REGULATION OF VASCULAR REMODELING BY ANGIOTENSIN-(1-9)

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ABSTRACT

The renin angiotensin system (RAS) is involved in the regulation of vascular remodeling induced by hypertension, vascular injury and inflammation. Angiotensin II (Ang II) is the most important peptide of the classical RAS pathway. Ang II exerts its biological action by binding to AT1 (AT1R) and AT2 (AT2R) receptors. The discovery of the homologous angiotensin I converting enzyme (ACE2), unmasked an intricated system of RAS regulation. In the counter-regulatory RAS pathway, Ang-(1-7) binds and activates Mas receptor (MasR), while Ang-(1-9) binds and activates AT2R. This review is focused in the inhibitory effects on vascular remodeling of ACE2, Ang-(1-9) and AT2R. Because of these vascular effects, the manipulation of the counter regulatory RAS emerges as a suitable therapeutical alternative to treat vascular diseases.
Keywords: ACE2, Ang-(1-9), AT2R, vascular remodeling, VSMC

RESUMEN

El sistema renina angiotensina (SRA) está involucrado en la regulación del remodelado vascular inducido por hipertensión, daño vascular e inflamación. La angiotensina II (Ang II) es el péptido más importante de la vía SRA clásica. Ang II ejerce su acción biológica al unirse a los receptores AT1 (RAT1) y AT2 (RAT2). El descubrimiento de la enzima convertidora de angiotensina I homóloga (ECA2), desenmascaró un intrincado sistema de regulación del SRA. En la vía SRA contrarreguladora, Ang-(1-7) se une y activa el receptor Mas (RMas), mientras que Ang-(1-9) se une y activa RAT2. Esta revisión se centra en los efectos inhibitorios sobre el remodelado vascular de ECA2, Ang-(1-9) y RAT2. Debido a estos efectos vasculares, la manipulación del SRA contrarregulador surge como una alternativa terapéutica adecuada para tratar enfermedades vasculares.
Palabras claves: ECA2, Ang-(1-9), RAT2. Remodelado vascular, CMLV

