The Therapeutic Potential of Angiotensin-(1-7) as a Novel Renin-Angiotensin System Mediator

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Abstract: In this review, we show Angiotensin-(1-7) as a novel Renin Angiotensin System mediator that antagonizes cardiovascular and proliferative effects of Angiotensin II and exerts complex renal actions. We also speculate the possibility of new drugs for the treatment of cardiovascular, genitourinary and hepatic diseases by interfering with ACE2-Angiotensin-(1-7)-Mas axis.

Keywords: Angiotensin-(1-7), Mas receptor, Angiotensin-converting enzyme 2, Angiotensin II, AVE 0991, Angiotensin antagonists, Renin-Angiotensin System.

INTRODUCTION

The Renin Angiotensin System (RAS) is classically conceived as a coordinated hormonal cascade in the control of cardiovascular, renal, and adrenal function that governs fluid and electrolyte balance and arterial pressure through Angiotensin (Ang) II actions [1]. Recent advances in cell and molecular biology, as well as physiological and pharmacological approaches, have generated exciting new concepts derived from the identification of new peptides, new enzymes that generate these peptides, novel receptors, new functions for these recently discovered mediators and for those already known, receptor-receptor interactions, and the local tissue RAS not requiring hormone secretion into the systemic circulation [2-4]. In this regard, the recognition of other biological active fragments of RAS metabolism such as Ang III, Ang IV and Ang-(1-7) [2-4], the Ang IV binding site insulin-regulated aminopeptidase [5], the angiotensin converting enzyme 2 (ACE2), an homologue of classical angiotensin converting enzyme (ACE) which forms Ang-(1-7) directly from Ang II and indirectly from Ang I [6-8], and the Ang-(1-7) G protein-coupled receptor Mas have improved the comprehension of the role of the RAS in normal physiology and disease states [9].

Among the novel RAS peptides, Ang-(1-7) is particularly interesting because many studies showed that this heptapeptide are functionally linked to the overall mechanisms that intrinsically regulate the function of the RAS in physiology and pathology [3,4,10]. In general, Ang-(1-7) opposes the vascular and proliferative effects of Ang II and also exerts complex renal actions [2,10,11,12]. Ang-(1-7) is formed from Ang II by prolylendopeptidase, prolyl-carboxipeptidase or ACE2 or directly from Ang I through hydrolysis by prolylendopeptidase and endopeptidase 24.11 and it is metabolized by ACE to Ang-(1-5) [6,7,12,13]. ACE inhibitors elevate Ang-(1-7) concentrations [12,14] by both increasing Ang I, the substrate for Ang-(1-7), as well by preventing Ang-(1-7) degradation. Recent studies suggest that, at least in part, the beneficial effects of ACE inhibitors in heart and kidney diseases may be attributed to Ang-(1-7) [15,16]. These findings are in keeping with the hypothesis that the RAS is capable to self-regulate its activity through the formation of Ang-(1-7) [3,4]. In addition, Kostenis *et al.* [17] recently demonstrated that the Ang-(1-7) G-protein coupled receptor Mas can hetero-oligomerize with Ang II type 1 receptor (AT_1) and by so doing inhibit the actions of Ang II. Indeed, receptor Mas acts *in vivo* as an antagonist of the AT_1 receptor [17]. We currently believe that RAS can act through two opposite arms: the major one responsible for the main actions of this system is constituted by the ACE-Ang II-AT_1 receptor axis and the other, a counterregulatory arm, is formed by the ACE2-Ang-(1-7)-Mas axis [16] as shown in Fig. **1**.

In this review, we discuss the role of the RAS at different sites, specifically focusing the actions of ACE2-Ang-(1-7)-Mas axis and its potential as a target for the development of new drugs for the treatment of cardiovascular, genitourinary and hepatic diseases.

