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REVIEW

ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis

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Recent advances have improved our understanding of the renin-angiotensin system (RAS). These have included the recognition that angiotensin (Ang)-(1-7) is a biologically active product of the RAS cascade. The identification of the ACE homologue ACE2, which forms Ang-(1-7) from Ang II, and the GPCR Mas as an Ang-(1-7) receptor have provided the necessary biochemical and molecular background and tools to study the biological significance of Ang-(1-7). Most available evidence supports a counter-regulatory role for Ang-(1-7) by opposing many actions of Ang II on AT_1 receptors, especially vasoconstriction and proliferation. Many studies have now shown that Ang-(1-7) by acting via Mas receptor exerts inhibitory effects on inflammation and on vascular and cellular growth mechanisms. Ang-(1-7) has also been shown to reduce key signalling pathways and molecules thought to be relevant for fibrogenesis. Here, we review recent findings related to the function of the ACE2/Ang-(1-7)/Mas axis and focus on the role of this axis in modifying processes associated with acute and chronic inflammation, including leukocyte influx, fibrogenesis and proliferation of certain cell types. More attention will be given to the involvement of the ACE2/Ang-(1-7)/Mas axis in the context of renal disease because of the known relevance of the RAS for the function of this organ and for the regulation of kidney inflammation and fibrosis. Taken together, this knowledge may help in paving the way for the development of novel treatments for chronic inflammatory and renal diseases.

Abbreviations

A-779, angiotensin-(1-7) Mas receptor inhibitor; Ang-(1-7), angiotensin-(1-7); Ang, angiotensin; ARBs, AT_1 receptor blockers; AT_1, angiotensin II receptor type 1; AT_2, angiotensin II receptor type 2; AVE 0991, angiotensin-(1-7) Mas receptor agonist; Mas, G-coupled protein receptor of angiotensin-(1-7); NEP, neutral endopeptidase; RAS, renin-angiotensin system; VCAM, vascular cells adhesion molecules

Introduction

For years, the renin-angiotensin system (RAS) was described as a linear hormonal system involved in blood pressure regulation and water balance. Within this view, angiotensinogen synthesized in the liver is converted into the inactive peptide angiotensin (Ang) I through the renin action, which is produced by the juxtaglomerular cells of the kidney (Hall *et al.*, 1990; Guyton, 1992). Subsequently, Ang I is cleaved by the ACE generating Ang II (Kokubu *et al.*, 1979) whose actions are mediated by two GPCRs, angiotensin II receptor type 1 (AT_1) and type 2 (AT_2; Inagami, 1998; Touyz and Berry, 2002). In this context, the central players of this system are represented by ACE, Ang II and AT_1 receptor.

In several disease states, activation of this axis, that is, ACE/Ang II/AT_1 receptor, causes deleterious effects, including vasoconstriction, inflammation, fibrosis, cellular growth and migration and fluid retention (Kim and Iwao, 2000; Mehta and Griendling, 2007). Based on this concept, key antihypertensive, cardiovascular and renoprotective drugs were developed. Two of them deserve to be mentioned: ACE inhibitors (ACEi) and AT_1 receptor blockers (ARBs). These drugs are widely used with well-documented effectiveness. Nevertheless, important limitations have been reported

related to the use of these RAS blockers. For instance, besides their potential side effects, the responses to ACEi treatment are influenced by gender and ethnic diversity and ARBs have limited efficacy in treatment of end-organ damage (Powers *et al.*, 2011).

It is now clear that the RAS is far more complex than previously conceived. In fact, it has been proposed that, in addition to the ACE/Ang II/AT_1 receptor branch, the RAS possesses a counter-regulatory axis composed by ACE2, angiotensin-(1-7) [Ang-(1-7)] and the Mas receptor. It has been suggested that the activity of the RAS and the actions of RAS blockers depend on the balance between the ACE/Ang II/AT_1 and ACE2/Ang-(1-7)/Mas axes (Ferreira and Santos, 2005). Landmark studies were crucial to establish this new concept. At the end of the 1980s, Schiavone and co-workers (Schiavone *et al.*, 1988) and Campagnole-Santos and colleagues (Campagnole-Santos *et al.*, 1989) provided preliminary evidence that Ang-(1-7) was a biologically active peptide of the RAS. Subsequently, several studies showed that Ang-(1-7) exerts relevant cardiovascular and renal effects (Benter *et al.*, 1993; 1995) and two pivotal discoveries clearly established Ang-(1-7) as an active RAS mediator. First, two independent research groups reported simultaneously in 2000 the existence and characterization of an enzyme homologue to ACE, the ACE2, which was established later as the main Ang-(1-7)-forming enzyme (Donoghue *et al.*, 2000; Tipnis *et al.*, 2000). Second, Santos and co-workers discovered that the G-protein coupled Mas is a functional receptor for Ang-(1-7) (Santos *et al.*, 2003a). Thus, Ang-(1-7) is now considered a biologically active member of the RAS, which binds to Mas inducing many beneficial actions, such as vasodilation, inhibition of cell growth, anti-thrombosis and anti-arrhythmogenic effects (le Tran and Forster, 1997; Santos *et al.*, 2003b; 2004; Grobe *et al.*, 2007; Mercure *et al.*, 2008; Nadu *et al.*, 2008; Ferreira *et al.*, 2010; Santiago *et al.*, 2010).

Ang-(1-7) is produced mainly through the action of ACE2, which has approximately 400-fold less affinity to Ang I than to Ang II. Therefore, Ang II is the major substrate for Ang-(1-7) synthesis (Ferrario, 1990; Donoghue *et al.*, 2000; Tipnis *et al.*, 2000; Vickers *et al.*, 2002). ACE2 can also form Ang-(1-7) less efficiently through hydrolysis of Ang I to Ang-(1–9) with subsequent Ang-(1-7) formation (Vickers *et al.*, 2002). It is important to highlight the key role of ACE2 in this new concept of the RAS since it degrades the vasoconstrictive/proliferative peptide Ang II to form the vasodilator/antiproliferative heptapeptide Ang-(1-7). This is a strategic finding that may be exploited for therapeutic purposes (Hernandez Prada *et al.*, 2008).

An important evidence for the relevance of the ACE2/Ang-(1-7)/Mas axis as a potential target to develop new therapeutic approaches comes from observations that administration of ACEi and ARBs causes substantial increases in plasma Ang-(1-7) levels (Iyer *et al.*, 1998b) and increases in ACE2 expression (Sukumaran *et al.*, 2011). These findings led to the assumption that part of the clinical benefits of ACEi and ARBs might be mediated by the ACE2/Ang-(1-7)/Mas axis (Chappell *et al.*, 1998b; Iyer *et al.*, 1998a; Davie and McMurray, 1999). Indeed, some effects of ACEi and ARBs can be blocked or attenuated by the Mas receptor antagonist, compound A-779 (Ang-(1-7) Mas receptor inhibitor), confirming the suggestion that the ACE2/Ang-(1-7)/Mas axis may be relevant for the actions of RAS blockers (Britto *et al.*, 1997).

Here, we review recent findings related to the function of the ACE2/Ang-(1-7)/Mas axis in regulating processes associated with acute and chronic inflammation, including leukocyte influx, fibrogenesis and proliferation of certain cell types. We will also discuss the involvement of the ACE2/Ang-(1-7)/Mas axis in the context of renal disease because of the known relevance of the RAS for the function of this organ and for the regulation of kidney inflammation and fibrosis. This knowledge may help in paving the way for the development of novel treatments for chronic inflammatory and renal diseases.

