Angiotensin-(1–7) in kidney disease: a review of the controversies

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ABSTRACT

Ang-(1–7) [angiotensin-(1–7)] is a biologically active heptapeptide component of the RAS (renin–angiotensin system), and is generated in the kidney at relatively high levels, via enzymatic pathways that include ACE2 (angiotensin-converting enzyme 2). The biological effects of Ang-(1–7) in the kidney are primarily mediated by interaction with the G-protein-coupled receptor Mas. However, other complex effects have been described that may involve receptor–receptor interactions with AT1 (angiotensin II type 1) or AT2 (angiotensin II type 2) receptors, as well as nuclear receptor binding. In the renal vasculature, Ang-(1–7) has vasodilatory properties and it opposes growth-stimulatory signalling in tubular epithelial cells. In several kidney diseases, including hypertensive and diabetic nephropathy, glomerulonephritis, tubulointerstitial fibrosis, pre-eclampsia and acute kidney injury, a growing body of evidence supports a role for endogenous or exogenous Ang-(1–7) as an antagonist of signalling mediated by AT1 receptors and thereby as a protector against nephron injury. In certain experimental conditions, Ang-(1–7) appears to paradoxically exacerbate renal injury, suggesting that dose or route of administration, state of activation of the local RAS, cell-specific signalling or non-Mas receptor-mediated pathways may contribute to the deleterious responses. Although Ang-(1–7) has promise as a potential therapeutic agent in humans with kidney disease, further studies are required to delineate its signalling mechanisms in the kidney under physiological and pathophysiological conditions.

INTRODUCTION

The RAS (renin–angiotensin system) prominently regulates cardiovascular and renal function. Although the eight-amino-acid product AngII (angiotensin II) is the most well-characterized component of the RAS, accumulating evidence suggests that other peptide products, including the heptapeptide Ang-(1–7) [angiotensin-(1–7)], exert biological effects. Ang-(1–7) is found in the circulation and in many tissues, including the heart and kidneys. Studies on the biological properties of Ang-(1–7) have led to a widespread consensus that

Key words: angiotensin, angiotensin-converting enzyme 2 (ACE2), diabetes, kidney, nephropathy, proteinuria.

Abbreviations: ACE, angiotensin-converting enzyme; ACE2-KO, ACE2-knockout; ACEi, ACE inhibitor; Ad-ACE2, ACE2-encoding adenoviral vector; AKI, acute kidney injury; Ang-(1–4), angiotensin-(1–4); Ang-(1–5), angiotensin-(1–5); Ang-(1–7), angiotensin-(1–7); AngI etc., angiotensin I etc.; AT1 receptor, AngII type 1 receptor; ARB, AT1 receptor blocker; BP, blood pressure; CKD, chronic kidney disease; ERK, extracellular-signal-regulated kinase; GFR, glomerular filtration rate; hrACE2, human recombinant ACE2; l-NAME, N\textsubscript{G}-nitro-arginine methyl ester; MAPK, mitogen-activated protein kinase; Mas-KO, Mas receptor knockout; NF-κB, nuclear factor κB; NOX, NADPH oxidase; Ns, nephrectomy; RAS, renin-angiotensin system; ROS, reactive oxygen species; RUPP, reduced uterine perfusion of pre-eclampsia; sEng, soluble endoglin; sFlt1, soluble fms-like tyrosine kinase-1; SHP, Src homology 2 domain-containing protein tyrosine phosphatase; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone SHR; STZ, streptozotocin; TGF, transforming growth factor; 2K1C, two kidney–one clip; UUO, unilateral ureteral obstruction; VEGF, vascular endothelial growth factor.

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it acts as a counter-regulatory peptide in the RAS, often opposing the vasoconstrictive and pro-proliferative actions of AngII. Recent years have witnessed a renewed interest in the role of Ang-(1–7) in renal pathophysiology, precipitated by two discoveries: (i) identification of the proto-oncogene Mas protein as a receptor for Ang-(1–7) in the kidney and other tissues [1], and (ii) cloning and characterization of ACE2 [ACE (angiotensin-converting enzyme) 2], a protein expressed in the kidney that degrades AngII and generates Ang-(1–7) [2,3].