Ang-(1-7) in the Cardiovascular System

Ang-(1-7) and its receptor Mas are present in the heart [9,18-20] and it is well-known that the heart is an important target for Ang-(1-7) actions. The interaction of this peptide with its receptor in the heart leads to vasodilation and an improvement in the cardiac function [21-23]. Furthermore, Ang-(1-7) also plays its beneficial effects indirectly through

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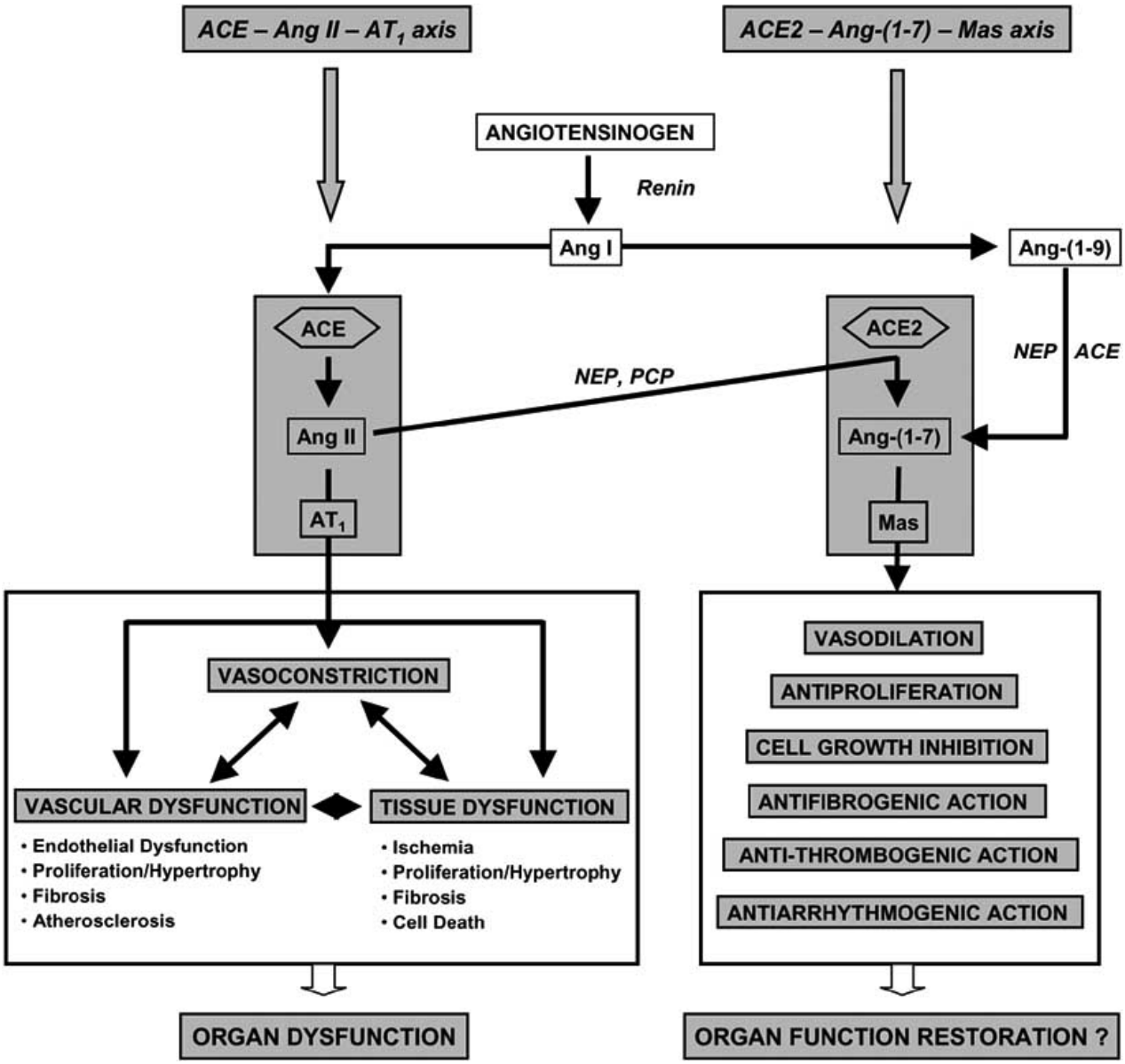