Introduction

Vascular smooth-muscle cells (VSMCs) are the main component of the artery's medial layer. These cells contract and thereby regulate blood vessel tone and consequently blood flow and pressure [1,2]. Pathological vascular remodeling is characterized by the narrowing of the vessel lumen, thickening of muscle intima layer, exacerbated extracellular matrix (ECM) production (fibrosis) and infiltration by immune cells [3,4]. In these diseases, changes in the VSMC phenotype have been extensively described [1]. In addition to increases on proliferation and migration rates, VSMC phenotypic switching also includes altered expression of contractile proteins, increased ECM component production, expression of pro-inflammatory cytokines and production of proteases [1]. Deregulation of neurohormonal mechanisms, including the renin–angiotensin system (RAS), which modulates endothelial and smooth muscle function and, ultimately, vessel wall structure, is involved in the genesis and progression of vascular diseases [2,3].
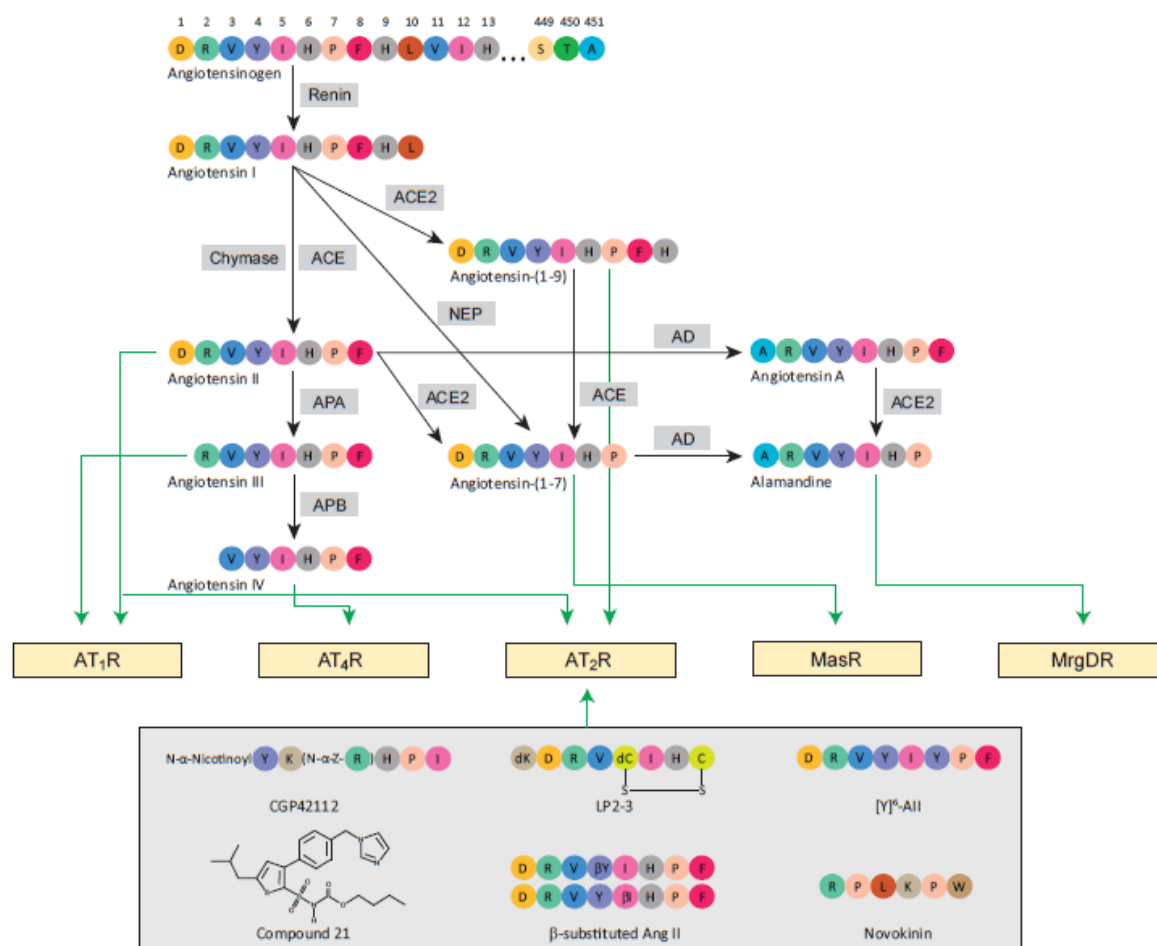
Renin angiotensin system (RAS)

RAS is a well-known regulator of blood pressure by controlling both salt and water homeostasis [4]. Moreover, the RAS is also involved in the vascular response to injury and inflammation [2,4]. Chronic activation of RAS leads to hypertension and triggers in vessels and other organs proinflammatory, prothrombotic, and atherogenic effects which are responsible for the hypertension-associated end-organ damage [4].

The classic RAS pathway (Figure 1) begins with angiotensinogen synthetized in the liver, which is cleaved by renin (EC 3.4.23.15), a protease released from the juxtaglomerular cells at the renal afferent arterioles after a decrease in Na^+ and/or Cl^- concentration in the glomerular ultrafiltrate at this level, or by adrenergic stimulation [3]. As result, a 10 amino acid peptide, angiotensin I (Ang I) is produced [5]. A zinc-dependent dicarboxypeptidase, the angiotensin converting enzyme I (ACE; EC 3.4.15.1), removes the last two amino acids from the carboxyl terminal of Ang I, generating the 8 amino acid peptide, Ang II. Ang II is also enzymatically generated from Ang I by chymase (EC 3.4.21.39) [5].

Ang II is the most important peptide of the classical pathway [4,5]. Ang II exerts its biological action by binding to G protein coupled receptors AT1 (AT1R) and AT2 (AT2R) [3,4]. The activation of AT1R by Ang II on VSMCs triggers vasoconstriction, on adrenal cortex stimulates aldosterone secretion, and on renal proximal tubules induces sodium reabsorption. AT1R activation also stimulates sympathetic neurotransmission [3,4]. In addition, signal transduction triggered by AT1R on VSMCs promotes NADPH and nuclear transcription factor−κB (NF-κB), eliciting oxidative stress, inflammation, and cell proliferation [2]. On the other hand, the activation of AT2R by Ang II generates opposite effects to those described for AT1R. AT2R activation induces vasodilation, normal endothelial function, natriuresis, anti-proliferative, anti-inflammatory and antifibrotic effects [6].