Many kidney diseases are characterized by activation of the renal RAS and excessive generation of AngII, leading to vasoconstriction, sodium and water retention and elevated BP (blood pressure), as well as non-haemodynamic responses, such as the generation of ROS (reactive oxygen species), inflammatory mediators and pro-fibrotic cytokines. These adverse effects of AngII are mediated by interaction with plasma membrane AT1 receptors (AngII type 1 receptors). Thus pharmacological inhibition of the production or actions of AngII (with ACEis (ACE inhibitors) or ARBs (AT1 receptor blockers)) now represents an effective strategy to delay the progression of proteinuric CKD (chronic kidney disease) in experimental models and humans [4,5]. As Ang-(1–7) may be a counterbalancing factor in the RAS, it is logical to pursue its potential therapeutic role as a protective agent in progressive CKD. In the present article, we review the controversies regarding the effects of endogenous or exogenous Ang-(1–7) in experimental and human kidney diseases. The effects of Mas gene deletion and ACE2 manipulation are also examined in this context, although our focus is on the specific role of Ang-(1–7). For comprehensive presentations on the effects of ACE2 in kidney disorders, the reader is referred to recent excellent reviews on the subject [6,7].

**ANG-(1–7) SIGNALLING IN THE KIDNEY**

Under physiological conditions, Ang-(1–7) is present in plasma in picomolar concentrations, comparable with circulating levels of AngII. Renal levels of Ang-(1–7) are relatively high [8], suggesting that the kidneys are a source of synthesis, and Ang-(1–7) is found in substantial amounts in the urine [10]. Indeed, Ferrario et al. [9] reported a significant decrease in urinary excretion of Ang-(1–7) in untreated subjects with essential hypertension, compared with healthy volunteers, providing initial evidence for a physiological role of Ang-(1–7) in human BP regulation.

The formation of Ang-(1–7) in the kidney was considered for years to be primarily the result of hydrolysis of the substrate decapeptide AngI (angiotensin I), mediated by tissue endopeptidases [prolyl oligopeptidase (EC3.4.21.26), neutral endopeptidase (EC3.4.24.11; neprilysin), and oligopeptidase (EC3.4.24.15)], although this has not been conclusively demonstrated in the kidney. With the discovery of the monocarboxypeptidase ACE2, a novel pathway for the generation of Ang-(1–7) has been uncovered. ACE2 is highly expressed in cells of the proximal tubule, and is also found in renal vascular cells and glomerular epithelial cells [6,10–12]. Cleavage of the Pro7–Phe8 bond of AngII by ACE2 occurs with high catalytic efficiency, leading to the direct generation of Ang-(1–7) [13]. In addition, ACE2 cleaves AngI to Ang-(1–9) (angiotensin-(1–9)), which can then undergo ACE-dependent degradation to Ang-(1–7), although this pathway occurs with relatively low efficiency. Once formed, Ang-(1–7) is subject to ACE-dependent degradation to the inactive peptide Ang-(1–5) (angiotensin-(1–5)) [14]. Thus, in SHR (spontaneously hypertensive rats), pharmacological ACE inhibition raises circulating levels of Ang-(1–7), and this has been implicated in the antihypertensive response [15]. In brush border membranes and in urine, Ang-(1–7) may also be hydrolysed by neprilysin to the inactive peptide Ang-(1–4) (angiotensin-(1–4)) [16] (Figure 1).

*In vivo* detection of Ang-(1–7) was first reported in rat brain, adrenal and plasma in 1989 by Chappell et al. [17]. Ang-(1–7) has since been identified as the endogenous ligand for the G-protein-coupled receptor Mas [1], which is expressed in many tissues, including kidney, heart, vasculature, brain, liver, spleen, testis and lung [18]. Within the kidney, the Mas receptor has been functionally localized to afferent arterioles, and is also detected immunohistochemically in proximal tubules, collecting ducts and the thick ascending limb of Henle [19–21]. Immunoreactive staining for Mas is observed within glomeruli from rat [21], but not sheep [17]. In mice with genetic deletion of the Mas receptor, radioligand binding of Ang-(1–7) in the kidney is eliminated [1].

The effects of Ang-(1–7)–Mas interactions on physiological functions in the kidney are complex and cell- or nephron-segment-specific. Ang-(1–7) dilates isolated preconstricted rabbit glomerular afferent arterioles, an effect blocked by treatment with the Ang-(1–7) antagonist A779 [20]. This vasodilatory effect appears to be mediated by release of NO (nitric oxide) [19]. In cultured VSMCs (vascular smooth muscle cells), Ang-(1–7) inhibits AngII-activated PKC (protein kinase C) and MAPKs (mitogen-activated protein kinases) [22,23], suggesting that it exerts anti-hypertrophic effects. Similarly, in cultured human endothelial cells, Ang-(1–7) inhibits AngII-stimulated phosphorylation of c-Src and ERK (extracellular-signal-regulated kinase) 1/2, and blunts AngII-stimulated NOX (NADPH oxidase) activity [24]. These effects of Ang-(1–7) are associated with phosphorylation of SHP (Src homology 2 domain-containing protein tyrosine phosphatase)-2 and are blocked by A779 [24].

