CMLS, Cell. Mol. Life Sci. 61 (2004) 2720–2727
1420-682X/04/212720-08
DOI 10.1007/s00018-004-4243-4
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CMLS Cellular and Molecular Life Sciences

Novel angiotensin peptides

C. M. Ferrario and M. C. Chappell

The Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Winston Salem, North Carolina 27157 (USA), Fax: +1 336 716 6644, e-mail: cferrari@wfubmc.edu

Abstract. Virtually all existing evidence on the function of angiotensin II (Ang II) in the regulation of tissue homeostasis and blood pressure regulation bears on the more restricted question of what other mechanisms or systems may amplify or inhibit the actions of this important peptide. Whereas there is evidence that Ang II may potentiate the effects of catecholamines, various cytokines and also growth factors, the repertoire of substances which may inhibit the actions of Ang II is more limited and has been restricted primarily to prostacyclin, bradykinin and nitric oxide. Advances in receptor pharmacology and introduction of selective antagonists to two of the receptor subtypes at which Ang II binds permitted a more critical examination of the functions of the renin angiotensin system in physiological and pathophysiological conditions, as well as uncovering the previously unsuspected possibility that within the biochemical pathways leading to the formation of the peptide the renin angiotensin system could process either its immediate precursor (angiotensin I) or the actual Ang II peptide into an alternative form, angiotensin-(1–7) [Ang-(1–7)], the function of which was to antagonize the effects of Ang II. We review here the biological actions of Ang-(1–7) and discuss how this discovery may change altogether the perception of how the renin angiotensin system functions in the regulation of tissue perfusion pressure and the regulation of salt and water metabolism.

Key words. Angiotensin peptides; ACE2; blood pressure; cardiac hypertrophy; heart failure; hypertension; renal function; renin angiotensin system.

Introduction

The receptors and cellular messengers mediating the biological actions of angiotensin II (Ang II) have occupied the attention of researchers for most of the two last decades. Despite evidence to the contrary, there was little interest to further explore the biochemical pathways participating in the generation of other angiotensin peptides. Most investigators were satisfied with the idea that renin and angiotensin converting enzyme (ACE) were all that was required to form Ang II and explain the diversity of mechanisms that could contribute to the regulation of peptide synthesis and metabolism. Reports showing that additional peptidases [1–6] participated in the formation of biologically active forms of angiotensin peptides drew small interest, partly because the established therapeutic efficacy of ACE inhibitors and the emerging similitude of effects of angiotensin receptor blockers on cardiac and vascular pathology suggested that there was no further need to explore the biochemical physiology of the renin angiotensin system (RAS).

This is no longer true today. The cloning and characterization of an ACE homologue, ACE2 [7, 8], has forced a revision of long-held concepts and stimulated a re-examination of the biochemical pathways of angiotensin peptide formation and actions. This renewed interest in the topic has forced consideration of how the vasodilator and antitrophic biological effects of the heptapeptide angiotensin-(1–7) [Ang-(1–7)] are functionally linked to the overall mechanisms that regulate intrinsically the function of the RAS in physiology and pathology. This review highlights the fundamentals of what we know of the actions of Ang-(1–7), the biochemical pathways and enzymes forming and degrading the peptide, as well as outline emerging areas where further research will be required to obtain a lucid understanding of the regulatory functions of the RAS.

* Corresponding author.

