharmacological modulation of the ACE2-ANG-(1-7)-Mas receptor axis. Continuous and dotted line represent a stimulatory and inhibitory action, respectively. Abbreviations: ACE: angiotensin-converting enzyme; ACEIs: angiotensin-converting enzyme inhibitors; ARBs: AT1-receptor blockers; At-RA: All-trans retinoic acid.

Considering the pivotal role of ACE2 in Ang-(1-7) bio-synthesis, an interesting therapeutic approach to enhance Mas receptor stimulation is the upregulation of this enzyme. Expression and activity of ACE2 are regulated by several endogenous factors. Cytokines, like interferon γ and inter-leukin 4 [81], Ang II [82], aldosterone [83, 84] and endo-thelin-1 [82] reduce ACE2 expression by a genomic effect. Ang II-induced downregulation of ACE2 is mediated by AT_1 receptor stimulation. Gallagher *et al.* [82] have demonstrated that both Ang II and endothelin-1 downregulates ACE2 ex-pression by a mechanism involving activation of the MAP kinases (ERK1 and/or ERK2).

Conversely, all-trans retinoic acid and 17β-estradiol have been found to up-regulate ACE2 protein and gene expression [85, 86]. In addition, Ang-(1-7) and atrial natriuretic factor have been shown to prevent Ang II-induced downregulation of ACE2 [82].

Considering regulation of ACE2 activity, several thera-peutic groups, including ACE inhibitors, AT_1 receptor blockers, vasopeptidase inhibitors and mineralocorticoid receptor blockers, are able to increase endogenous Ang-(1-7) levels. ACE inhibitors increase endogenous levels of Ang-(1-7) by 25-fold through different mechanism [15], including increase of Ang-(1-7) synthesis by upregulation of ACE2 and greater disposition of precursors, and decrease of Ang-(1-7) degradation [87]. Several works demonstrate that en-hancement of endogenous Ang-(1-7) by ACE inhibitors is involved, at least in part, in several beneficial effects of this therapeutic group, including blood pressure lowering effect [25, 88-90], antithrombotic effect [91], and baroreflex im-provement [92], among others. Conversely, van der Wouden *et al.* [93] have found that Ang-(1-7) plays no major role in the anti-proteinuric and blood pressure lowering effects of ACE inhibitors in an experimental model of nephrosis.

Similarly to ACE inhibitors, AT_1 receptor antagonists also enhance Ang-(1-7) endogenous levels by as much as 25-fold [9]. For this therapeutic group, Ang-(1-7) increment could be explained by the fact that Ang II affinity for AT_1 receptors is 1000 times higher than its affinity to ACE2. Therefore, after blockade of AT_1 receptors, Ang II would accumulate and be converted to Ang-(1-7) by ACE2. An-other explanation is the fact that AT_1 receptor antagonists induce overexpression and increased activity of ACE2 [15]. As described for ACE inhibitors, Ang-(1-7) also participates partially in the blood pressure lowering effect of AT_1 recep-tor antagonists [89].

It is important to mention that enhancement of Ang-(1-7) levels also seems to contribute to the beneficial effects of aldosterone antagonists, such as spironolactone, in patients with heart failure. For instance, Keidar *et al.* [83] showed that chronic treatment of heart failure with spironolactone dramatically enhances ACE2 activity and its mRNA expres-sion by 300% and 654%, respectively.

In addition, Choudhary *et al.* [94] have described that all-trans retinoic acid prevents development of cardiac remodeling in aortic banded rats by upregulation of expression of ACE2 and through inhibition of the expression of cardiac and renal renin, angiotensinogen, ACE, and AT_1 receptor.

More recently, direct inhibitors and activators of ACE2 have been developed. Although the inhibitor of ACE2, MLN-4760, has no therapeutic role, it has been used to define the role of the enzyme in diabetic nephropathy, considering that 16-week administration of the inhibitor worsened albuminuria in db/db mice [95]. Hernandez Prada *et al.* [96], by *in silico* approach, selected a xanthenone (1-[[2-(dimethylamino) ethyl]amino]-4-(hydroxymethyl)-7-[[(4-methylphenyl) sulfonyl] oxy] 9H-xanthen-9-one) that is able to enhance ACE2 activity in a dose dependent manner. Administration of this compound in spontaneously hypertensive rats reduces blood pressure and reverses cardiac and renal fibrosis [96]. In addition, chronic administration of the xanthenone prevents pulmonary hypertension and right ventricular hypertrophy induced by monocrotaline injection [97].