Fig. (1). The general effects produced by the activation of the opposing Renin Angiotensin System arms, ACE-Ang II-AT1 axis and ACE2-Ang-(1-7)-Mas axis.

bradykinin (BK) potentiation or by opposing Ang II actions [11,24]. Ang-(1-7) was first localized in the heart by Santos *et al.* (1990) in dogs [25]. In this report, immunoreactive Ang-(1-7) was demonstrated in aortic root, coronary sinus, and right atrium. In coronary bed of rats, Ang I is extensively metabolized leading to the generation of Ang II, Ang III, Ang IV, and Ang-(1-7) [26,27]. Recently, Averill *et al.* (2003) found that Ang-(1-7) immunoreactivity was limited to cardiac myocytes and absent in interstitial cells and vessels of rat hearts [20]. Myocardial infarction induced by left coronary artery occlusion significantly augmented the Ang-(1-7) immoreactivity in the ventricular tissue surrounding the infarcted area [20]. Ang-(1-7) is also formed in human cardiac circulation, apparently through an ACE-dependent pathway [28]. In addition, Mas mRNA was detected in mouse and rat hearts [18]. These studies suggest that Ang-(1-7) and its receptor are co-localized in the heart and this peptide can be locally generated.

The novel angiotensin-converting enzyme homologue, ACE2, is closely involved in the Ang-(1-7) production. This enzyme can form Ang-(1-7) from Ang II [29] or less efficiently through hydrolysis of Ang I to Ang-(1-9) [6] with subsequent Ang-(1-7) generation through ACE and neutral endopeptidase (NEP) hydrolysis [13]. However, it has been suggested that endopeptidase-mediated metabolism of Ang I is more important than ACE2-mediated metabolism of Ang II for Ang-(1-7) production in arterial and coronary sinus blood of subjects with heart failure [30].

In the heart, ACE2 is highly expressed [31] where it plays an important regulatory role [28,32-36]. The ACE2 gene maps to hypertension-related quantitative trait loci (QTLs) on the X chromosome in three different rats models

of hypertension and its deletion produced abnormal heart function providing a consistent evidence for a cardiac role of this enzyme in the cardiovascular system [32]. It should be mentioned that the role of ACE2 in the heart appears to depend on the species background [33,34]. Contrasting with the putative beneficial role of ACE2 in the heart, its overexpression in the heart leads to hypotension, progressive conduction disturbances, and sudden death [35]. During pathological circumstances, ACE2 appears to play a crucial role in the heart function. Thus, Ishiyama *et al.* (2004) demonstrated that after AT_1 receptor blockade and coronary artery ligation, cardiac ACE2 mRNA expression was upregulated in Lewis normotensive rats [37]. ACE2 mRNA also increased in the border/infarct area at day 3 after myocardial infarction in rats and in failing human hearts [38]. Both ACE2 mRNA expression [39] and ACE2 protein and activity [28,38] are significantly augmented in failing human heart. In addition, ACE2 from failing human heart can be associated with integrin β1 by producing a protein complex that showed high catalytic activity with Ang II but not with Ang I as substrate [36].

Ang-(1-7) elicits several beneficial effects in the cardiac muscle and vasculature which reinforce the relevance of the ACE2-Ang-(1-7)-Mas axis in the cardiovascular system. This peptide produced a significant increase in cardiac output and stroke volume in anesthetized Wistar rats [40], decreased the incidence and duration of ischemia-reperfusion arrhythmias [22] apparently by activating the sodium pump [41] and improved the post-ischemic contractile function [42] in isolated perfused rat hearts. These effects were completely blunted by the Ang-(1-7) receptor Mas antagonist, A-779. In keeping with these data, chronic infusion of Ang-(1-7) improved endothelial aortic function and coronary perfusion and preserve cardiac function in an experimental rat model of heart failure [23]. Additionally, transgenic rats [TGR(A1-7)3292] which present a 2.5-fold increase in plasma Ang-(1-7) concentration, showed a slight but significant increase in daily and nocturnal dP/dt, a less pronounced cardiac hypertrophy induced by isoproterenol, a reduced duration of reperfusion arrhythmias, and an improved postischemic function in isolated perfused hearts [43]. Ang-(1-7) also reduces the growth of cardiomyocytes by a mechanism presumably involving its receptor Mas [44,45].