Ang-(1–7) forming and degrading enzymes

The classical established major axis of the RAS results in the generation of Ang II through sequential cleaving of angiotensinogen by renin and angiotensin I (Ang I) by ACE. A second axis of the system exists for which Ang-(1–7) is its product. This second pathway comprises two separate arms – one that directly generates Ang-(1–7) from Ang I through hydrolysis of the Pro^7–Pro^8 bond in the C-terminal domain of the peptide (fig. 1). Formation of Ang-(1–7) from Ang I by the endopeptidases, neprilysin (EC 3.4.24.11), prolyl-oligopeptidase (EC 3.4.21.26) and thimet endopeptidase (EC 3.4.24.15) appears to be tissue-specific with neprilysin being primarily responsible for Ang-(1–7) production in the circulation and vascular endothelium, whereas the two alternate enzymic pathways are more active in tissues such as the brain, kidney and smooth muscle [2, 4–6]. The second arm of this axis generates Ang-(1–7) by hydrolysis of Ang II via the mono-peptidase ACE2, a newly discovered ACE homologue and a member of the M2 gluzincin family [7, 8]. This last process in the biochemical cascade of the RAS connects the two active axes of the RAS by regulating the rate of Ang II disposition and Ang-(1–7) formation. Indeed, both ACE and ACE2 may represent fulcrums at which the biological effect of the pleiotropic actions of Ang II and Ang-(1–7) are regulated since ACE metabolizes Ang-(1–7) into Ang-(1–5) while ACE2 hydrolyses Ang II into Ang-(1–7). This interpretation is supported by our findings that the cardiac dysfunction found in ACE2 knockout mice is reversed in mice deficient in both ACE2 and ACE [9], whereas in neural cells in culture addition of Ang II reduces ACE2 messenger RNA (mRNA) [10], while the Ang II antagonists losartan and olmesartan increase ACE2 mRNA in the rat ischemic myocardium [11].

The newer biochemical pathways now established confirm our previous hypothesis of a counterregulatory mechanism that within the RAS modulates the actions of the opposing peptides Ang II and Ang-(1–7) in the control of tissue perfusion and homeostasis [12–16]. As elaborated by Yagil and Yagil [17], a built-in system of negative regulation of Ang II by Ang-(1–7) through their effects on ACE and ACE2 allows for an inner balance between the pressor – trophic effects of Ang II and the opposing depressor-antitrophic actions of Ang-(1–7). This is not a new concept since there are multiple other examples of biological regulation of peptide biotransformation systems, as illustrated in one of our earlier papers [18].

The ying-yang functions of Ang II and Ang-(1–7)

It is hardly necessary to document in detail the many actions of Ang II in the regulation of body fluid volumes,

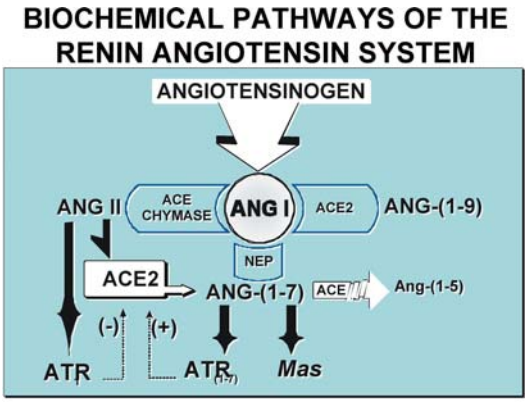

Figure 1. Schematic diagram of the key biochemical steps involved in the generation of angiotensin II (Ang II) and angiotensin-(1–7) [Ang-(1–7)]. Biotransformation of angiotensinogen I by renin into the decapeptide angiotensin I (Ang I) initiates the cascade where the prohormone is cleaved into the octapeptide Ang II primarily by angiotensin converting enzyme (ACE), the nonapeptide Ang-(1–9) by angiotensin-converting enzyme 2 (ACE2) and into Ang-(1–7) by the action of several tissue specific endopeptidases (NEP). A chymase pathway for Ang I to Ang II conversion has been demonstrated, although its role in pathology remains to be established. Ang II and Ang-(1–7) can then be converted into Ang-(1–7) and Ang-(1–5) by the actions of ACE2 and ACE, respectively. The active peptides are denoted to interact with the subtype receptors type 1 (AT_1-R), the non-AT_1/AT_2 Ang-(1–7) ($AT_{(1-7)}$R), and the *mas* (*Mas*) receptors, respectively. Broken arrow lines with (–) and (+) symbols illustrate potential feedback mechanisms of ACE2 gene regulation as interpreted from initial studies in our laboratory [10, 11].

blood pressure control mechanisms and homeostasis in general, as there is a voluminous literature on this subject. Suffice to say that Ang II plays a critical role in control of fluid volumes, blood pressure regulation and cardiovascular remodeling through direct effects on protein synthesis, cell growth and cell differentiation, induction of growth-promoting genes and modulation of the synthesis of radical oxygen species, autocoids, prostanoids and cytokines.