Several years ago, Andreatta-van Leyen et al. [25] demonstrated that Ang-(1–7) activated PLA2
Figure 1  Mechanisms for the formation and degradation of Ang-(1–7) in the kidney

The pathways for formation of Ang-(1–7) in kidney tissue are depicted: (i) ACE2-dependent degradation of AngII, or ACE2-mediated cleavage of AngI, which can then be converted into Ang-(1–7) by ACE, or (ii) prolyl oligopeptidase (POP)-, neprilysin (NEP)- or thimet oligopeptidase (TOP)-mediated cleavage of AngI. Ang-(1–7) is metabolized by ACE to Ang-(1–5) or by neprilysin or aminopeptidase (AP) to generate Ang(1–4). Ang-(1–7) binds with high affinity to the G-protein-coupled receptor Mas. At high concentrations, Ang-(1–7) may bind to and activate the AT1 receptor. The Mas receptor can act as a physiological antagonist of the AT1 receptor.

(phospholipase A2) in proximal tubular cells, an effect associated with inhibition of transcellular sodium transport. In isolated perfused rat proximal straight tubules, Ang-(1–7) causes a biphasic effect on fluid absorption, with high concentrations (10^{-8} M) decreasing transport and low concentrations (10^{-12} M) stimulating transport, via binding to AT1 receptors [26]. Lara et al. [27–29] have subsequently uncovered interactions between Ang-(1–7) and AT1, AT2 and Mas receptors in adult pig kidney proximal tubules, which have an impact on basolateral Na^+ -ATPase activity and net sodium reabsorption. Thus Ang-(1–7) reverses the stimulatory effect of AngII on Na^+ -ATPase activity via interaction with an A779-sensitive receptor [27]. However, Ang-(1–7) also stimulates activity via interaction with the AT1 receptor and inhibits Na^+ -ATPase via binding to the AT2 receptor, with the subsequent activation of cGMP [28,29]. These in vitro studies highlight the potential for complex interactions among angiotensin peptides and their receptors in the proximal tubule, which could result in activation of common downstream signalling events.

Notwithstanding these effects on sodium transport, evidence suggests that Ang-(1–7) inhibits AngII-mediated growth responses in the proximal tubule. In cultured rat proximal tubular cells, Ang-(1–7) potently inhibits AngII-stimulated phosphorylation of ERK1/2, p38 MAPK and JNK (c-Jun N-terminal kinase), an effect reversed by pre-treatment with A779 [30]. Ang-(1–7) also prevents AngII-induced production of TGF (transforming growth factor)-β1 in proximal tubular cells [30]. In cultured pig kidney tubular cells (LLC-PK1), Ang-(1–7) inhibits glucose-induced phosphorylation of p38 MAPK, an effect attributed to increased activity of SHP-1 [31]. Ang-(1–7) attenuates high-glucose-induced TGF-β1 production, but has no effect on enhanced fibronectin or collagen levels in these cells [31]. These findings indicate that Ang-(1–7) attenuates AngII- and glucose-induced MAPK activation via stimulation of SHP-1 in proximal tubular cells. The inhibition of TGF-β1 production suggests that Ang-(1–7) may partially protect against glucose-induced injury in the diabetic kidney. However, in rat kidney epithelial cells (NRK), high concentrations of Ang-(1–7) (10^{-5} M, in the absence of RAS activation or high glucose) stimulate markers of epithelial–mesenchymal transformation, as well as the expression of TGF-β1 and CTGF (connective tissue growth factor), effects blocked by A779 [32]. In contrast, in the presence of high glucose, Ang-(1–7) (10^{-5} M) attenuates epithelial–mesenchymal transition and TGF-β1 production in NRK cells [33]. Thus, in the proximal tubule, the protective effects of Ang-(1–7) may be confined to states of RAS activation or a high-glucose environment.