However, the physiological implications of this system have continued to expand over time [5]. In the classical RAS, new peptides have been described, including Ang III, Ang IV and Ang-(1-12) (Figure 1). Ang III is synthetized through the action of aminopeptidase A (EC 3.4.11.7) on Ang II. These 7 amino acids long peptide activates AT1R and AT2R and appears to be the main peptide involved in vasopressin and atrial natriuretic peptide release [7]. However, no direct action of Ang III on VSMCs has been yet described.

Figure 1. Classical and counter-regulatory renin-angiotensin pathways. In the classical system, renin cleaves angiotensinogen to produce angiotensin I (Ang I). Ang I is cleaved by angiotensin-converting enzyme (ACE) or chymase to form Ang II, which can act through the activation of the type-1 Ang II receptor (AT1R) and type-2 Ang II receptor (AT2R). Ang II can be further proteolyzed by *aminopeptidase A* (APA) to form Ang III, which also acts through AT1R. Moreover, Ang III can be cleaved by *aminopeptidase B* (APB) to generate Ang IV, which binds to AT4R. Ang I can also be cleaved by angiotensin-converting enzyme 2 (ACE2) and *neutral endopeptidase* (NEP) to produce Ang-(1–9) and Ang-(1–7), respectively. Ang-(1–9) activates AT2R. Ang-(1–7) binds to the Mas receptor (MasR). Ang-(1–7) can also be formed from cleavage of Ang II by ACE2 and be further metabolized to alamandine. Alternatively, Ang II may be processed by aspartate decarboxylase (AD) to produce Ang A, which can be converted to alamandine by ACE2. Alamandine binds and activates *the Mas-related G-protein coupled receptor (MrgDR)*.

Ang IV is generated from Ang III by aminopeptidase B (EC 3.4.11.6) [8]. Ang IV binds to AT4R, which has been identified as the transmembrane enzyme, insulin-regulated membrane aminopeptidase (IRAP) [9]. In VSMC, Ang IV is involved in vascular inflammation because it activates NF-κB and up-regulates the monocyte chemokine monocyte chemoattractant protein-1, intercellular adhesion molecule-1, interleukin 6 and tumor necrosis factor alpha [10].

Ang-(1-12) functions as an intracrine/paracrine substrate for local production of Ang II and therefore it is responsible for the locally produced Ang II-dependent actions. Ang II generation from Ang-(1-12) does not require renin and ACE but chymase [11]. Ang-(1-12) binds to AT1R and causes vasoconstriction, accumulation of inflammatory markers in the sub-endothelial region of blood vessels and activates VSMC proliferation [12].

Angiotensin A (Ang A) is an octapeptide highly similar to Ang II, only differing in the N-terminal in which the Asp1 is decarboxylated into Ala1 [13]. This peptide has the same affinity for the AT1R and AT2R as Ang II [13]. This peptide can be hydrolyzed by ACE2 to form the heptapeptide alamandine. Alamandine can also be produced through decarboxylation of Asp1 into Ala1 of Ang-(1–7) [14]. Alamandine acts through the activation of MgrD receptor [14].

ACE2 and vascular remodelling

The discovery of the homologous angiotensin I converting enzyme (ACE2; EC 3.4.17.23), added a greater complexity to the classical RAS axis [5]. The current evidence suggests that this new axis has counter-regulatory effects to the classical RAS [5]. ACE2 differs from ACE both in substrate specificity and in function. ACE2 is a zinc-dependent monocarboxypeptidase which is 40% similar to ACE. ACE2 produces Ang-(1-9) and Ang-(1-7) from the hydrolysis of Ang I and Ang II, respectively. Moreover, Ang-(1-7) can also be synthetized from Ang-(1-9) through the action of ACE [5]. Ang-(1-7) can be converted to Ang-(1-5) by ACE or by neutral endopeptidase (neprilysin; EC 3.4.24.11) [5]. Ang-(1-7) binds to and activates Mas receptor (MasR), while Ang-(1-9) binds to and activates AT2R [5].