The opposite, however, may not be true for Ang-(1–7), since it is only recently that a growing body of studies has began to confirm the work that my colleagues and I have carried out since we first showed in 1988 that Ang-(1–7) had a comparable activity to Ang II in hypothalamic-hypophysial explants [19]. The vasodilator properties of Ang-(1–7) are best demonstrated in situations in which the RAS is activated, such as sodium-volume depletion [20, 21], two-kidney one-clip hypertension [22] and [mRen2]27 transgenic hypertensive rats. In isolated precapillary resistance vessels, piglet pial arterioles, the renal glomeruli, aorta and canine coronary arteries, Ang-(1–7) increases lumen diameter at doses equivalent to Ang II-induced contractions [23–37]. The lower vasodilator dose threshold obtained in isolated perfused arterial vessels suggests that Ang-(1–7) is acting as a local modulator of vascular tone [31]. The vasodilator effects

of Ang-(1–7) are mediated by a non-AT_1/AT_2 receptor that stimulates local release of nitric oxide and vasodilator prostaglandins [22–24, 26, 27, 32, 37–43]. Furthermore, the vascular relaxant properties of Ang-(1–7) modulate the vasodilator activity of bradykinin, amplifying the vasodilator effects of this autocoid. A recent innovative study demonstrated the vasodilator effect of Ang-(1–7) in mature skin and sponge-induced neovasculature in mice [44, 45]. Blood flow increased in the presence of Ang-(1–7) and was blocked by the selective Ang-(1–7) antagonist [D-Ala^7] Ang-(1–7), nitric oxide synthase inhibitors or indomethacin. Treatment with an AT_2 receptor antagonist failed to alter the growth of newly formed vessels, although it prevented vasodilation in the pre-existing vessels. These studies suggest that Ang-(1–7) may act as an angiogenic factor during wound healing, chronic inflammatory processes, and tumor growth and development.

The issue of whether the depressor effects of Ang-(1–7) counteract systemic and vascular effects of chronic rises in plasma Ang II was addressed in the whole animal and in hypertensive subjects. In normal rats activation of the RAS due to either mild or severe sodium volume depletion increases plasma Ang-(1–7) and Ang II levels while having no effect on blood pressure [20, 21]. However, systemic administration of a selective Ang-(1–7) antibody or D-Ala^7-Ang-(1–7) in this condition induces an elevation of blood pressure, revealing that the heptapeptide may be opposing a pressor effect of increased Ang II activity in response to salt restriction. The vasodepressor action of Ang-(1–7) contributes to the antihypertensive action of ACE inhibitors and Ang II receptor blockers (ARBs). In both the spontaneously hypertensive rat (SHR) and [mRen2]27 transgenic hypertensive rats, plasma and urinary excretion rates of Ang-(1–7) are augmented following oral administration of lisinopril alone or in combination with losartan [46, 47]. The antihypertensive response to drug administration is reduced by ~30–40% when Ang-(1–7) synthesis is prevented by injection of a neprilysin inhibitor, an Ang-(1–7) antibody, or D-Ala^7-Ang-(1–7). In keeping with the concept that Ang-(1–7) balances Ang II activity, Ang-(1–7) levels are augmented in essential hypertensive patients medicated with captopril and in salt-sensitive hypertensive subjects given omapatrilat, a dual ACE and neprilysin inhibitor [48–50]. Collectively, these data suggests that a deficit in Ang-(1–7) synthesis or activity may contribute to the evolution of human hypertension with dysregulation of the intrinsic control mechanism due to genetic or acquired changes in the activity of Ang-(1–7)-forming or degrading enzymes. We speculate that ACE or even ACE2 polymorphisms may contribute to loss of balance by either excess metabolism of Ang-(1–7) or reduced Ang-(1–7) formation. This possibility is suggested by the recent observation that in essential hypertensive subjects with the DD-ACE genotype (higher ACE activity), plasma levels of Ang-(1–7) are four times lower than in patients with the II-ACE genotype (lower ACE levels), whereas plasma Ang II levels are similar in both groups [51]. While other possibilities exist, the finding that ACE inhibition does increase Ang-(1–7) levels associated with a positive response in terms of blood pressure control and diuresis might suggest that the imbalance may result from 'insufficient' formation of the heptapeptide. The investigation of this hypothesis is worth pursuing.