In contrast with the inhibitory effects of Ang-(1–7) on AngII- and high-glucose-induced signalling in proximal tubular cells, the effects of Ang-(1–7) in glomerular mesangial cells are variable, with activation of growth-stimulatory pathways in some cases. Zimpelmann and Burns [34] have shown that incubation of a human mesangial cell line with Ang-(1–7) caused a rapid and significant increase in arachidonic acid release, as well as phosphorylation of MAPKs. The stimulatory effect of Ang-(1–7) was blocked by A779, but was unaffected by AT1 or AT2 receptor antagonism [34]. Furthermore, Ang-(1–7) did not prevent AngII- or high-glucose-induced p38 MAPK phosphorylation. Ang-(1–7) also stimulated production of TGF-β1, fibronectin and collagen IV in these cells, effects blocked by inhibition of p38 MAPK. Similarly, in a rat mesangial cell line,
Ang-(1–7) (10^{-12} to 10^{-8} M) was found by Liu et al. [35] to stimulate ERK1/2 phosphorylation, an effect blocked by A779, but not by AT1 or AT2 receptor antagonists. ERK phosphorylation by Ang-(1–7) was dependent upon the production of cAMP and was independent of NOX [35]. On the other hand, in primary cultures of rat mesangial cells, Ang-(1–7) inhibits high-glucose-stimulated NOX activation [36]. Furthermore, attenuation of high-glucose-stimulated NOX activity by human recombinant ACE2 was partly prevented by A779 in these cells, suggesting a protective role for Ang-(1–7) [36]. In primary cultures of mouse mesangial cells, Moon et al. [37] showed that Ang-(1–7) attenuated AngII-induced MAPK phosphorylation, as well as expression of TGF-β1, fibronectin and collagen IV. Furthermore, Ang-(1–7) prevented the AngII-induced expression of NOX subunits, including p47^{phox}, p67^{phox}, and NOX-4. In summary, these findings suggest variable responses to Ang-(1–7) in cultured mesangial cells, depending on species or culture conditions. Stimulation of growth-stimulatory pathways has been observed in passaged cell lines, whereas, in primary cultures, results suggest inhibition of AngII- or high-glucose-induced pro-fibrotic pathways.

Glomerular podocytes are epithelial cells that interdigitate along the filtration barrier and prevent leakage of large-molecular-mass substances (such as proteins) into the urinary space. In proteinuric kidney diseases, such as diabetic nephropathy, podocyte injury can culminate in apoptosis and loss of podocytes in the urine. Although it appears that podocytes express ACE2 [13] and are able to generate Ang-(1–7) in culture [38], there is currently no information on the effects of Ang-(1–7) on podocyte signalling or on AngII-mediated responses in these cells. This is clearly an area that requires further study, given the overall importance of the podocyte in progressive nephropathies.

To add to the complexity of Ang-(1–7) signalling mechanisms in the kidney, the Mas receptor contains a nuclear localization sequence, and nuclear fractions from sheep renal cortices demonstrate specific binding of Ang-(1–7) [19]. Moreover, isolated cortical nuclei generate NO in response to Ang-(1–7) in a dose-dependent fashion [19]. Isolated nuclei also contain components of the RAS, suggesting the possibility for the generation of Ang-(1–7) in the nuclear compartment, with subsequent basal production of NO. In this regard, Ang-(1–7) has been localized by immunohistochemistry to nuclei of rat mesangial cells [39].

To summarize, information on Ang-(1–7)/Mas signalling in the kidney is complex and emphasizes the need for more detailed studies at the cellular level. This complexity may underlie conflicting in vivo data on the role of Ang-(1–7) in the regulation of renal haemodynamics or sodium and water handling. For instance, some studies have found no effect of Ang-(1–7) on renal blood flow [40], others have documented vasodilatory responses [20], and others reported that high concentrations of Ang-(1–7) induce vasoconstriction in the hydronephrotic kidney, perhaps via AT1 receptor activation [41]. To some extent, variable responses may be explained by relative levels of Ang-(1–7) and AngII, and an interaction between Mas and AT1 receptor signalling, as noted above. In this regard, in mammalian cells co-transfected with DNA for the Mas receptor and the AT1 receptor, a hetero-oligomeric complex forms between both proteins, which is associated with the inhibition of AngII actions [42]. Thus the Mas receptor may act as a physiological antagonist of AT1 receptor signalling, whereas, alone, it may only mediate weak effects. In addition, in the proximal tubule, Ang-(1–7) signalling may be impacted by the ability of megalin to internalize the heptapeptide [43]. Moreover, the recent demonstration of rapid Mas receptor desensitization and internalization by Ang-(1–7), with targeting to early endosomes, suggests that cell responsiveness to Ang-(1–7) is dynamic and could vary dramatically depending on the state of activation of the RAS [44]. Finally, several Mas-related genes (mrgs) exist that encode G-protein-coupled receptors, reflecting the molecular diversity of these proteins [45]. However, the binding affinity of Ang-(1–7) to various subtypes of the Mas family and signalling pathways are unknown.