Concerning the effects of the Ang-(1-7) in the coronary vasculature, its actions are often opposite to those induced by the Ang II [21,46]. Ang-(1-7) elicited vasorelaxation in isolated canine [21] and porcine [46] coronary artery rings. In isolated perfused mouse hearts, this peptide produced complex vascular effects involving interaction of its receptor with AT_1 and AT_2-related mechanisms, leading to vasodilation [47]. The mechanisms of the coronary actions of Ang-(1-7) are not completely clear, but likely involve release of prostacyclin and/or NO [21,46,47]. At higher concentrations however, Ang-(1-7) facilitated reperfusion arrhythmias and induced a concentration-dependent decrease in coronary flow in isolated perfused rat hearts [26]. A similar finding was observed in isolated hamster hearts [48]. These observations suggest that high local concentrations of Ang-(1-7) exert deleterious effects in the heart possibly through activation of NADPH oxidase [49] or through a direct interaction with AT_1 receptor [11].

The presence of a specific binding site for Ang-(1-7) in the heart was first suggested by studies demonstrating that cardiac Ang-(1-7) effects are fully blocked by the specific Ang-(1-7) antagonist A-779 [22,40,42]. Recently, the identification of the G-protein-coupled receptor Mas as a specific binding site for Ang-(1-7) confirmed these previous pharmacological findings [9]. In this way, Ang-(1-7)-induced vasodilation was completely blocked in isolated heart from Mas-deficient mice [47]. In addition, in keeping with the cardioprotective role of Ang-(1-7), isolated hearts of Mas-deficient mice presented a marked decrease in systolic tension and in positive and negative dT/dt. A significant decrease in the heart rate and an increase in the coronary vascular resistance were also observed [47]. Furthermore, the left ventricular pressure, positive dP/dt, and cardiac output were significantly lower in Mas-knockout mice as assessed by means a micro conductance catheter placed into the left ventricle [17]. A reduced heart rate variability associated with an increased sympathetic tone was also found in Mas-deficient mice [50]. These findings indicate that the interaction of Ang-(1-7) with its specific receptor Mas is an important step for the actions of this peptide in the heart.

In addition, Ang-(1-7) can also exerts its cardiac beneficial effects by means an amplification of the vasodilator actions of BK. Similar to other vascular beds [51,52], it has been shown that Ang-(1-7) increases BK-induced vasodilation responses in isolated perfused rat hearts [53] and in isolated canine coronary arteries [21]. Another indirect mechanism that could be involved in the Ang-(1-7) effects is its ability to antagonize the Ang II responses [54-56]. In this regard, we have found that chronic infusion of Ang-(1-7) reduces heart Ang II levels through an AT_1-independent mechanism involving an increase in the ACE2 mRNA expression [57].

In summary, Ang-(1-7) exerts many cardioprotective effects against heart failure such as improvement of endothelial aortic function and coronary perfusion, improvement in postischemic function [23,43], reduction of cardiac hypertrophy induced by isoproterenol [43], reduction of reperfusion arrhythmias [43], and significant elevation of cardiac output and stroke volume [40]. Additionally, the finding that isolated hearts from Mas-knockout mice presented a lower cardiac output and an increase in coronary vascular resistance corroborated the importance of Ang-(1-7)-Mas axis in the preservation of cardiac function [47]. Thus, in the future, the activation of ACE2-Ang-(1-7)-Mas axis in the heart and blood vessels appears to be an useful approach for the treatment of chronic heart failure, coronary diseases and arrhythmias.