Ang-(1–7) and renal and cardiac function

The actions of Ang-(1–7) in both the kidney and the heart illustrate the intrinsic role of Ang-(1–7) as a paracrine hormone.

Ang-(1–7) and renal function

Ang II causes differential constriction of diverse segments of the renal microvasculature, sodium and water retention, and growth-promoting action through stimulation of transporters in the proximal epithelium and profibrotic factors, respectively. For Ang-(1–7), most actions are in opposition to those of Ang II. Ang-(1–7) infusions into the renal artery lead to diuresis and natriuresis accompanied by increased glomerular filtration rate (GFR) [29, 48, 52–56]. Ang-(1–7) induces dilation of pre-constricted afferent arterioles [33], while Ang-(1–7) and Ang-(3–7) are potent inhibitors of Na^+,K^+-ATPase activity in isolated convoluted proximal tubules and the renal cortex [54]. Actions at the Na^+/H^+ exchanger may also occur [57]. Ang-(1–7) inhibits transcellular flux of sodium in renal tubular epithelial cells associated with activation of phospholipase A_2 [57, 58]. Furthermore, inhibition of sodium transport by Ang I is markedly potentiated by captopril, suggesting either a shift in processing pathways to Ang-(1–7) or reduced metabolism of the peptide in the proximal tubules of the kidney. In normotensive rats, intrarenal administration of Ang-(1–7) attenuated tubular sodium reabsorption, but had no effect on the vascular effect of exogenous Ang II [25]. In the SHR omapatrilat produces a chronic and pronounced diuresis associated with large increases in urinary excretion of Ang-(1–7) [48] and enhanced Ang-(1–7) and ACE2 staining in renal tubules and collecting ducts. Because urine becomes markedly hyposmotic in SHR rats medicated with omapatrilat, Ferrario et al. [48] suggested that Ang-(1–7) may have an important action in modulation of vasopressin function within the renal tubules. In keeping with this finding, Magaldi et al. [59] reported that Ang-(1–7) interacts via its receptor with the vasopressin V(2) system through a mechanism involving adenylate-cyclase activation.