The ACE2/Ang-(1-7)/Mas axis regulates leukocyte recruitment and activation

Many studies have shown that the RAS plays a relevant role in the pathogenesis of inflammatory diseases (Owen and Campbell, 1998; Ruiz-Ortega *et al.*, 2001; Suzuki *et al.*, 2003; Ma *et al.*, 2010; Sukumaran *et al.*, 2011; 2012; Capettini *et al.*, 2012). In this context, most pro-inflammatory actions of the RAS appear to be due to the effects of Ang II. Indeed, Ang II activates several cell functions and molecular signalling pathways related to tissue injury, inflammation and fibrosis, including calcium mobilization, free radical generation, activation of protein kinases and nuclear transcription factors, recruitment of inflammatory cells, adhesion of monocytes and neutrophils to endothelial and mesangial cells, up-regulation of adhesion molecules and stimulation of expression, synthesis and release of cytokines and chemokines (Ruiz-Ortega *et al.*, 2001; Suzuki *et al.*, 2003; Ma *et al.*, 2010; Sukumaran *et al.*, 2011; 2012; Capettini *et al.*, 2012). Most of these actions of Ang II are mediated by the AT_1 receptor and it has been suggested that blockade of the AT_1 may be useful for the treatment of inflammatory diseases, as reviewed elsewhere (Ruiz-Ortega *et al.*, 2001; 2006; Benicky *et al.*, 2009; Kon *et al.*, 2011; Capettini *et al.*, 2012).

More recently, several studies have shown that the counter-regulatory ACE2/Ang-(1-7)/Mas axis may also influence inflammatory responses. Indeed, there is now much evidence demonstrating that Ang-(1-7) modulates negatively leukocyte migration, cytokine expression and release, and fibrogenic pathways (Grobe *et al.*, 2007; Guo *et al.*, 2008; Ferreira *et al.*, 2009; Yamazato *et al.*, 2009; Shenoy *et al.*, 2010; Silveira *et al.*, 2010a; Thomas *et al.*, 2010; Sriramula *et al.*, 2011; Agarwal *et al.*, 2012; Barroso *et al.*, 2012; El-Hashim *et al.*, 2012; Jiang *et al.*, 2012; Jin *et al.*, 2012; Sukumaran *et al.*, 2012) (Figure 1, Table 1). For example, studies by Sukumaran and co-workers (Sukumaran *et al.*, 2011; 2012) have shown that the ACE2/Ang-(1-7)/Mas axis is activated and is relevant for the anti-inflammatory effects of the ARBs Telmisartan and Olmesartan in a rat model of autoimmune myocarditis. ARBs increased ACE2, Ang-(1-7) and Mas expression in line with reduction of pro-inflammatory cytokines as TNF-α, IFN-γ, IL-1β, IL-6 and increase of the anti-inflammatory cytokine, IL-10 (Sukumaran *et al.*, 2011; 2012). These anti-inflammatory effects were associated with less

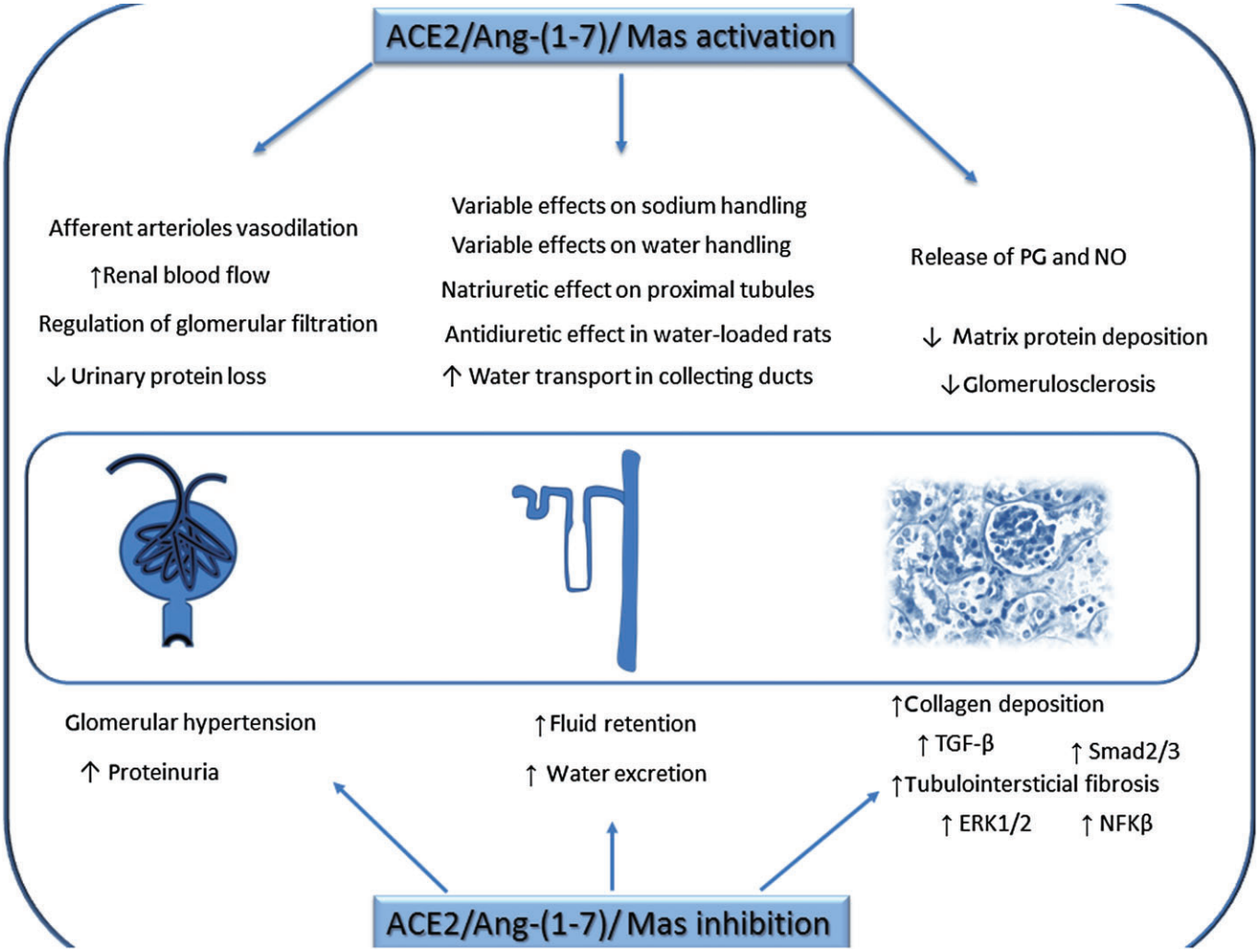

Figure 1
Schematic representation for the role of ACE2-Ang-(1-7)-Mas receptor axis on renal tissue.

myocardial fibrosis and down-regulation of PI3K, phospho-Akt, phospho-p38 MAPK, phospho-JNK, phospho-ERK and phospho-MAPK-2, but formal demonstration of the role of the ACE2/Ang-(1-7)/Mas axis was not provided (Sukumaran *et al.*, 2011; 2012).

In the brain, infusion of Ang-(1-7) into the lateral ventricle of Sprague-Dawley rats subjected to permanent middle cerebral artery occlusion was associated with local decrease of oxidative stress, suppression of NF-κB activity and reduction of pro-inflammatory cytokines (Jiang *et al.*, 2012). Accordingly, it has been reported that the overexpression of ACE2 in paraventricular nucleus attenuated Ang II-induced increase in the expression of TNF-α, IL-1β and IL-6 (Sriramula *et al.*, 2011). Up-regulation of IL-10, ACE2 and Mas expression was also reported in spontaneous hypertensive rats subjected to exercise of moderate intensity (Agarwal *et al.*, 2012).