Therapeutic use of Ang-(1-7) is theoretically precluded considering its short plasma half-life in rodents (about 10 min) by a rapid proteolysis due to ACE activity and its poor bioavailability and absorption by oral or topical routes, considering its peptidergic nature and its high water-solubility [7]. However, the plasma half-life is approximately 30 minutes in humans [8]. Promising strategies have been proposed to increase the therapeutic potential of Ang-(1-7), including the use of liposomes as sustained and controlled release systems and cyclodextrins to improve peptide bioavailability by oral route [98]. Santos *et al.* [98] have demonstrated that inclusion of Ang-(1-7) in liposomes prolongs its biological activity by preventing its degradation and by the slow release of Ang-(1-7) from the liposome. However, it is important to mention that this strategy to improve Ang-(1-7) half-life has only been assessed after intracerebroventricular administration. Therefore, further studies are needed to establish the ability of liposome for sustained liberation of the peptide after systemic administration.

The design of non-peptidic receptor Mas agonists is probably the most attractive therapeutic strategy in order to enhance the beneficial physiological actions of the heptapeptide. In 2001, Aventis Pharma designed the first nonpeptide and orally active analog of Ang-(1-7), AVE 0991 (5-Formyl-4-methoxy-2-phenyl-1-[[4-[2-(ethylaminocarbonylsulfonamido)-5-isobutyl-3-thienyl]-phenyl]-methyl]-imidazole). AVE 0991 has a 10-fold higher affinity for Mas receptor than Ang-(1-7) [73]. Although the pharmacokinetic properties of this new chemical entity have not been studied, it is well known that AVE 0991 mimics Ang-(1-7) effects in several organs [99]. AVE 0991 evokes similar effects with regard to Ang-(1-7) in cultured bovine aortic endothelial cells [100], kidneys [101], and vessels [102, 103]. In addition, Benter *et al.* [64] showed that chronic administration of Ang-(1-7) or its synthetic analog, AVE 0991, significantly improved the ischemia-reperfusion recovery in isolated perfused spontaneously hypertensive rat hearts treated with L-NAME. More recently, Ferreira *et al.* [104] have demonstrated the ability of AVE 0991 to attenuate post ischemic heart failure in a model of myocardial infarction induced by left coronary artery ligation. In addition, AVE 0991 also exerts a cardioprotective

role on cardiac remodeling induced by cardiac β-adrenoceptor overstimulation [105]. Cardiac beneficial effects of AVE 0991 were also demonstrated in a preclinical model of type I diabetes mellitus. Chronic administration of the agonist prevented diabetic rats from severe systolic dysfunction without affecting glycemia [106]. Pharmacological actions of AVE 0991 are resumed in Table **1**.

Table 1.   Pharmacological Effects of the Non-Peptide Mas Receptor Agonist AVE 0991

Alteration of circadian rhythm of blood pressure
Antidiuresis
Attenuation of cardiac remodeling
Blood pressure lowering effect
Endothelium dependent vasodilatation
Improvement of endothelial function
Improvement of baroreflex sensitivity
Prevention of diabetic-induced cardiovascular dysfunction
Prevention of diabetic cardiomyopathy

LIMITATIONS

Although a large amount of evidence, mainly from preclinical studies, supports a possible therapeutic role of drugs aimed at increasing activity of ACE2-Ang-(1-7)-Mas axis in the treatment of hypertension, diabetes, heart failure, among other cardiovascular diseases, there are several factors that could limit this new therapeutic strategy.

In first place, as mentioned above, most physiological actions of Ang-(1-7) are biphasic, and, consequently, higher doses of the heptapeptide would produce activation of AT_1 receptor, probably reducing the beneficial effects of Mas receptor stimulation. Therefore, there is a need to clearly define the most adequate dosing of receptor Mas agonists in different clinical situations. Moreover, to the best our knowledge, selectivity of the non-peptide AVE 0991 for Mas receptor is actually unknown.