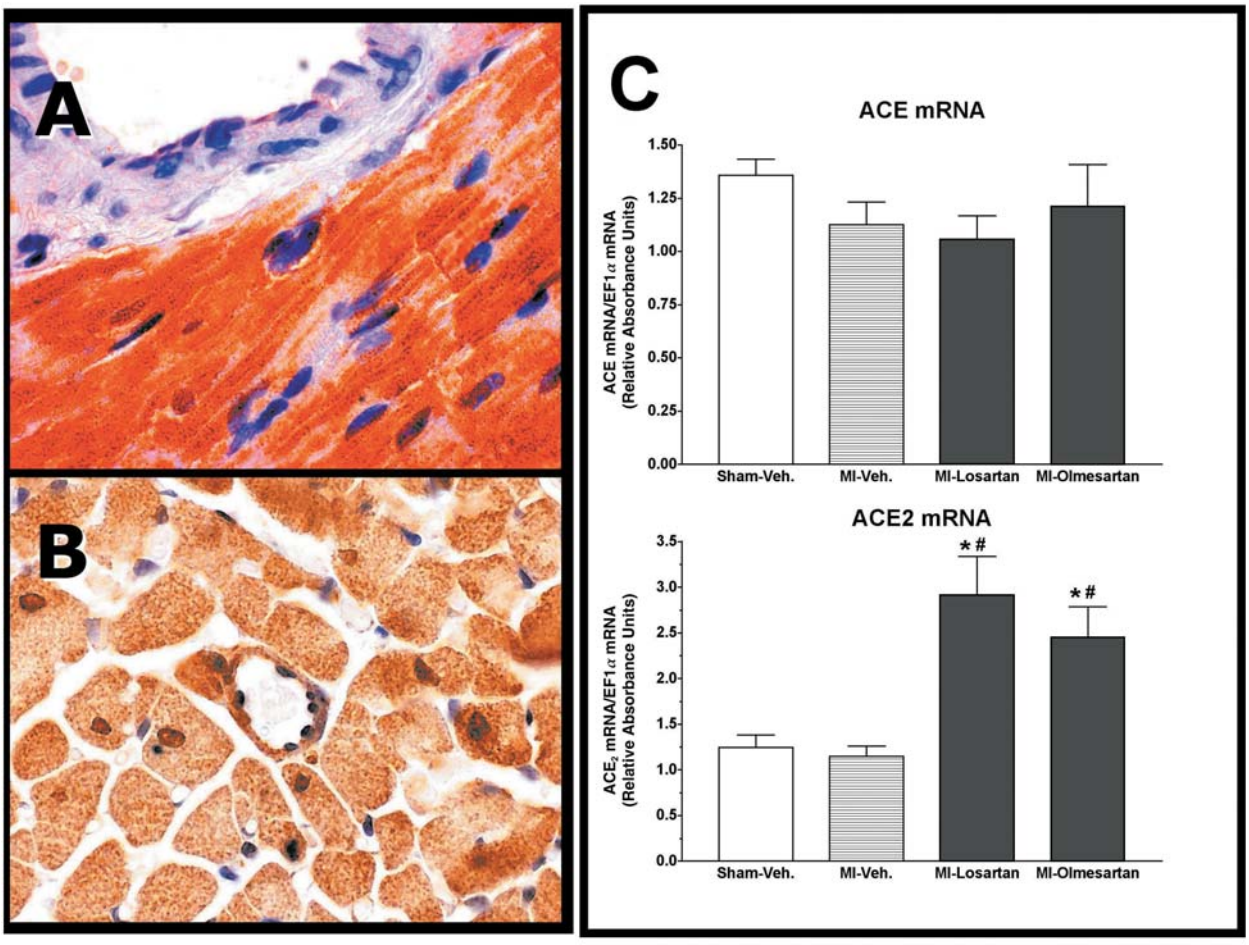

Figure 2. Immunocytochemical visualization of Ang-(1–7) (*A*) and ACE2 (*B*) in the rat left ventricle. Note that Ang-(1–7) staining is restricted to cardiac myocytes, whereas a more extensive staining which includes coronary vessels is visualized for ACE2 (data from our reference [63] and unpublished observations, respectively). Panel *C* illustrates increased ACE2 but not ACE mRNAs in the heart of Lewis rats medicated with either losartan or olmesartan for 28 days after coronary artery ligation. (MI, myocardial infarction. Data adapted from our reference [11]).

Ang-(1–7) and cardiac function

A role of Ang-(1–7) as a modulator of cardiac function and remodeling is beginning to emerge as another site at which the heptapeptide may influence ventricular contractility or even cardiac remodeling. The RAS plays a key role in structural and functional remodeling in hypertension and myocardial infarction (MI), and Ang II is implicated as a major determinant in these processes [60]. Ang II stimulates progression of cardiac hypertrophy and fibrosis in the rat ischemic heart failure model [61], while Ang II blockade prevents the development of left ventricular remodeling and hypertrophy after MI [62].