Ang-(1-7) in the Renal System

Ang-(1-7) is present in the kidney at concentrations that are comparable to Ang II [58]. The processing pathways for Ang-(1-7) in the circulation and kidney are distinct. In the circulation, the endopeptidase neprilysin is the major enzyme that produces Ang-(1-7) from Ang I or Ang-(1-9). In the kidney, neprilysin may contribute to both the synthesis as well as the degradation of Ang-(1-7). This enzyme cleaves Ang I to Ang-(1-7) and also metabolizes the peptide at Tyr^4-Ile^5 bond to form Ang-(1-4) and Ang-(5-7) [59,60]. Ang-(1-7) is the primary product obtained in preparations of isolated

proximal tubules and exists in urine at higher concentrations than Ang II [60]. The heptapetide is also present in the distal convoluted tubules and collecting ducts [61]. In addition, Chappell *et al.* demonstrated that the distribution of ACE2 within renal tubules is similar to that of Ang-(1-7) [12]. This finding was a preliminary evidence for the direct conversion of Ang II to Ang-(1-7) in the kidney. In keeping with these observations, Ferrario *et al.* showed a role for ACE2 in Ang-(1-7) formation from Ang II in the kidney of normotensive rats as primarily reflected by the increased ACE2 activity measured in renal membranes from the kidney of rats given either lisinopril or losartan [62]. These data further suggest that increased levels of Ang-(1-7) in the urine of animals after ACE inhibition or AT_1 receptor blockade reflect an intrarenal formation of this heptapeptide [62].

Many studies have addressed the complex Ang-(1-7) renal actions [3,11,12,24,58,63]. Differences between species, local and systemic concentrations of Ang-(1-7), nephron segment, level of RAS activation and sodium and water status can be responsible for discrepant results concerning renal effects of Ang-(1-7) [3,11,24,63]. A diuretic/natriuretic action of Ang-(1-7) has been described in several *in vitro* [64-66] and *in vivo* experimental models, mostly by inhibition of sodium reabsorption at proximal tubule [65,67,68]. On the other hand, our group and others have shown an antidiuretic/antinatriuretic effect induced by Ang-(1-7) [63,69-77]. Hence, Magaldi *et al.* (2003) found that, at nanomolar concentration, Ang-(1-7) increased the water conductivity in intramedullary collecting ducts [72]. At picomolar concentration, the heptapeptide increased fluid and bicarbonate reabsorption in the proximal straight tubules [69]. In addition, intratubular Ang-(1-7) application at a concentration of 10^{-8} M augmented fluid reabsorption in micropunctured Henle´s loop [70] and increased the water transport in the inner medullary collecting duct probably through an interaction between Mas and V_2 receptors [71,72]. We have also reported that Ang-(1-7) has a potent antidiuretic activity in water-loaded rats [71,73,74] and mice [9,75] probably mediated by its specific G-protein coupled receptor Mas [9,71,74,76]. These data were in accordance with the renal effects produced by the selective Ang-(1-7) antagonists, A-779 [71,76] and D Pro^7-Ang-(1-7) [77], which promote an increase in basal urinary volume associated with higher glomerular filtration rate and water excretion than control rats. More recently, these findings were further corroborated by the data obtained with the oral Ang-(1-7) receptor Mas agonist, the compound AVE 0991 [75,78]. In water-loaded C57BL/6 mice, AVE 0991 produced a significant reduction in urinary volume, associated with an increase in urinary osmolality [75]. The Ang-(1-7) antagonist, A-779, completely blocked the antidiuretic effect of AVE 0991 [75]. As observed previously for Ang-(1-7) [9], the antidiuretic effect of AVE 0991 after water load was blunted in Mas-knockout mice [75]. *In vitro* receptor autoradiography in C57BL/6 mice showed that the specific binding of ^{125}I-Ang-(1-7) to mouse kidney slices was displaced by AVE 0991, whereas no effects were observed in the binding of ^{125}I-angiotensin II or ^{125}I-angiotensin IV [75].

In a spite of the controversial and complex findings, it has become clear that Ang-(1-7) participates in renal handling of sodium and water. In this regard, the agonists and antagonists of Ang-(1-7)- Mas Axis could be useful tools for the modulation of sodium and water excretion in physiological and pathological conditions such as arterial hypertension, nephrogenic insipidus diabetic, nephrotic syndrome, chronic renal failure, chronic heart failure, ascitis and hepatorrenal syndrome. Indeed, the levels of Ang-(1-7) in plasma, renal tissue and urine are altered during physiological and pathophysiological conditions, including those associated with changes in blood pressure, blood volume and sodium intake [10,12,14,15,79].