### HYPERTENSIVE RENAL INJURY

An activated RAS has been implicated in the development and maintenance of certain forms of hypertension [46]. Since Ang-(1–7) stimulates NO production and vasodilation, it may have therapeutic benefit in hypertension and hypertensive renal injury. In vivo studies in experimental animals have tested the effects of administration of Ang-(1–7), with doses generally ranging between 200 and 600 μg/kg of body weight per day [47–50]. In normotensive pithed rats that have impaired BP regulatory responses, systemic injection of Ang-(1–7) causes reductions in BP [51]. A transient decrease in BP has also been observed with intravenous infusion of Ang-(1–7) in SHR [52]. Interestingly, in SHR, the antihypertensive effects of combined ACEi and ARB treatment are associated with elevated plasma levels of Ang-(1–7), and Ang-(1–7) may partly mediate the response, since treatment with an antibody against Ang-(1–7) induces a significant elevation in systolic BP [15]. Thus, in animal models of hypertension, endogenous Ang-(1–7) appears to play an important counter-regulatory role to AngII, tending to normalize BP. In further support of this concept, in the 2K1C (two kidney-one clip) model of renovascular hypertension, administration of Ang-(1–7) has no effect on BP, whereas A779 exacerbates BP elevation [53]. Similarly, genetic
deletion of the Mas receptor is associated with increased BP responses in the 2K1C model [54].

A protective role for Ang-(1–7) in hypertension-induced renal injury is strongly supported by several studies. In SHR, chronic inhibition of NO synthesis with l-NAME (N\(^{\text{O}}\)-nitro-l-arginine methyl ester) induces endothelial dysfunction, severe BP elevation and end-organ damage [47]. In l-NAME-treated SHR, chronic administration of Ang-(1–7) (576 μg/kg of body weight per day) significantly reduced BP and proteinuria, was associated with lower histological grades of hypertensive vascular injury in the kidneys and restored mesenteric vascular responsiveness to constrictor and vasodilator stimuli [47]. The effects of Ang-(1–7) are similar to those of the ACEi captopril in this model. Furthermore, although treatment with the COX (cyclo-oxygenase) inhibitor indomethacin blocks the beneficial effects of Ang-(1–7) on BP in l-NAME-treated SHR, it does not reverse the protective effects on proteinuria, vascular reactivity or renal vascular morphology. Thus the BP-lowering effects of Ang-(1–7) probably involve the release of prostaglandins, whereas other pathways mediate proteinuria-lowering and improved endothelial function in this model, independent of BP.

The effect of endogenous Ang-(1–7) on BP and proteinuric responses in SHR chronically treated with l-NAME has been studied by Benter et al. [55]. Administration of the Ang-(1–7) antagonist A779 to SHR treated with l-NAME exacerbated BP, but did not increase proteinuria further. On the other hand, A779 treatment significantly inhibited the reduction in BP associated with use of the ACEi captopril or the vasodilator hydralazine [55]. Reductions in proteinuria by hydralazine or captopril were also attenuated by A779 (although the effects of captopril did not achieve statistical significance). These studies suggest that endogenous Ang-(1–7) contributes to the BP-lowering and renoprotective effects of these agents in severe hypertension due to NO deficiency.

SHRSP (stroke-prone SHR) is a model of severe hypertension accompanied by nephron injury and insulin resistance. Giani et al. [56] have studied the effects of exogenous administration of Ang-(1–7) in SHRSP subjected to salt loading. Of interest, Ang-(1–7) (600 μg/kg of body weight per day) for 2 weeks reduced elevated plasma glucose and triacylglycerol (triglyceride) levels, suggesting that Ang-(1–7) may improve insulin sensitivity. Ang-(1–7) treatment was associated with reductions in systolic BP and proteinuria, and decreased tubulointerstitial fibrosis and glomerular extracellular matrix deposition [56]. Renal expression of the pro-inflammatory cytokines IL-6 (interleukin-6) and TNF-α (tumour necrosis factor-α), and NF-κB (nuclear factor κB) was significantly reduced in Ang-(1–7)-treated SHRSP. Finally, Ang-(1–7) completely restored the expression of the podocyte protein nephrin, suggesting a return of glomerular functional and/or structural integrity. Whether this is due to reductions in BP associated with Ang-(1–7) or to direct effects on the podocyte is unclear. These studies nevertheless provide persuasive evidence for a protective effect of Ang-(1–7) in hypertensive nephropathy.