ACE2 is distributed in most tissues but with higher expression in heart, kidney, nasal mucosa, lung, small intestine, brain, and testis; while ACE is mainly expressed in the endothelium throughout the vasculature [15]. Both are plasma membrane bound enzymes that can be released into the circulation by shedding [16]. A dysregulation in this system, by increasing the classical pathway or by decreasing the counter-regulatory axis, can contribute significantly to the pathophysiology of cardiovascular diseases [5].

ACE2 is expressed in VSMC, and vascular injury induces the decrease of ACE2 mRNA and protein, without changes in ACE. Moreover, the neointimal formation induced by vascular injury is further increased in ACE2 knockout mice [17]. In spontaneously hypertensive stroke-prone rats (SHRSP), the transgenic overexpression of human ACE2 in VSMC reduces blood pressure and improves endothelial function [18]. Conversely, in ACE2 knockout aorta, an increased vascular remodelling and fibrosis is observed in response to chronic subcutaneous Ang II injection [19].

In an atherosclerosis mice model, ACE2 deficiency resulted in significantly larger vascular lesions in both aortic atherosclerotic plaques and arterial neointima formation [20]. Additionally, in this model, the inhibition of ACE2 with MLN-4760 also resulted in an increased vascular inflammation and atherosclerotic plaque formation [21]. On the other hand, the overexpression of ACE2 in mice and rabbit atherosclerosis models inhibits the evolution of atherosclerotic plaques [22,23].

All data obtained so far, indicate that the increase of ACE2 in VSMC is associated with a decrease in vascular remodelling and confers protection against several vascular diseases, including hypertension and atherosclerosis. Strategies, such as the administration of Ang receptor blockers (ARB), increases vascular ACE2 [24], suggesting that beneficial vascular effect of these drugs could be mediated by ACE2 induction. Another strategy is the administration of recombinant human soluble ACE2 (rhACE2). In a phase 1 clinical trial, it was shown that administration of rhACE2 in doses of 100-1,200 µg/kg is well tolerated by healthy human subjects. rhACE2 showed a dose-independent terminal elimination half-life in the range of 10 h. Moreover, despite marked changes in angiotensin system peptide concentrations, cardiovascular effects were absent, suggesting the presence of effective compensatory mechanisms in healthy volunteers [25]. This therapeutical approach was also explored as a potential therapy to treat human pulmonary arterial hypertension. In a phase 1 clinical trial with 5 patients, rhACE2 was well tolerated, associated with improved pulmonary haemodynamic and reduced markers of oxidant and inflammatory mediators. Unfortunately, no assessment of pulmonary artery remodelling parameters was performed [26]. Further strategies should be explored to confirm that activation of VSMC ACE2 could be a valid therapeutical strategy to treat vascular diseases.

Ang-(1-9) and vascular remodelling

Administration of Ang-(1-9) with osmotic minipumps shows a dose response reduction on blood pressure associated with a decrease in vascular remodeling in SHR [27]. Reduction of vascular remodeling induced by Ang-(1-9) is also described in other models of hypertension, including the Ang II infusion pump [28] and the DOCA-salt hypertensive model [29]. In these models, Ang-(1-9) infusion also decreases aortic intima-media thickness [28,29]. In a pulmonary arterial hypertension crotaline model, Ang-(1-9) reduces right ventricular weight and systolic pressure. It also diminishes proinflammatory cytokines levels and attenuates fibrosis and endothelial damage [30]. In VSMC, Ang-(1-9) decreases proliferation, migration and contractile protein decrease induced by platelet derived growth factor-BB. These effects are mediated by an Ang-(1-9)/AT2R-dependent reduction

of Akt phosphorylation that activates FoxO1 transcription factor [27]. Taking together, all these data suggest that Ang-(1-9) has a direct action of VSMCs inhibiting its dedifferentiation. This effect could be a novel target to be exploited as a treatment for vascular diseases.