It has been demonstrated that genetic ACE2 deficiency increased vascular inflammation and atherosclerosis in ApoE knockout mouse by increasing gene expression of vascular cells adhesion molecules (VCAM), cytokines, chemokines and MMP (Thomas *et al.*, 2010). In agreement with the latter concept, Jin *et al.* (2012) also showed that loss of ACE2 resulted in greater increases in Ang II-induced mRNA expression of inflammatory cytokines and chemokines in aorta of ACE2-deficient mice. This was associated with activation of NADPH oxidase activity, production of superoxide, profilin-1 expression and activation of the Akt-ERK-eNOS signalling pathway (Jin *et al.*, 2012). Activation of the ACE2/Ang-(1-7)/Mas axis was also shown to modulate the expression of pro-inflammatory cytokines in a model of pulmonary hypertension. Indeed, there was decreased expression of TNF-α, IL-1β, IL-6, MCP-1 and TGF-β and increased expression of the anti-inflammatory cytokine, IL-10 (Ferreira *et al.*, 2009; Yamazato *et al.*, 2009; Shenoy *et al.*, 2010).

Recruitment of leukocytes into tissues is a coordinated event that involves the timely and precise production and expression of chemokines (and other chemoattractants) and cell adhesion molecules (Petri *et al.*, 2008; Menezes *et al.*, 2011; Williams *et al.*, 2011; Sanz and Kubes, 2012). Several *in vitro* studies have shown that the RAS modulates the migra-

Table 1

ACE2/Ang-(1-7)/Mas receptor axis and modulation of inflammation

Organ/model	Compounds or strategy used	Effects	References
Kidney: ischaemia/reperfusion injury	Ang-(1-7) or AVE0991	↓ Neutrophil influx; ↓ CXCL; ↓ IL-6, TNF-α, ET-1.	Barroso *et al.*, 2012; Giani *et al.*, 2012
Brain: cerebral ischaemia	Ang-(1-7)	↓ Oxidative stress; ↓NF-Kβ activity; ↓ Pro-inflammatory cytokines.	Jiang *et al.*, 2012
Brain: exercise training	Moderate exercise	↑ IL-10, ACE2 and Mas receptor	Agarwal *et al.*, 2012
Brain: PVN (paraventricular nucleus of hypothalamus)	Overexpression of ACE2	↓ TNF-α, IL-1β , IL-6; ↑ IL-10	Sriramula *et al.*, 2011
Lungs: pulmonary fibrosis and hypertension	ACE2 activator; Lentiviral packaged Ang-(1–7), ACE2 cDNA transfer; ACE2 overexpression	↓ TNF-α, IL-1β, IL-6, MCP-1, TGF-β; ↑ IL-10.	Ferreira *et al.*, 2009; Shenoy *et al.*, 2009; Yamazato *et al.*, 2009
Joints: antigen-induced arthritis (AIA)	Ang-(1-7); AVE0991	↓ Leukocytes rolling and adhesion; ↓ Neutrophils influx; ↓ TNF-α , IL-1β, CXCL1; ↓ Hypernociception intensity.	Silveira *et al.*, 2010a
Kidney: type 2 diabetes model	Ang-(1-7)	↓TNF-α, IL-6.	Giani *et al.*, 2012
Kidney: unilateral ureteral obstructive nephropathy	ACE2 deficiency	↑ TNF-α, IL-1β and MCP-1	Liu *et al.*, 2012b
Vascular endothelium	ACE2 deficiency	↑ Vascular inflammation and atherosclerosis; ↑ Expression adhesion molecules (VCAM); ↑ TNF-α, MCP-1, IL-6; ↑ MMPs.	Thomas *et al.*, 2012
Aorta	ACE2 deficiency	↑ TNF-α, IL-1β, IL-6, MCP-1; ↑ Activation of NADPH oxidase; ↑ Superoxide production; ↑ Profilin-1 expression; ↑ AKT-ERK-eNOS signalling.	Jin *et al.*, 2012
Heart	ACE2 deficiency	↑ Neutrophil accumulation, IL-1β, IL-6, MCP-1, MMPs	Oudit *et al.*, 2007

AVE 0991 is a Mas receptor agonist and mimics the effects of Ang-(1-7) (Wiemer G *et al.*, 2002).
CXCL, chemokine receptor; ET-1, endothelin 1; MCP-1, monocyte chemoattractant protein 1.

tion and function of leukocytes (Piqueras *et al.*, 2000; Nabah *et al.*, 2004; Mateo *et al.*, 2006; Nabah *et al.*, 2007; Souza and Costa-Neto, 2012). Ang II contributes significantly to the process of leukocyte migration *in vivo* by modifying the interaction of leukocytes with endothelial cells (Piqueras *et al.*, 2000; Alvarez *et al.*, 2001; Mateo *et al.*, 2006; Nabah *et al.*, 2007; Silveira *et al.*, 2010a; Company *et al.*, 2011). Consistently with the latter findings, inhibition of ACE or blockade of AT_1 receptors decreases leukocyte endothelial cell interactions in various models of inflammation. Recently, our group demonstrated that administration of Ang-(1-7) or a synthetic analogue, AVE 0991 (Ang-(1-7) Mas receptor agonist), decreased rolling and adhesion of leukocytes to the microvascular endothelium at inflamed joints in a model of antigen-induced arthritis (Silveira *et al.*, 2010a). Blockade of leukocyte adhesion was associated with decreased neutrophil influx into the joints and improvement of joint hypernociception (Silveira *et al.*, 2010a). Altogether, these studies suggest that the ACE2/Ang-(1-7)/Mas axis counter-regulates the actions of the ACE/Ang II/AT_1 axis in the context of leukocyte recruitment.

Macrophages are believed to play a key role in development and progression of atherosclerosis, a pathological process in which there is altered production of many pro-inflammatory cytokines and up-regulation of adhesion molecules within atherosclerotic plaques (Libby, 2009). Few studies have reported that the ACE2/Ang-(1-7)/Mas axis decreases macrophage function (Thomas *et al.*, 2010; Souza and Costa-Neto, 2012). Mas transcripts are up-regulated in macrophages after LPS exposure and Ang-(1-7) was able to regulate mRNA levels of pro-inflammatory cytokines IL-6 and TNF-α and to decrease the phosphorylation levels of Src kinases (Souza and Costa-Neto, 2012). Using macrophages isolated from the bone marrow of ACE2 knockout mice, there was increased expression of the adhesion molecule VCAM-1 and high levels of various cytokines, including TNF-α, CCL2

and IL-6, following LPS induction (Thomas *et al.*, 2010). Moreover, the overexpression of ACE2 inhibited expression of MCP-1 induced by Ang II in macrophages and this effect seemed to be mediated by increased levels of Ang-(1-7) (Guo *et al.*, 2008). Altogether, these studies clearly show that Ang-(1-7) can activate Mas receptor on the surface of leukocytes and usually inhibit their pro-inflammatory function (Figure 1).

The ACE2/Ang-(1-7)/Mas axis regulates fibrogenesis and remodelling

In addition to regulating leukocyte influx and parameters of acute inflammation (Table 1), there is good evidence that Ang II contributes significantly to fibrogenesis and organ remodelling, both of which are features of chronic inflammation (Table 2). Ang II acting on AT_1 receptors promotes hypertrophy (Sadoshima and Izumo, 1993) and stimulates fibroblast proliferation and expression of extracellular matrix proteins (Rosenkranz, 2004). The latter actions of ACE/Ang II/AT_1 axis appear to be blocked by ACEi and ARBs. Indeed, the beneficial effects of these RAS blockers in the context of chronic renal and cardiovascular diseases are, at least in part, attributed to inhibition of tissue fibrosis and remodelling (Lindholm *et al.*, 2002; Weir, 2007; Gerc and Buksa, 2010; Tocci and Volpe, 2011). In contrast to the fibrogenic and proliferative actions of the ACE/Ang II/AT_1 axis, it has been suggested that the ACE2/Ang-(1-7)/Mas axis exerts anti-fibrogenic and anti-proliferative actions (Tallant and Clark, 2003; Gallagher and Tallant, 2004; Iwata *et al.*, 2005; Tallant *et al.*, 2005; Su *et al.*, 2006; Pereira *et al.*, 2007), as detailed below. Thus, imbalances between the two axes of the RAS plays a major role in the pathogenesis of several fibrotic diseases (Santos *et al.*, 2003b; Tallant *et al.*, 2005; Kuba *et al.*, 2006; Lubel *et al.*, 2009; Ferreira *et al.*, 2012).