In second place, further research is needed to completely characterize Ang-(1-7) pharmacodynamics. Although identification of Mas receptor as cellular target of the heptapeptide greatly improves knowledge of physiological actions of Ang-(1-7), it is important to mention that some effects of Ang-(1-7) seem to be mediated by multiple receptors pathways and also blocked by administration of antagonists of Ang II and bradykinin receptors [3]. Therefore, concerns arise regarding the advantage of Mas receptor agonists in patients under treatment with AT_1 receptor blockers.

Strategies aimed at increasing ACE2 activity to enhance Ang-(1-7) biosynthesis suffer from potential therapeutic limitations. Although Ang II is considered to be the main substrate for ACE2, this enzyme is also involved in the degradation of several other peptides, including apelin-13, neurotensin, kinestensin, dynorphin and bradykinin fragments [107]. Increased metabolism of dynorphin could be related to adverse effect such as hyperalgesia, considering the role of this peptide in central and peripheral regulation of pain [108]. In addition, neurotensin exerts brain actions as a pri-

mary neurotransmitter or neuromodulator of classical neurotransmitters [109], and therefore administration of ACE2 activators could induce neurological adverse effects. Moreover, ACE2 also serves as the cellular entry point for the severe acute respiratory syndrome (SARS) virus [110], and therefore there are strong concerns regarding the safety of ACE2 up regulation.

In addition, we doubt that enhancers of Ang-(1-7)-Mas axis could potentiate beneficial effects of well established cardiovascular therapies. In this way, ACE inhibitors, AT_1 receptor antagonists and aldosterone antagonists have been used along several years in the treatment of different cardiovascular diseases, including hypertension and chronic heart failure [111, 112]. Moreover, these therapeutic groups have demonstrated to reduce mortality associated to chronic heart failure in several controlled clinical trials and are recommended as first-line therapy in different stages of heart failure [112]. As previously mentioned, it has been shown that ACE inhibitors, AT_1 receptor antagonists and aldosterone antagonists are able to increase ACE2 activity and/or Ang-(1-7) levels and several lines of evidence have demonstrated that enhancement of the heptapeptide endogenous levels is partially involved in the beneficial effects of ACE inhibitors and AT_1 receptor antagonists [25, 88-92].

In addition, beneficial effects of ACE inhibitors are also mediated through an increment of bradykinin, PGI_2 and an enhancement of NO disposition [113]. As previously mentioned, most physiological actions of Ang-(1-7) partially involve these endogenous compounds.

Considering these aspects, it is improbable that exogenous administration of Ang-(1-7) agonists or increase of endogenous levels of the heptapeptide by means of direct ACE2 activation could increase cardiovascular beneficial effects in patients treated with traditional drugs acting on the RAS, considering that these strategies share similar mechanism of actions.

CONCLUSIONS

Nowadays, several investigational lines have clearly demonstrated a protective role of Ang-(1-7) on the cardiovascular system. Increasing endogenous Ang-(1-7) levels limits the deleterious effect of Ang II through activation of the Mas receptor and by up regulating the action of several cellular mediators, including NO, prostaglandins and bradykinin, among others. In addition, Mas receptor activation seems to regulate the activity of Ang II receptors, such as AT_1 and AT_2 receptors, considering that several beneficial effects of Ang-(1-7) are prevented after blocked of these receptors. Activation of Mas receptor, either by enhancement of endogenous Ang-(1-7) concentrations or by application of a Mas receptor agonist, could have a therapeutic role in the treatment of several cardiovascular diseases.

Although several lines of evidence have clearly demonstrated that enhancement of Ang-(1-7) endogenous levels contributes to the beneficial effect of established RAS blockers, including ACEIs, AT_1 receptor antagonists and aldosterone receptor blockers, the therapeutic role of specific agents aimed to increase Mas receptor activation, such as ACE2 modulators or non-peptide Mas receptor agonists, has not been established yet.

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