Gender may be important in defining the renal effects of Ang-(1–7) in hypertension. In female rats with hypertension due to renal wrap, ovariectomy is associated with exacerbated renal injury compared with oestrogen-replete females [57]. Chronic treatment of rats with Ang-(1–7) (576 μg/kg of body weight per day) prevents glomerulosclerosis and tubulointerstitial fibrosis associated with ovariectomy in this model, without reducing BP [57]. Ovariectomized rats with renal wrap hypertension have significantly reduced renal cortical expression of ACE2, an effect reversed by oestrogen treatment. These results suggest that oestrogen-deficient females may be at increased risk of hypertensive renal injury, secondary to reduced renal ACE2 and the consequent diminished generation of Ang-(1–7). The protective effects of Ang-(1–7) could be related to a reduction in renal NOX activity. Thus, in diabetic SHR, treatment with Ang-(1–7) decreases elevated levels of renal NOX activity, without lowering mean BP [58]. Reductions in renal catalase activity and expression of PPAR-γ (peroxisome-proliferator-activated receptor-γ) are also restored by Ang-(1–7) treatment in diabetic SHR, suggesting that Ang-(1–7) signalling in the kidney opposes the damaging effects of NOX activation in this model [59].

**DIABETIC NEPHROPATHY**

In the diabetic kidney, AngII acts on AT\(_1\) receptors to promote adverse signalling pathways, including ROS generation, MAPK activation and generation of TGF-β1. These effects of AngII accelerate podocyte loss, glomerulosclerosis and tubulointerstitial fibrosis [60]. Although Ang-(1–7) exerts cardioprotective effects in experimental diabetes [48,61,62], the effects of endogenous or exogenous Ang-(1–7) on the course of diabetic nephropathy remain incompletely characterized (Figure 2).

Some insights into the effect of endogenous Ang-(1–7) in diabetic nephropathy have arisen from animal studies focusing on the role of ACE2. The effects of diabetes on renal expression of ACE2 are somewhat controversial. Tikellis et al. [63] found that tubular ACE2 mRNA and protein expression were significantly decreased in male rats with STZ (streptozotocin)-induced diabetes after 24 weeks. Similarly, proximal tubular expression of ACE2 mRNA and protein was reduced in male C57BL/6 STZ-diabetic mice, which was associated with a reduction in kidney cortical levels of Ang-(1–7).
Figure 2 Protective role of Ang-(1–7) in experimental diabetic nephropathy

Diabetic nephropathy is associated with a decrease in the ACE2/ACE ratio in the kidney, which leads to elevated levels of AngII and decreased Ang-(1–7). In diabetes, enhanced renal vasoconstrictive responses to AngII are inhibited by Ang-(1–7). In mesangial cells, high glucose and AngII activate MAPK, enhance the expression of TGF-β1, fibronectin and collagen IV and stimulate NOX. In a high-glucose environment, these effects could be inhibited by Ang-(1–7). In the presence of high glucose or AngII, Ang-(1–7) blocks MAPK signalling via SHP-1 activation in proximal tubular cells. Podocyte expression of ACE2/ACE is decreased in diabetic nephropathy, although the effects of diminished Ang-(1–7) generation are unknown. The overall effect of Ang-(1–7) in the diabetic kidney is to diminish generation of ROS and pro-fibrotic cytokines such as TGF-β1. As a consequence, the progression of diabetic nephropathy is attenuated. Continuous arrows indicate stimulation, and broken lines indicate inhibition.

[64]. Paradoxically, renal ACE2 activity levels were increased in that study, an effect ascribed to possible contamination by elevated plasma levels of ACE2 in diabetes [64]. Recently, Yamaleyeva et al. [65] reported increased circulating ACE2 in male and female diabetic mRen2.Lewis rats. Female diabetic rats were protected against a diabetes-induced decline in renal cortical ACE2 activity compared with diabetic male rats, indicating gender-specific regulation of renal ACE2 in diabetes [65]. Wysocki et al. [66] reported increased ACE2 activity in renal cortices of female STZ-diabetic mice and mice with Type 2 diabetes (db/db), compared with non-diabetic controls, and this was associated with increased kidney ACE2 protein expression. No change in ACE2 mRNA expression occurred with diabetes, suggesting a post-transcriptional mechanism to enhance cortical ACE2 expression.