In models of cardiac fibrosis and remodelling, Grobe *et al.* (2007) showed that administration of Ang-(1-7) prevented cardiac fibrosis induced by Ang II in Sprague-Dawley rats (Grobe *et al.*, 2007). In trained two-kidney one-clip hypertensive rats, administration of Ang-(1-7) decreased fibrosis and it was accompanied by up-regulation of Mas, AT_2 and endothelial NOS phosphorylation in the heart (Shah *et al.*, 2012). ACE2-deficient mice, which have lower levels of Ang-(1-7), exhibited early cardiac hypertrophy (Oudit *et al.*, 2007) and adverse ventricular remodelling after myocardial infarction (Kassiri *et al.*, 2009). ACE2 deficiency also resulted in progressive cardiac fibrosis in models of aging and cardiac pressure overload (Crackower *et al.*, 2002; Yamamoto *et al.*, 2006; Nakamura *et al.*, 2008). In contrast, ACE2 overexpression reversed cardiac hypertrophy and fibrosis in mice (Huentelman *et al.*, 2005; Der Sarkissian *et al.*, 2008). The anti-proliferative and anti-fibrotic effect of Mas activation in the heart may also involve modulation of several extracellular matrix proteins (Gava *et al.*, 2012). Neonatal and adult Mas-deficient mice showed significantly higher levels of collagen types I and III and fibronectin and reduced levels of collagen IV in both right ventricle and AV valves (Gava *et al.*, 2012).

There is now a large body of evidence suggesting that the presence of Ang II and AT_1 receptors are required for experimental lung fibrosis (Uhal *et al.*, 2012). There is also evidence that the ACE2/Ang-(1-7)/Mas axis may have a regulatory role in the context of pulmonary fibrosis. In this regard, ACE2 mRNA, protein and enzymatic activity were decreased in human idiopathic pulmonary fibrosis and in both rat and mouse models of experimental lung fibrosis induced by bleomycin (Li *et al.*, 2008). Blockade of ACE2 in mice increased pulmonary Ang II levels and enhanced bleomycin-induced lung fibrosis through a mechanism dependent on AT_1 receptors (Li *et al.*, 2008). In the latter study, the role of Ang-(1-7) was not investigated.

More recently, Ang-(1-7) was shown to attenuate ovalbumin-induced leukocyte influx in airways spaces, perivascular and peribronchial inflammation, fibrosis and goblet cell hyper/metaplasia in a murine model of asthma (El-Hashim *et al.*, 2012). Mechanistically, Ang-(1-7) reduced the phosphorylation of ERK1/2 and IκB-α and, consequently, decreased the activation of NF-κB in ovalbumin-challenged mice (El-Hashim *et al.*, 2012). It will be interesting to investigate whether such effects of Ang-(1-7) will be reproduce in other models of chronic pulmonary inflammation in which fibrogenesis is more prominent.

TGF beta (TGF-β1) has been reported to be the most potent pro-fibrotic cytokine (Annes *et al.*, 2003; Goodwin and Jenkins, 2009). Extensive evidence suggests a direct link between the RAS and TGF-β, indicating that TGF-β1 acts downstream of Ang II (Rosenkranz, 2004). Thus, Ang II stimulates TGF-β1 mRNA and protein expression by cardiomyocytes and cardiac fibroblasts (Campbell and Katwa, 1997; Gray *et al.*, 1998) and the treatment with ACEi or ARBs decreased TGF-β1 levels in hypertrophied (Kim *et al.*, 1996) and infarcted hearts (Sun *et al.*, 1998; Yu *et al.*, 2001). Recently, other studies have evaluated the role of ACE2/Ang-(1-7)/Mas axis in modifying expression of TGF-β and key components of the TGF-β pathway (Iwata *et al.*, 2005; Grobe *et al.*, 2007; Zeng *et al.*, 2009; Shenoy *et al.*, 2010; Marques *et al.*, 2012) (Table 2). Ang-(1-7) decreased TGF-β1 mRNA levels in cultured cardiac fibroblasts (Iwata *et al.*, 2005), reduced plasma levels of TGF-β1 in rat model of myocardial infarction (Grobe *et al.*, 2007) and improved vascular remodelling via down-regulation of TGF-β and inhibition of the Smad2 pathway (Zeng *et al.*, 2009). The blockade of Mas with A-779 increased liver tissue levels of TGF-β1 in a rat model of hepatic fibrosis (Pereira *et al.*, 2007). Recently, it has been shown that a p.o. formulation including Ang-(1-7) in hydroxypropyl β-cyclodextrin produced improvement of diastolic and systolic functions and reduction of the expression of fibrosis scar markers (TGF-β and collagen type I) in a rat model of myocardial infarction induced by left coronary artery occlusion (Marques *et al.*, 2012). In another study, intratracheal administration of lentiviruses expressing Ang-(1-7) or ACE2 induced overexpression of Ang-(1-7) in lungs of rats given bleomycin. More importantly, there was reduced expression of TGF-β and significant reduction of pulmonary fibrosis (Shenoy *et al.*, 2010). Altogether, these studies suggest that regulation of TGF-β synthesis appears to contribute significantly to the anti-fibrogenic effects of the ACE2/Ang-(1-7)/Mas axis.

In addition to blocking TGF-β synthesis, several studies have evaluated the capacity of the ACE2/Ang-(1-7)/Mas axis to regulate TGF-β-independent molecular pathways believed

Table 2

ACE2/Ang-(1-7)/Mas receptor axis and modulation of fibrogenesis and remodelling

Organ/model	Compounds or strategy used	Effect	References
Kidney: type 2 diabetes model	Ang-(1-7)	↓ Mesangial expansion; ↓ TGF-β and fibronectin; ↓ mRNA and NOX activity.	Moon *et al.*, 2011
Kidney: proximal tubular cells	Ang-(1-7)	↓ Phosphorylation of ERK1/2, p38 MAPKs and JNK.	Su *et al.*, 2006
Kidney: type 2 diabetes model	Ang-(1-7)	↓ Renal fibrosis; ↓ Renal oxidative stress.	Giani *et al.*, 2012
Kidney: unilateral ureteral obstruction	ACE2 gene deletion	↑ Tubulointerstitial fibrosis; ↑ ERK1/2; ↑ TGF-β/Smad2/3; ↑ NF-κB.	Liu *et al.*, 2012b
Heart: cardiac fibrosis	Ang-(1-7)	↓ Myocyte hypertrophy; ↓ Interstitial fibrosis.	Grobe *et al.*, 2007
Heart: cardiac fibrosis and hypertension	Ang-(1-7)	↓ Increases in myocyte, diameter and cardiac fibrosis.	Shah *et al.*, 2012
Heart: cardiac hypertrophy	ACE2 deletion	↑ Ventricular dilation; ↓ Intrinsic myocardial contractility.	Oudit *et al.*, 2007
Heart: ventricular remodelling after myocardial infarction	ACE2 deletion	↑ Mortality rate; ↑ Ventricular remodelling; worsening ventricular function; ↑ NADPH oxidase activity; ↑ Neutrophil infiltration.	Kassiri *et al.*, 2009
Heart: cardiac fibrosis in aging and cardiac pressure overload	ACE2 deletion	↑ Perivascular and interstitial fibrosis and disarray.	Yamamoto *et al.*, 2006
Heart: cardiac hypertrophy and fibrosis	ACE2 cDNA; ACE2 overexpression	↓ Cardiac hypertrophy and fibrosis; ↓ Myocardial tissue damage; ↑ LV wall motion and contractility; ↓ LV wall thinning and cardiac dysfunction.	Der Sarkissian *et al.*, 2008; Huentelman *et al.*, 2005
Heart: ventricle and AV valves	Mas deletion	↑ Collagen I and III and fibronectin; ↓ Reduce collagen IV; ↓Systolic tension and ↓ Posterior wall thickness; ↓ Left ventricular end-systolic dimension.	Gava *et al.*, 2012
Lung: pulmonary hypertension	ACE2, Ang-(1-7)	↓ TGF-β; ↓ Fibrosis.	Shenoy *et al.*, 2010
Lung: ovalbumin-induced asthma model	Ang-(1-7)	↓ Lymphocytes, neutrophils and eosinophils; ↓ Cellular infiltration; ↓ Fibrosis.	El-Hashim *et al.*, 2012
Heart	Mas deletion	↑ Collagen I, collagen III; ↑ Fibronectin; ↓ Collagen IV.	Santos *et al.*, 2006
Liver: hepatic fibrosis	A-779	↑ TGF-β1; ↑ Ishak score; ↑ Hydroxyproline.	Pereira *et al.*, 2007