Evidence for a local action of Ang-(1–7) emanates from anatomical, biochemical and physiological studies performed in both rats and human tissue. Using selective Ang-(1–7) antibodies, intense positive immunoreactive Ang-(1–7) staining was visualized in the rat's heart located only within the cytoplasm of myocytes and with a clear predominance within the right and left ventricles [63]. Furthermore, Ang-(1–7) staining was increased significantly in the viable myocardium of the left ventricle within 28 days post-MI (fig. 2, panel A). Correlative studies of ACE2 staining in the rat heart denote a similar presence of ACE2 in myocytes but also in the endothelium of coronary vessels (fig. 2, panel B). Evidence that Ang-(1–7), found by immunocytochemistry, is produced locally was investigated by measuring the recovery of the heptapeptide in the effluent of microdialysis tubes placed within the canine left ventricle. These experiments showed high Ang-(1–7) levels in the interstitial fluid collected from the ventricular tissue during an infusion of Ang II or Ang I [64]. Recently, angiotensinase activity in left and right ventricular membranes from idiopathic dilated cardiomyopathy, primary pulmonary hypertension and non-failing human hearts was measured with radiolabeled Ang I or Ang II. Ang-(1–7)-forming activity from ^{125}I-Ang I was inhibited by thiorphan, an inhibitor of neprilysin, while Ang-(1–7) activity generated by ^{125}I-

Ang II as substrate was inhibited by the ACE2-specific inhibitor C16 [65]. The importance of the ACE pathway in Ang-(1–7) formation was shown in the heart of transplant recipients in whom intracoronary infusion of labeled Ang I in the presence of enalapril produced substantial elevations in Ang-(1–7) from the blood emerging from the coronary sinus [66]. These recent studies in humans agree with our previous findings in the canine heart where high coronary sinus Ang-(1–7) levels were detected after acute occlusion of a coronary artery [67].

What role Ang-(1–7) plays in heart function may be derived from studies showing that the intravenous infusion of Ang-(1–7) lasting for 8 weeks and commencing 2 weeks after induction of MI in rats induced a marked regression of left ventricular failure [68]. One possible mechanism for the beneficial effects of Ang-(1–7) on cardiac function post-MI derives from experiments showing that Ang-(1–7) attenuates reperfusion arrhythmias in the isolated rat heart [69, 70]. In addition, recent studies performed in our laboratory showed that the reversal of cardiac hypertrophy by chronic administration of either losartan or olmesartan post-MI was associated with upregulation of cardiac ACE2 mRNA (fig. 2, panel C) in the presence of high levels of circulating Ang II and Ang-(1–7) [11]. The increase in ACE2 mRNA in the viable myocardium had no effect on ACE mRNA. These data suggest a differential regulation of the two arms of the RAS occurring following blockade of AT_1 receptors. Given that blockade of AT_1 receptors is associated with increases in circulating Ang II due to inhibition of AT_1-receptor internalization of the ligand, the possibility existed that the upregulation of ACE2 mRNA resulted from an action of Ang II on cardiac AT_2 receptors. This was not the case, since concomitant administration of the AT_2 receptor blocker PD 12319 for the last 3 days of treatment with losartan did not eliminate the increase in cardiac ACE2 mRNA [11]. Therefore, these data suggest that ACE2 upregulation in the viable myocardium of rats given losartan is mediated by disinhibition of cellular actions of AT_1 receptors on the ACE2 gene or activation of cardiac Ang-(1–7) receptors. Although Gallagher et al. [10] reported recently that Ang II downregulates ACE2 mRNA in astrocytes in culture, the situation may not apply to the intact organism or at least to tissues such as the heart. Reminiscent of the concept of the two arms of the RAS, our findings suggest instead that ACE2 mRNA may be under the control of both Ang II and Ang-(1–7), each having an opposite effect on ACE2 gene transcription. This suggestion is derived from the observation of increased cardiac ACE2 mRNA after chronic blockade with losartan post-MI [11]. While questions remain as to the nature of the receptor mediating the cardiovascular actions of Ang-(1–7), the recent identification of Ang-(1–7) as an endogenous ligand for the G-protein-coupled receptor *mas* [71] provides an opportunity to dissect the mechanism by which AT_1 and *mas* receptors may modulate the activity of Ang II and Ang-(1–7) on ACE2. At present, the approach employed in our studies of the effects of AT_1 blockade on cardiac ACE2 provide a new insight into the biochemical mechanisms that may underscore regulation of ACE2 in vivo.