Within the glomerulus, podocyte ACE2 expression decreases in experimental diabetes, which is accompanied by enhanced expression of ACE [12,67]. An increase in the ACE/ACE2 ratio in the diabetic glomerulus could promote the production of AngII and decreased levels of Ang-(1–7). An increase in the ACE/ACE2 ratio is also supported by renal biopsy studies from humans with diabetic nephropathy, which reveal increased glomerular and tubular immunostaining for ACE and decreased glomerular and tubular ACE2 compared with healthy controls [68]. Similarly, Reich et al. [69] found a
significant decrease in ACE2 mRNA expression in laser captured glomeruli and proximal tubules from kidney biopsies of patients with Type 2 diabetes and diabetic nephropathy, which was associated with an increase in ACE mRNA in both compartments. By immunohistochemistry, ACE2 protein expression was significantly reduced in proximal tubules from diabetic subjects, but glomerular ACE2 protein expression was below the limits of detection for immunohistochemical comparison. Taken together, these findings support the notion that Ang-(1–7) levels may be suppressed in the diabetic kidney, particularly within the glomerulus, secondary to an increase in the ACE/ACE2 ratio.

The effects of blocking ACE2 activity on the course of experimental diabetic nephropathy have been studied by Batlle and co-workers [12,67]. In STZ-diabetic mice, treatment with the ACE2 inhibitor MLN-4760 led to an augmentation in urinary albumin excretion, mesangial expansion, and fibronectin and collagen I staining in the tubulointerstitium compared with control STZ-diabetic mice. Effects of ACE2 inhibition on BP or renal AngII or Ang-(1–7) levels were not assessed in that study, although treatment with MLN-4760 did not significantly alter plasma AngII levels [67]. In db/db diabetic mice, MLN-4760 accelerated diabetic renal injury, with enhanced urinary albumin excretion and glomerular fibronectin staining compared with control diabetic mice. Treatment of diabetic mice with the ARB telmisartan reversed the adverse effects associated with ACE2 inhibition, supporting a major role for AT1 receptor-mediated pathways in the accelerated injury response [12]. However, a potential protective role for increases in circulating or renal levels of Ang-(1–7) that might occur with ARB treatment cannot be excluded. Furthermore, a decrease in renal Mas receptor expression has been reported in STZ-diabetic mice and ARB treatment appears to restore renal Mas levels [70]. Accordingly, it is conceivable that the protective effects of ARBs in diabetic nephropathy may involve both inhibition of AT1 receptor activation, as well as enhancement of the actions of Ang-(1–7) by this mechanism.

The ACE2-KO (ACE2-knockout) mouse has been an important tool to study the progression of diabetic nephropathy. Shiota et al. [71] reported that STZ-diabetic ACE2-KO mice developed albuminuria at an earlier stage and to a greater extent than diabetic wild-type mice. Furthermore, diabetic ACE2-KO mice exhibited enhanced glomerular and tubulointerstitial injury compared with diabetic wild-type mice [71]. Treatment with the ARB olmesartan did not affect BP and only partially corrected the albuminuria and glomerular and tubulointerstitial injury scores in diabetic ACE2-KO mice. The incomplete attenuation of renal injury by AT1 receptor blockade indirectly suggests that a deficiency of Ang-(1–7) may contribute to diabetic nephropathy. By contrast, treatment of Akita Type 1 diabetic ACE2-KO mice with the ARB irbesartan completely normalized urinary albumin excretion, suggesting a primary role for increased AngII as the mediator of injury, rather than diminished levels of Ang-(1–7) [72]. In contrast with these studies, Tikellis et al. [64] reported that indices of renal hypertrophy and fibrogenesis were reduced in diabetic ACE2-KO mice or diabetic mice treated with an ACE2 antagonist, supporting a possible adverse effect of Ang-(1–7). In summary, although ACE2-KO studies have been highly informative, characterization of the specific role of Ang-(1–7) in renoprotection will clearly require alternate strategies, including studies in diabetic mice with deletion of the Mas receptor gene.

The effects of enhancing ACE2 activity have been examined in experimental diabetes. In a novel study by Oudit et al. [36], treatment of Akita diabetic mice with hrACE2 (human recombinant ACE2) caused a reduction in urinary albumin excretion, glomerular volume and renal cortical NOX activity compared with control diabetic mice. hrACE2 treatment significantly reduced AngII levels in the plasma and renal cortex, whereas Ang-(1–7) levels were only elevated in the renal cortex. A reduction in BP was observed with hrACE2, which could have contributed to the protective renal effects. However, in separate experiments in cultured rat mesangial cells, Ang-(1–7) attenuated glucose-induced NOX activity, suggesting that Ang-(1–7) may prevent pathological ROS generation in the diabetic kidney [36]. In agreement with these studies, treatment of STZ-diabetic rats with a single intravenous injection of rat Ad-ACE2 (ACE2-encoding adenoviral vector) attenuated elevations in creatinine clearance, urinary albumin excretion and glomerulosclerosis compared with untreated STZ-diabetic rats [73]. Induction of diabetes was associated with increases in BP and renal AngII levels, whereas renal Ang-(1–7) levels decreased. Ad-ACE2 normalized BP, as well as renal AngII and Ang-(1–7) levels [73]. Taken together, delivery of recombinant ACE2 or ACE2 gene therapy appears to be protective in diabetic nephropathy. However, whether this is due primarily to increased degradation of AngII or production of Ang-(1–7) remains unclear.