A-779 is a Mas receptor antagonist (Santos RA *et al.*, 1994).
LV, left ventricle.

to be important for fibrogenesis. For example, treatment of cardiac fibroblasts with Ang-(1-7) reduced Ang II- or ET-1-stimulated increase in phospho-ERK1 and -ERK2 and, in contrast, Ang-(1-7) increased the immunoreactivity and mRNA of dual-specificity phosphatase (McCollum *et al.*, 2012). Ang-(1-7) administration also blocked Ang II-stimulated phosphorylation and activation of ERK1 and ERK2 and MAPK activity in cardiac myocytes (Tallant *et al.*, 2005). Moreover, incubation of cardiac fibroblasts with ET-1 increased COX-2 and PG synthase mRNAs, while Ang-(1-7) blocked the increase of both enzymes, suggesting that the heptapeptide regulates the balance between proliferative and anti-proliferative PGs

(McCollum *et al.*, 2012). Treatment of cardiac fibroblasts with recombinant human ACE2 or ACE2 activators reduced collagen production and Ang II-mediated increase in phospho-MAP kinases. Both effects were blocked by the Mas receptor antagonist A-779 (Zhong *et al.*, 2010; Ferreira *et al.*, 2011). These studies suggest that Ang-(1-7) may block key signalling pro-fibrogenic events initiated by Ang II, endothelins and other pro-fibrogenic molecules.

Many studies have reported that Ang-(1-7) exerts antiproliferative actions at different tissues (Tallant and Clark, 2003; Gallagher and Tallant, 2004; Iwata *et al.*, 2005; Tallant *et al.*, 2005; Santos *et al.*, 2006; Su *et al.*, 2006; Pereira *et al.*, 2007). The antiproliferative effects of Ang-(1-7) in vascular smooth muscle cells (Tallant *et al.*, 2005), liver tissue (Pereira *et al.*, 2007) and cardiomyocytes (Iwata *et al.*, 2005) seem to be mediated by Mas. In support of the latter possibility, Mas-deficient mice exhibited impairment of heart function associated with changes in collagen expression towards a pro-fibrotic profile (Santos *et al.*, 2006). The molecular mechanisms that underlie these antiproliferative action of Ang-(1-7) include stimulation of PGs and cAMP production and inhibition of MAP kinases (Tallant and Clark, 2003). For example, Ang-(1-7) caused inhibition of growth of human lung cells via reduction in serum-stimulated phosphorylation of ERK 1 and ERK 2 (Gallagher and Tallant, 2004). In cultured vascular smooth muscle cells, Ang-(1-7) inhibited Ang II-activated protein kinase C and MAPK (Tallant *et al.*, 2005). Similarly, in cultured human endothelial cells, Ang-(1-7) inhibited Ang II-stimulated phosphorylation of c-Src and ERK 1/2, and blunted Ang II-stimulated NADPH oxidase activity (Sampaio *et al.*, 2007). These effects of Ang-(1-7) were associated with phosphorylation of Src homology 2 domain containing protein tyrosine phosphatase-2 and blocked by the Mas antagonist A-779 (Sampaio *et al.*, 2007).

As the ERK cascade is activated in response to different stimuli, such as growth factors, cytokines or DNA-damaging agents, the stimulation of the ACE2/Ang-(1-7)/Mas axis could also be effective in halting glomerulosclerosis. In this regard, Su *et al.* (2006) showed that Ang-(1-7) inhibits Ang II-stimulated phosphorylation of ERK1/2, p38 MAPKs and JNK in culture rat proximal tubular cells, an effect reversed by pretreatment with A-779 (Su *et al.*, 2006). Ang-(1-7) also prevented Ang II-induced production of TGF-β1 in proximal tubular cells (Su *et al.*, 2006). In cultured pig kidney tubular cells, Ang-(1-7) inhibits glucose-induced phosphorylation of p38 MAPK, an effect attributed to the increased activity of SHP-1 (Gava *et al.*, 2009). Ang-(1-7) attenuates high-glucose-induced TGF-β1 production, but has no effect on enhanced fibronectin or collagen levels in these cells (Gava *et al.*, 2009). Thus, the generation of Ang-(1-7) by proximal tubular ACE2 could counteract the proliferative effects of locally produced Ang II (Su *et al.*, 2006).

The ACE2/Ang-(1-7)/Mas axis in renal physiology

Many studies have shown the role of Ang-(1-7) in regulating renal haemodynamics, glomerular filtration and tubular resorption (see reviews by Simoes e Silva and Flynn, 2012; Zimmerman and Burns, 2012; Figure 2). Renal effects of Ang-(1-7) are complex and may not always be opposite to those elicited by Ang II. While Ang II markedly raises efferent glomerular arteriolar resistance and does not change afferent arteriolar resistance, Ang-(1-7) directly and indirectly vasodilates afferent arterioles and increases renal blood flow by acting via Mas with release of PGs and NO (Ren *et al.*, 2002; Sampaio *et al.*, 2003; Stegbauer *et al.*, 2004; Botelho-Santos *et al.*, 2007). As observed for Ang II acting at the proximal tubule, Ang-(1-7) also exerts differential effects according to peptide concentration and nephron site (Simoes e Silva and Flynn, 2012; Zimmerman and Burns, 2012 for review). *In vitro* studies and findings in anaesthetized animals have suggested that Ang-(1-7) acts as a natriuretic/diuretic hormone by directly inhibiting sodium reabsorption (DelliPizzi *et al.*, 1994; Vallon *et al.*, 1997; Lopez Ordieres *et al.*, 1998; Handa, 1999; Burgelova *et al.*, 2002) or by modulating Ang II actions (Burgelova *et al.*, 2002; Lara Lda *et al.*, 2006). In contrast, other studies showed that Ang-(1-7) has an antidiuretic effect in water-loaded animals, probably acting at distal nephron sites via a Mas-mediated increase in water transport (Santos and Baracho, 1992; Garcia and Garvin, 1994; Santos *et al.*, 1996; 2001; Simoes e Silva *et al.*, 1997; 1998; Baracho *et al.*, 1998; Magaldi *et al.*, 2003; Pinheiro *et al.*, 2004; Ferreira *et al.*, 2006). In addition, acute and chronic administration of Mas antagonists increased basal urinary volume, water excretion and glomerular filtration rate in normotensive and in spontaneous hypertensive rats (Santos *et al.*, 1996; 2003a; Simoes e Silva *et al.*, 1998; Magaldi *et al.*, 2003). Indeed, 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 these divergent effects on renal function (Figure 2).