AT2R and vascular remodeling

Ang-(1-9), Ang II, and Ang III are described as the endogenous ligand of AT2R [5]. However, several other AT2R agonist have been described. CGP42112, the first available synthetic AT2R agonist, behaves as a full agonist both *in vitro* and *in vivo* [31], although it has been criticized for the lack of selectivity [32]. Other synthetic AT2R agonist are Compound 21 (C21), a nonpeptide small molecule agonist (Vicore Pharma, Gothenburg, Sweden) [33]; LP2-3, a cyclic Ang II derivative (Lanthio Pharma, Groningen, the Netherlands) [34]; several Ang II based peptides obtained by single beta-amino acid substitution [35]; [Y]6-AII, another Ang II-derived peptide [36], and Novokinin, a small peptide that was designed based on ovokinin, a vasorelaxing peptide present in egg albumin [37].

AT2R activation induces vasodilation in several arteries, including coronary, cerebral, mesenteric, uterine, and renal arteries [38]. However, to unmask the vasodilator action of AT2R, experiments must be done by suppressing the vasoconstrictor action of AT1R with an AT1R blocker before and during AT2R stimulation [39]. On the other hand, in AT2R deficient mice, an increase in the basal blood pressure and an augmented blood pressure reactivity upon Ang II infusion as compared to wild type mice are observed [40]. Moreover, the chronic infusion of Ang II to transgenic mice that overexpressed AT2R in VSMC, completely abolished the AT1-mediated pressor effect [41].

Chronically elevated blood pressure causes vascular remodeling characterized by decreased luminal area and thickened medial layer, associated to VSMC hypertrophy, increased VSMC migration, proliferation, extracellular matrix protein synthesis and secretion; and inhibition of apoptosis [28,42]. Using AT2R-deficient mice, it was demonstrated that AT2R mediates an inhibitory effect on femoral, aorta and coronary arteries remodeling in response to pressure overload [43]. The effects of direct AT2R stimulation on vascular remodeling induced by hypertension was studied using C21 [44]. In hypertense animals, AT2R activation prevented the development of vascular hypertrophy and fibrosis [44]. However, in wild type animals, AT2R-dependent inhibition of vascular remodeling effects can be observed mostly when AT1R is blocked. In pulmonary hypertension, C21 attenuates the progression of lung fibrosis and pulmonary hypertension in monocrotaline [45] and bleomycin [46] models. Both C21 and CGP42112 also inhibit neointimal formation induced by vascular injury [45,47]. Taking together, these data indicates that AT2R activation inhibits vascular remodeling. However, whether Ang-(1-9) can mimic most of these AT2R-dependent vascular effects still remains to be elucidated.

Perspectives

The main action described for the counter regulatory RAS is focused to the regulation of the classical RAS. Because the dysregulation of classical RAS is associated with several vascular diseases, including arterial hypertension, pulmonary hypertension, vascular remodeling, and atherosclerosis, the activation of counter regulatory RAS emerges as a suitable therapeutical alternative to treat these diseases [5]. Although preclinical studies performed *in vitro* and *in vivo* shows promising results, much work should still be performed to achieve practical clinical application of counter regulatory RAS components. One of the major limitations to overcome will be to increase the low half-life of different angiotensin peptides in plasma [48]. Therefore, new pharmaceutical formulations and/or the development of new molecules that activates AT2R will be required. Some advances in this field have been already described. Molecules such as C21 (AT2R agonist) and rhACE2 have already been used in clinical trials (Table 1). However, this is a still open area of work that will require further exploration.

Acknowledgements

Conflict of interest

M.C. is a co-inventor on patent applications PCT/CL2012/000016 and CL200803736 related to the use of Ang-(1-9) as a cardioprotective, anti-cardiac remodeling and anti-hypertensive compound.

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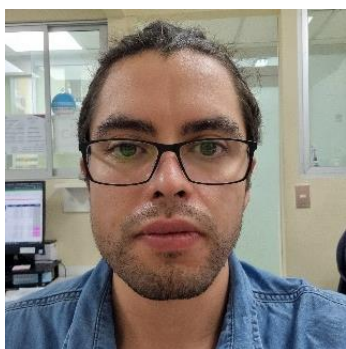
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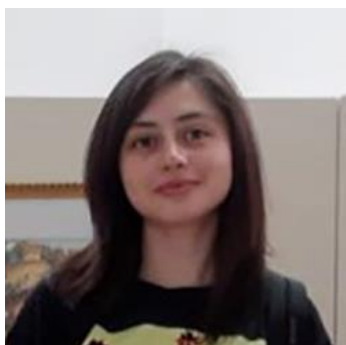
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