Ang-(1–7) in the reproductive system

Emerging evidence suggests that Ang-(1–7) plays a role in the regulation of the physiological response to pregnancy since circulating and urinary levels of Ang-(1–7) increased throughout gestation [55]. In age and race matched 3rd trimester preeclampsia subjects, plasma levels of Ang-(1–7) were significantly decreased compared with normal pregnant subjects [55]. In the rat, pregnancy was associated with increased renal concentrations of Ang I and Ang-(1–7), as well as augmented excretion of the heptapeptide [72]. Moreover, in mesenteric vessels the vasodilatory response to Ang-(1–7) was significantly enhanced in pregnant versus virgin animals [72]. Consistent with the concept that ACE2 is closely linked to the expression of Ang-(1–7), Brosnihan et al. [73] have also shown that the expression of ACE2 is markedly increased in the proximal tubules of the kidney during pregnancy. Thus, the regulation of ACE2 activity provides an immediate and powerful balance between Ang II and Ang-(1–7) within the kidney that may effectively dampen the pro-hypertensive arm of the RAS. During pregnancy, upregulation of ACE2 may provide sufficient buffering to prevent the expected increase in blood pressure with prolonged activation of the RAS. However, a deficit in ACE2 activity may culminate in severe hypertension that occurs in preeclampsia. Indeed, the identification of the regulatory factors that govern the expression of ACE2 in the setting of pregnancy and preeclampsia are clinically relevant.

Summary

As first pointed out by Ryan [74], linear peptides such as Ang I and Ang II, having no repeating amino acids, have 54 and 35 possible lower homologs, respectively, all of which must be accounted for in any attempt to determine the exact mechanisms of their disposal and function. Ang-(1–7), a product of both Ang I and Ang II, functions to antagonize the actions of Ang II by acting primarily through binding to a non-AT_1/AT_2 receptor and also the *mas* receptor. Biologically, Ang-(1–7) acts to dilate blood vessels through cellular mechanisms that result in increased production of vasodilator prostaglandins and nitric oxide, as well as amplifying the intrinsic actions of bradykinin [75]. In the kidney, Ang-(1–7) tubular actions leading to natriuresis and diuresis result from activation

of a number of transporters involved in water and electrolyte absorption through the renal tubules and collecting ducts. New data suggest that Ang-(1–7) may also modulate the effects of tubular vasopressin via an effect on its V2 receptor. In the heart, Ang-(1–7) may counteract the hypertrophic, pro-fibrotic and pro-thrombotic [76, 77] effects of Ang II as well as increase blood flow within the myocardium. Ang-(1–7) may also contribute to the antihypertensive effects of ACE inhibitors and ARBs [46–78] by two different mechanisms. Inhibition of ACE increases blood and tissue concentrations of Ang-(1–7) by preventing ACE-mediated peptide degradation and increased substrate (Ang I) availability. Blockade of Ang II receptors increases blood and tissue levels of Ang-(1–7) by: (i) increasing Ang I substrate availability by interruption of an AT_1-mediated negative feedback on renin, and (ii) augmenting the rate of Ang II conversion into Ang-(1–7) via increased ACE2 expression and activity. In the context of this proposal, both ACE and ACE2 represent critical steps at which modulation of angiotensin peptide functions are precisely regulated, the specific mechanisms of which need to be investigated. Genomic studies are also urgently needed to determine whether polymorphisms in the ACE2 gene or the genes for Ang-(1–7)-forming enzymes acting on the Ang I substrate may illuminate further the intrinsic mechanisms by which the synthesis and metabolism of these two active peptides are influenced. While there is a current tendency to consider ACE2 as simply an angiotensinase, that is an enzyme that inactivates Ang II, this generalized terminology obscures the potential importance of this constituent of the renin angiotensin system since the product generated from its action is biologically active and functionally important. We doubt that any investigator would consider ACE simply as an angiotensinase.

Acknowledgement: Supported in part by NIH Grants RO1 HL 056973 and PO1 HL 51952. American Heart Association grants AHA 0151521U and AHA 0355741U.

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