The intrarenal levels of Ang II and Ang-(1-7) are regulated in accordance with homeostatic needs. Ang-(1-7) is present in the kidney at concentrations that are comparable to Ang II (Chappell *et al.*, 1998a; 2001; 2004; Allred *et al.*, 2000; Ferrario *et al.*, 2002; 2005). Ang-(1-7) is the main product obtained in isolated proximal tubules (Chappell *et al.*, 2001) and it is also present in distal convoluted tubules and collecting ducts (Ferrario *et al.*, 2002). The processing pathways for Ang-(1-7) synthesis are different in the circulation and in the kidney. In the circulation, neutral endopeptidase (NEP) is one of the major enzymes that produce Ang-(1-7) from Ang I or Ang-(1–9) (Chappell *et al.*, 1998b), while in the kidney, NEP may contribute to both the synthesis as well as the degradation of Ang-(1-7) (Allred *et al.*, 2000; Chappell *et al.*, 2001). It appears that ACE2 is the main enzyme responsible for the conversion of Ang II into Ang-(1-7) in the kidney (Chappell *et al.*, 2004; Ferrario *et al.*, 2005). It has been demonstrated that the distribution of ACE2 within renal tubules is similar to that of Ang-(1-7) (Chappell *et al.*, 2004) and rats receiving either lisinopril or losartan increased ACE2 activity and urinary levels of Ang-(1-7) (Ferrario *et al.*, 2005). It was hypothesized that ACE inhibition or AT_1 receptor blockade might increase intrarenal formation of Ang-(1-7) through ACE2 activation (Ferrario *et al.*, 2005). It should also be pointed that there are gender differences in renal activity of ACE2 and in the mRNA expression for this enzyme at renal tissue. Ji and co-workers (Ji *et al.*, 2008) showed that ovariectomy decreased ACE2 protein and mRNA expression in

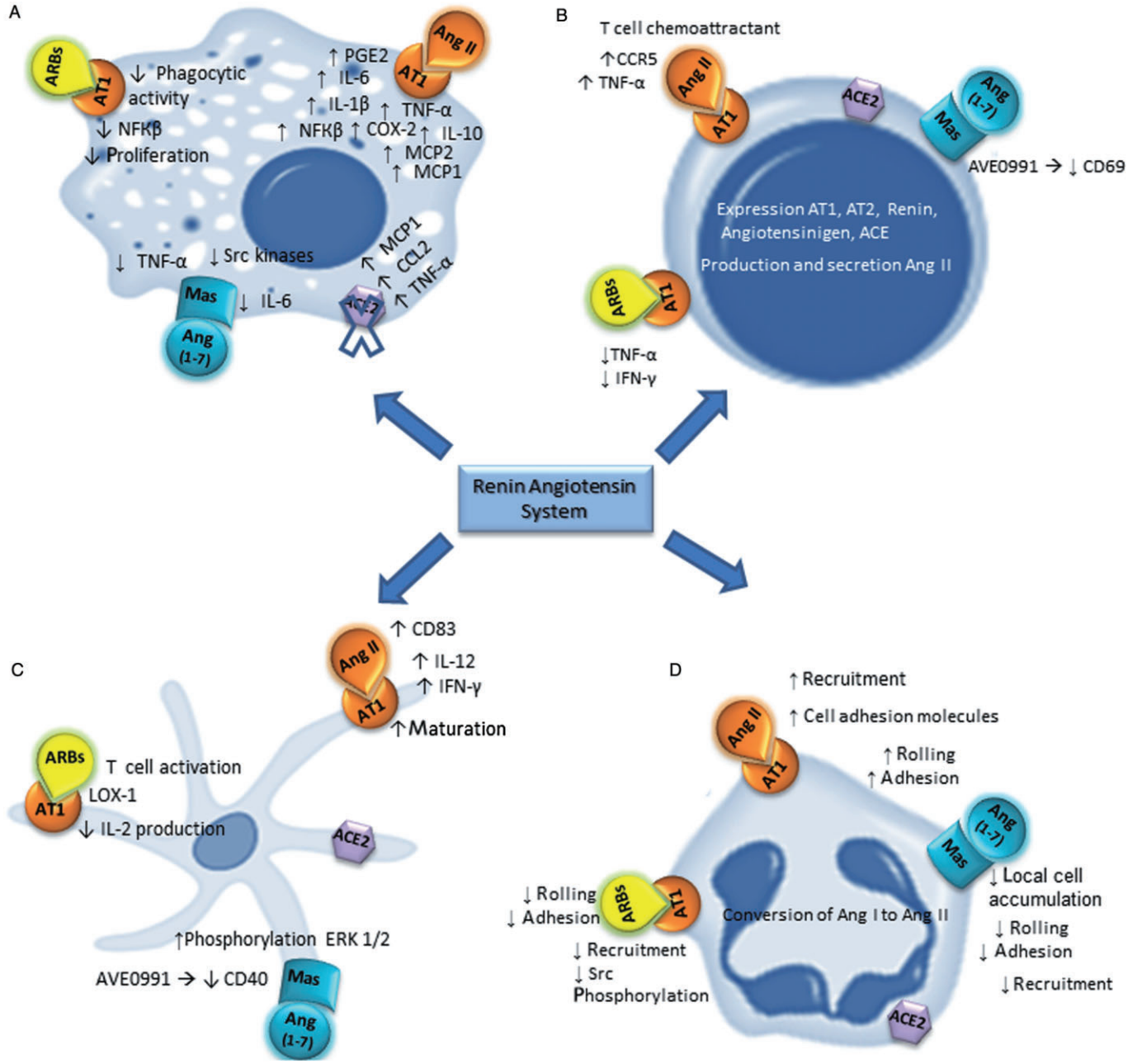

Figure 2

Schematic representation of the RAS effects on inflammatory cells. Main components of the RAS are present on macrophages (A), lymphocytes (B), dendritic cells (C) and neutrophils (D) and modulate inflammatory response by affecting cytokine production and release, cellular migration and signalling pathways.

hypertensive rats, while 17-beta-estradiol replacement prevented these effects. In addition, the infusion of Ang-(1-7) attenuated renal injury, which was exacerbated by ovariectomy in this experimental model (Ji *et al.*, 2008). More recently, higher ACE2 activity was detected in the kidney of male mice compared to females, a difference which was linked to the presence of 17-beta-estradiol in the ovarian hormone milieu (Liu *et al.*, 2010). Therefore, changes in 17-beta-estradiol probably lead to fluctuations in renal ACE2 activity in women during puberty, pregnancy and menopause with potential physiological implications.

Immunohistochemical data have shown a similar distribution for Ang-(1-7), ACE2 and Mas within the kidney (Chappell *et al.*, 2004), placing the key components together for activation and activity. Consistent with the latter finding, it has been shown that the biological effects of Ang-(1-7) in the kidney are primarily mediated by Mas (Santos *et al.*, 2003b; Pinheiro *et al.*, 2004; 2009). Mas-deficient mice have

fluid retention, glomerular hyperfiltration, microalbuminuria, increased collagen deposition and mRNA overexpression of AT_1 receptor and TGF-β in renal tissues (Pinheiro *et al.*, 2009). These results indicate that the lack of Mas may lead to RAS imbalance with unopposed actions of the ACE/Ang II/AT_1 axis in the kidney.