Beside important tubular actions, Ang-(1-7) is also involved in renal hemodynamic regulation. The ability of the kidney to generate high intratubular and interstitial concentrations of Ang II and Ang-(1-7) allows the kidney to regulate intrarenal levels of these angiotensins in accord with the homeostatic needs for the regulation of renal hemodynamics and tubular reabsorption and the regulation of sodium balance. When the RAS is inappropriately stimulated, high intrarenal Ang II levels, acting on AT_1 receptors, can contribute to both systemic and glomerular capillary hypertension, which can induce hemodynamic injury to the vascular endothelium and glomerulus [80,81]. In addition, direct profibrotic and proinflammatory actions of Ang II may also promote kidney damage [80,81]. On the other hand, Ren *et al*. reported that Ang-(1-7) induced dilatation of pre-constricted renal afferent arterioles in rabbits [82] and Sampaio at al. showed that an infusion of low concentrations of Ang-(1-7) increased renal blood flow in rats, but the high doses of this heptapeptide produced an opposite effect [40]. Ang-(1-7) also attenuated the effect of Ang II-induced pressor responses and Ang II-enhanced noradrenaline release to renal nerve stimulation in rat isolated kidney [83]. These results opened the possibility that Ang-(1-7) can also act as a physiological regulator of intraglomerular pressure, probably opposing the hypertensive and fibrogenic effects of Ang II.

The renoprotective effects of ACE inhibitors and AT_1 receptor blockers clearly involve multiple pathways including anti-proliferative and anti-fibrogenic actions. As already mentioned, the increased concentrations of Ang-(1-7) could, at least in part, contribute to the mechanism of action of these drugs. However, a protective role of Ang-(1-7) in renal fibrosis is only speculative. In this regard, many studies have shown that Ang-(1-7) exerts inhibitory effects on vascular and cellular growth mechanism [44,84-87]. The molecular mechanisms for the anti-proliferative response to Ang-(1-7) include stimulation of prostaglandin and cAMP production as well as inhibition of mitogen-activated protein (MAP) kinases [85]. In addition, Mas receptor mediates the anti-proliferative effect of Ang-(1-7) in vascular smooth muscle cells [44] and in cardiomyocytes [45]. More recently, Gallagher and Tallant reported the inhibition of human lung cell growth by Ang-(1-7) through a reduction of the serum-stimulated phosphorylation of extracellular signal-regulated kinase (ERK) 1 and ERK2 [86]. Since the ERK cascade is activated in response to different stimuli, such as growth factors, cytokines or DNA damaging agents, the stimulation of Ang-(1-7)-Mas axis, for instance by the administration of AVE 0991, could be effective to halt glomerulosclerosis. However, further studies are necessary to confirm this possibility.

Angiotensin-(1-7) and the Male Reproductive System

Similarly to ACE, ACE2 is highly expressed in the endothelium of heart, kidney and testis of humans and mammals [35]. Douglas *et al*. (2004) has shown that ACE2 expression in testis is restricted to the Leydig cells in the rat and to Leydig and Sertoli cells in humans [87]. These authors also suggest a role for ACE2 in the control of testicular function, possibly by regulating steroidogenesis or some other Leydig cell function [87]. To date, the mRNA for the Ang-(1-7) Mas receptor [9] is highly expressed in Leydig cells [3,18,88]. The presence of ACE2 and Mas receptor in Leydig cells raises the possibility that Ang-(1-7) could also be involved in the regulation of male fertility. The recent characterization of the nonpeptide agonist AVE 0991 [75,78] will allow the elucidation of a putative physiological role of the ACE2-Ang-(1-7)-Mas axis in male reproductive system.