The ACE2/Ang-(1-7)/Mas axis in renal diseases

Experimental studies

There is now good recent evidence that Ang-(1-7) may exert a protective role in the context of experimental models of renal diseases (Pinheiro *et al.*, 2009; Silveira *et al.*, 2010b; Zhang *et al.*, 2010; Giani *et al.*, 2011; Barroso *et al.*, 2012; Liu *et al.*, 2012b; Xue *et al.*, 2012). For example, infusion of Ang-(1-7) reduced glomerulosclerosis by opposing Ang II effects in experimental glomerulonephritis (Zhang *et al.*, 2010). Recently, we reported that administration of the Mas agonist, AVE 0991, had renoprotective effects in experimental acute renal injury, as seen by improvement of function, decreased tissue injury, prevention of local and remote leukocyte infiltration and reduced release of the chemokine CXCL1 (Barroso *et al.*, 2012). In adriamycin-induced nephropathy, p.o. administration of AVE 0991 also improved renal function parameters, reduced urinary protein loss and attenuated histological changes (Silveira *et al.*, 2010c). The administration of Ang-(1-7) for 12 weeks in 5/6 nephrectomized male C57Bl/6 mice reduced blood pressure, attenuated elevations in plasma urea and creatinine and preserved cardiac function (Li *et al.*, 2009). On the other hand, despite reducing blood pressure to the same extend as Ang-(1-7), the antihypertensive agent hydralazine was not able to improve renal function and to avoid cardiac changes, thereby suggesting that renoprotection obtained with Ang-(1-7) was not mediated merely by the control of hypertension (Li *et al.*, 2009).

The effects of exogenous administration of Ang-(1-7) on the course of experimental diabetes are also particularly interesting. Ang-(1-7) caused significant reduction in urinary protein excretion without affecting blood pressure in male adult STZ-diabetic rats compared with untreated diabetic animals (Benter *et al.*, 2007). Renal arteries isolated from untreated STZ-diabetic rats exhibited enhanced vasoconstrictive responses to norepinephrine, endothelin-1 and Ang II, which were significantly attenuated by the administration of Ang-(1-7) (Benter *et al.*, 2007). In a subsequent study, the same research group treated diabetic spontaneous hypertensive rats with identical dose of Ang-(1-7) and obtained for a second time a significant reduction in urinary protein excretion with no changes in mean arterial pressure (Benter *et al.*, 2008). Furthermore, Ang-(1-7) inhibited renal nitrogen oxide (NOX) activity in these animals, suggesting that the heptapeptide normalized the vascular responses to vasoconstrictors and prevented renal NOX-induced oxidative stress (Benter *et al.*, 2008). In an experimental model of type 2 diabetes, the KK-A^y/Ta mouse, the treatment with Ang-(1-7) also attenuated the stimulatory effects of Ang II in mesangial expansion, TGF-β and fibronectin mRNA and NOX activity, thus counteracting Ang II-induced glomerular injury (Moon *et al.*, 2011). In Zucker diabetic fatty rats, Ang-(1-7) induced reduction in triglyceridemia, proteinuria, and systolic blood pressure together with restoration of creatinine clearance (Giani *et al.*, 2012). Additionally, Ang-(1-7) reduced renal fibrosis, attenuated renal oxidative stress and decreased renal immunostaining of IL-6, TNF-α, ED-1, HIF-1α and NGAL to values similar to those displayed by control animals (Giani *et al.*, 2012). A few studies have evaluated the molecular mechanisms of the renoprotection elicited by Ang-(1-7). For example, it has been shown that both AT_1 and Mas receptors were co-distributed in renal mesangial cells of rats and that Ang-(1-7), through the binding to Mas, counteracted the stimulatory effects of Ang II on ERK1/2 and TGF-β_1 pathways mediated by AT_1 receptors (Xue *et al.*, 2012). In conjunction, available studies suggest that the renoprotective effects of Ang-(1-7) seem to involve the modulation of oxidative stress, inflammation and fibrosis at renal tissue.

Many studies have also suggested a protective role for ACE2 in models of renal damage or disease (Oudit *et al.*, 2006; Wysocki *et al.*, 2006; Ye *et al.*, 2006; Soler *et al.*, 2007; Wong *et al.*, 2007; Dilauro *et al.*, 2010). Acquired or genetic ACE2 deficiency exacerbated renal damage and albuminuria in experimental models, possibly facilitating the damaging effects of Ang II (Oudit *et al.*, 2006; Wysocki *et al.*, 2006; Ye *et al.*, 2006; Soler *et al.*, 2007; Wong *et al.*, 2007; Dilauro *et al.*, 2010). In addition, chronic blockade of ACE2 with the enzyme inhibitor MLN-4760 in control or diabetic mice produced albuminuria and matrix protein deposition (Soler *et al.*, 2007). More recently, it has been shown that ACE2 was down-regulated in the renal cortex of mice that underwent subtotal nephrectomy and presented proteinuria via an AT_1 receptor-dependent mechanism (Dilauro *et al.*, 2010). Accordingly, renal expression of ACE2 was also reduced in an experimental model of renal ischaemia/reperfusion (Silveira *et al.*, 2010b). In a model of unilateral ureteral obstruction, the deletion of ACE2 gene resulted in a fourfold increase in the ratio of intrarenal Ang II/Ang-(1-7) and these changes were associated with the development of progressive tubulointerstitial fibrosis and inflammation with high levels of TNF-α, IL-1β and MCP-1 (Liu *et al.*, 2012b). Enhanced renal fibrosis and inflammation were attributed to marked increase in intrarenal Ang II signalling (AT_1/ERK1/2), TGF-β_1/Smad2/3, and NF-κB signalling pathways (Liu *et al.*, 2012b). Recently, it was shown that dual RAS blockade normalized ACE2 expression and prevented hypertension, albuminuria, tubulointerstitial fibrosis and tubular apoptosis in Akita angiotensinogen-transgenic mice (Lo *et al.*, 2012). Taken together, these findings suggest that decreased ACE2 activity may be involved in the pathogenesis of kidney disease, possibly by disrupting the metabolism of angiotensin peptides (Ferrario, 2011; Simoes e Silva and Flynn, 2012). Taking into account the enzymatic properties of the two ACEs and of the two main mediators Ang II and Ang-(1-7), an increased ACE/ACE2 activity ratio would lead to increased Ang II generation and high catabolism of Ang(1-7), while a decreased ratio will decrease Ang II and increase Ang-(1-7) levels (Ferrario, 2011; Simoes e Silva and Flynn, 2012).

A few reports have suggested that Ang-(1-7) may exacerbate renal injury paradoxically in certain experimental conditions, suggesting that dose or route of administration, state of activation of the local RAS, cell-specific signalling or non-