Angiotensin-(1-7) and the Renin Angiotensin System in the Liver

A growing body of evidence indicates that the RAS is clearly involved in hepatic fibrosis [89-94]. Ang II induces contraction and proliferation of hepatic stellate cells, which is considered the principal effector of hepatic acinar fibrosis [94-96]. Ang II also increases transforming growth factor beta 1 ($TGF\beta_1$) and collagen I gene expression *via* AT_1 receptors [90,92]. $TGF\beta_1$ induces the activation of hepatic stellate cells, which enhance the expression of $TGF\beta_1$. Thus, there is a formation of autocrine and paracrine loops that assure the continuous production of this fibrogenic cytokine [95,97]. Moreover, the importance of the RAS in hepatic fibrosis has also been suggested by studies in animals and humans in which the treatment with ACE inhibitors and AT_1 blockers had beneficial effects on liver injury [89-91,93,98].

The precise meaning of RAS activation during the development of liver injury remains unclear. The primary action of the RAS is to regulate vascular tone and renal salt excretion, although Ang II has been shown to have a number of blood pressure-independent actions including mitogenic and trophic effects on cell growth [24,99,100]. Resembling the kidney [99,101,102] and the heart [99,103], many studies provide evidence for a potential pathway through which Ang II might mediate and exacerbate liver injury [95,97]. As pointed out above, enhanced levels of circulating Ang II could trigger the hepatic fibrogenic cascade by activating the stellate cells through AT_1 receptors [94] and stimulating the $TGF\beta_1$ [89,91,95,97]. Accordingly, Bataller *et al.* recently reported that increased systemic Ang II augments hepatic fibrosis and promotes inflammation, oxidative stress and trombogenic events in an experimental model of liver damage, the bile duct ligation [104]. In addition, Paizis *et al.* showed a general increase in expression of ACE and other RAS components in liver from bile duct ligated rats [92]. More recently, the same group observed an upregulation of ACE2 and its widespread expression throughout the liver both in bile duct ligated animals and in human cirrhosis [105]. These authors believe that ACE2 upregulation may facilitate the degradation of Ang II and the formation of Ang-(1-7) that can be one of the factors responsible for the vasodilation of cirrhosis [105]. Another possibility is that the elevated expression and activity of ACE2 during hepatic injury could promote Ang-(1-7) synthesis that may therefore represent a more general counter-regulatory response to the multiple deleterious effects of increased local Ang II production. Since the chronic treatment with ACE inhibitors and following long-term administration of AT_1 blockers raise several-fold the levels of Ang-(1-7) [10,11,14], the modulation of the Ang-(1-7)-Mas axis in liver injury could be a useful tool for further understanding of the pathophysiological mechanisms and, possibly, the treatment of liver fibrosis.

CONCLUDING REMARKS

Chronic degenerative diseases, such as heart failure, end-stage renal disease and hepatic cirrhosis, are clearly related to RAS abnormal activation. Drugs that interfere with the actions of RAS, such as ACE inhibitors and Ang II type I receptor blockers (ARB) produce an improvement, although sometimes limited, of cardiovascular, renal and hepatic diseases [1,15,95,101,103]. In this article, we revised the importance of Ang-(1-7) as a novel RAS mediator that antagonizes cardiovascular and proliferative effects of Ang II and also exerts complex renal actions. The importance of Ang-(1-7) is substantiated by the finding of elevated serum peptide levels in human diseases [4,10,79] and after treatment with ACE inhibitors and ARB [3,4,15]. The identification of the ACE homologue enzyme, ACE2, which forms Ang-(1-7) from Ang II [6-8], and the G-protein coupled receptor Mas as an Ang-(1-7) receptor [9] have provided biochemical and molecular basis for its biological effects.

The availability of Ang-(1-7) Mas receptor agonists and antagonists such as the recently described nonpetide oral agonist, the compound AVE 0991 [75,78], and the well known antagonist, the peptide analogue A-779 [9,11,71,76], is providing new insights for the understanding of the role of Ang-(1-7) in physiological conditions as well as in human diseases. Growing evidence suggests that interference with ACE2-Ang-(1-7)-Mas axis may constitute a promising approach for the treatment of cardiovascular, renal and hepatic diseases.

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