Mas-mediated pathways may contribute to these deleterious responses (Zimmerman and Burns, 2012). For instance, Esteban *et al*. (Esteban *et al*., 2009) reported that renal deficiency of Mas diminished renal damage in unilateral ureteral obstruction and in ischaemia/reperfusion injury, and that the infusion of Ang-(1-7) to wild-type mice elicited an inflammatory response. In other animal models of renal diseases, discrepant results have been also reported. While Zhang *et al*. (2010) showed that a 5 day infusion of Ang-(1-7) improved glomerulosclerosis in a rat model of glomerulonephritis, Velkoska *et al*. (2011) verified that a 10 day infusion of the same concentration of Ang-(1-7) in rats with subtotal nephrectomy was associated with deleterious effects on blood pressure and heart function. Although these findings are conflicting, cell-specific signalling pathways associated with Ang-(1-7) in the kidney could play a role in the variable response. Hence, in the proximal tubule, Ang-(1-7) displays growth inhibitory properties and antagonizes the effects of Ang II (Su *et al*., 2006), whereas, in human mesangial cells, the heptapeptide seems to stimulate cell growth pathways by increasing arachidonic acid release and by MAPK phosphorylation (Zimpelmann and Burns, 2009). These effects of Ang-(1-7) were blocked by A-779, but unaffected by AT_1 or AT_2 receptor antagonism (Zimpelmann and Burns, 2009). Furthermore, Ang-(1-7) did not prevent Ang II- or high glucose-induced p38 MAPK phosphorylation, as occurred in proximal tubular cells. Ang-(1-7) also stimulated production of TGF-β1, fibronectin and collagen IV in these cells, effects blocked by inhibition of p38 MAPK (Zimpelmann and Burns, 2009). Results obtained in rat mesangial cells are also divergent. While Liu *et al*. (2012a) reported that Ang-(1-7) stimulates ERK1/2 phosphorylation via Mas activation, Oudit and co-workers (Oudit *et al*., 2010) showed that Ang-(1-7) inhibits high glucose-stimulated NOX activation. In addition, the Mas antagonist, A-779, partly prevented the attenuation of high-glucose-stimulated NOX activity by human recombinant ACE2 in these cells, suggesting a protective role for Ang-(1-7) (Oudit *et al*., 2010). Accordingly, in primary cultures of mouse mesangial cells, Moon *et al*. (2011) showed that Ang-(1-7) attenuated Ang II-induced MAPK phosphorylation, and expression of TGF-β1, fibronectin and collagen IV. In summary, these findings suggest variable responses to Ang-(1-7) in cultured mesangial cells, depending on species or culture conditions. Inhibition of Ang II- or high-glucose-induced pro-fibrotic pathways has been detected in primary cultures, whereas, in passage cell lines, findings suggest stimulation of growth and fibrogenic pathways. It must be said, however, that majority of experiments do suggest an overall renoprotective effect of administering Ang-(1-7) *in vivo*.

Clinical studies

Ang-(1-7) can be measured in plasma and urine samples collected in healthy subjects and in patients with diverse clinical conditions (Luque *et al*., 1996; Ferrario *et al*., 1998; Azizi and Menard, 2004; Simoes e Silva *et al*., 2004; 2006; Kocks *et al*., 2005; Nogueira *et al*., 2007; Vilas-Boas *et al*., 2009). Changes in blood pressure, extracellular volume, sodium intake and renal function were able to modify the levels of Ang-(1-7) measured in plasma, renal tissue and urine (Luque *et al*., 1996; Ferrario *et al*., 1998; Azizi and Menard, 2004; Simoes e SIlva *et al*., 2004; 2006; Kocks *et al*., 2005; Nogueira *et al*., 2007; Vilas-Boas *et al*., 2009). In addition, the concentration of the heptapeptide may differ in plasma and urine samples of the same subject, as reported by Ferrario and co-workers (Ferrario *et al*., 1998). In the latter study, untreated adults with primary hypertension exhibited lower urinary levels of Ang-(1-7) than normotensive controls, which were inversely correlated with blood pressure (Ferrario *et al*., 1998). In paediatric patients, we detected significant differences in circulating Ang II and Ang-(1-7) levels in renovascular disease and in primary hypertension (Simoes e Silva *et al*., 2004). Children with renovascular disease had plasma Ang II levels higher than plasma Ang-(1-7) and the successful correction of renal artery stenosis brought circulating levels of angiotensins back to values detected in healthy subjects (Simoes e Silva *et al*., 2004). On the other hand, patients with primary hypertension had selective elevation of plasma Ang-(1-7), while levels of Ang I and Ang II were similar to those in healthy subjects and the treatment of hypertension did not change circulating angiotensins (Simoes e Silva *et al*., 2004). The physiopathological meaning of the selective elevation of plasma Ang-(1-7) in primary hypertension is still unknown and raises the question whether this is a compensatory mechanism to oppose deleterious effects of Ang II or whether Ang-(1-7) at supra physiological concentrations may lead to sodium and water retention.

Recently, Mizuiri and co-workers (Mizuiri *et al*., 2011) demonstrated that renal biopsies from patients with IgA nephropathy had significantly reduced glomerular and tubulointerstitial immunostaining for ACE2 compared with healthy controls. On the other hand, glomerular ACE staining was increased. These findings raise the possibility that an upward shift in the intrarenal ACE/ACE2 ratio favouring increased synthesis of Ang II and reduced Ang-(1-7) might lead to progressive nephron loss in this condition (Mizuiri *et al*., 2011). In paediatric patients exhibiting chronic kidney disease, higher levels of plasma Ang-(1-7) and Ang II were also detected in hypertensive patients when compared to normotensives at the same stage of renal disease (Simoes e Silva *et al*., 2006). While the presence of hypertension increased plasma concentrations of both peptides, the progression to end-stage renal disease was accompanied by more pronounced elevation only in Ang-(1-7) levels, suggesting a deviation in RAS metabolism towards Ang-(1-7) synthesis (Simoes e Silva *et al*., 2006). Whether the elevation in plasma Ang-(1-7) provides a counter-regulatory mechanism against Ang II-mediated vasoconstriction or nephron injury in human chronic kidney disease remains to be determined.

Anti-proliferative and anti-fibrogenic pathways clearly contribute to the renoprotection obtained with ACEi and ARBs (Schmieder *et al*., 2011). An altered balance between Ang II and Ang-(1-7) might be relevant for the mechanism of renoprotective actions of ACEi and ARBs, as chronic treatment with these medications increases plasma levels of Ang-(1-7) (Azizi and Menard, 2004; Kocks *et al*., 2005; Simoes e Silva and Flynn, 2012). Studying healthy subjects, Kocks *et al*. (2005) showed that, during ACE inhibition, administration of a low-sodium diet did not affect plasma levels of Ang II but induced a significant elevation in Ang-(1-7) concentration.

Consequently, the combination of ACE inhibition and low-sodium diet appeared to shift the balance between Ang-(1-7) and Ang II towards Ang-(1-7), which in turn might contribute to the therapeutic benefits of ACE inhibition (Kocks *et al.*, 2005). Another relevant aspect that needs to be considered is the complex interaction between Mas and AT_1 receptor. Kostenis and co-workers showed that Mas can hetero-oligomerize with AT_1 and, by so doing, inhibit the actions of Ang II (Kostenis *et al.*, 2005). Thus, Mas may act as a physiological antagonist of AT_1 receptor signalling.

Concluding remarks

The discovery that Ang-(1-7) offsets the major biological effects of Ang II has contributed to the realization that the RAS is composed of two opposing axes. The first axis is constituted by the enzyme ACE, with Ang II as the end product, and the AT_1 receptor as the main effector mediating the biological actions of Ang II. The second axis results from ACE2-mediated hydrolysis of Ang II, leading to production of Ang-(1-7), with Mas receptor as the main effector conveying the vasodilator, antiproliferative, anti-inflammatory and anti-fibrotic effects of Ang-(1-7). Activation of the ACE2/Ang-(1-7)/Mas axis decreases inflammatory cell function and fibrogenesis in diverse models of human diseases. In the context of experimental models of renal diseases, most studies suggest that the ACE2/Ang-(1-7)/Mas axis has a protective role. The renoprotective effects of Ang-(1-7) seem to involve the modulation of oxidative stress, leukocyte influx and activation, and fibrosis in renal tissues. The few data provided by human studies also indicate a beneficial role for the activation of this alternative RAS axis in patients with renal diseases. In addition, it has hypothesized that the beneficial effects of ACEi and ARBs in renal diseases might involve, at least in part, the elevation of plasma Ang-(1-7) levels.

Further studies are clearly needed to elucidate the mechanisms by which both RAS axes precisely act in cooperation to modulate inflammation, fibrosis and proliferation in renal disease and other disease states. Nevertheless, current knowledge do support the possibility that drugs which mimic or enhance the function of the ACE2/Ang-(1-7)/Mas axis may be beneficial for the treatment of chronic diseases with inflammatory, fibrotic and proliferative components. Clinical trials with such drugs will eventually demonstrate if this possibility will turn into useful therapeutics.

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Conflict of interest

The authors declare no conflict of interest.

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