The effects of exogenous administration of Ang-(1–7) on the course of experimental diabetes are particularly interesting. In adult male STZ-diabetic rats, Ang-(1–7) (576 μg/kg of body weight per day) caused a significant reduction in urinary protein excretion compared with untreated diabetic rats, although effects on BP were not reported [48]. Renal arteries isolated from untreated STZ-diabetic rats exhibited enhanced vasoconstrictive responses to noradrenaline (norepinephrine), ET-1 (endothelin-1) and AngII, which were significantly attenuated in rats treated with Ang-(1–7) [48]. In a subsequent study, Benter et al. [58] treated diabetic SHR with Ang-(1–7) (576 μg/kg of body weight per day)
and reported a significant reduction in urinary protein excretion compared with untreated STZ-diabetic rats, with no changes in mean arterial pressure. Renal NOX activity was significantly elevated in SHR and to a greater extent in diabetic SHR, an effect completely inhibited by Ang-(1–7) treatment [58]. These compelling findings suggest that Ang-(1–7) exerts protective effects in STZ-diabetes, which may be due to normalization of vascular responses to vasoconstrictors and prevention of renal NOX-induced oxidative stress.

The KK-A+/Ta mouse spontaneously develops many of the features of Type 2 diabetes, including hyperglycaemia, hyperinsulinaemia, obesity, microalbuminuria and renal lesions such as mesangial cell hyperplasia and segmental sclerosis [74]. In recent studies, Moon et al. [37] treated KK-A+/Ta mice with either AngII, Ang-(1–7), AngII plus Ang-(1–7) or a combination of AngII, Ang-(1–7) and A779. Although Ang-(1–7) alone did not affect urinary albumin excretion, Ang-(1–7) attenuated AngII-stimulated albuminuria. AngII delivery significantly increased mesangial expansion, renal TGF-β1 and fibronectin mRNA, as well as the expression of NOX subunits in the kidney, including p22phox, p47phox and NOX-4. Co-treatment with Ang-(1–7) and AngII prevented these effects and attenuated AngII-stimulated ROS production in isolated glomeruli. Moreover, the effects of Ang-(1–7) were reversed by treatment with A779, suggesting a role for Mas receptors in the protective response. Thus, although exogenous Ang-(1–7) did not directly modify the course of diabetic nephropathy, it effectively counteracted AngII-induced glomerular injury in this model. In addition, the amount of Ang-(1–7) delivered in these studies was relatively low (360 μg/kg of body weight per day); dose could therefore be an important factor that modulates responses to AngII in diabetic nephropathy.

In summary, exogenous Ang-(1–7) exerts protective effects in diabetes by normalizing urinary protein excretion, renal NOX activity and vascular responsiveness. Nonetheless, not all studies have been in agreement with this conclusion. Shao et al. [75] reported that infusion of Ang-(1–7) (600 μg/kg of body weight per day) in STZ-diabetic rats led to increased weight loss and significantly elevated urinary protein excretion compared with untreated diabetic rats. Furthermore, renal mRNA for TGF-β1 and the AT1 receptor were elevated, whereas AT2 and Mas receptor mRNA levels were significantly reduced in animals that received Ang-(1–7). It is certainly difficult to reconcile these findings with the results of other studies on exogenous Ang-(1–7). However, it is noted that circulating AngII levels were increased in rats treated with Ang-(1–7) in this study, for unclear reasons [75]. Furthermore, Ang-(1–7) was infused intravenously and at a relatively high dose, raising the possibility of non-specific adverse signalling. In this regard, at high concentrations, Ang-(1–7) binds to the AT1 receptor, suggesting that adverse effects may be due to AT1 receptor signalling [76]. Additional studies determining the optimal dose and route of administration of Ang-(1–7) in diabetic nephropathy are therefore required before evaluation of its therapeutic potential in human disease.

**NON-DIABETIC KIDNEY DISEASE**

The effects of exogenously administered Ang-(1–7) in experimental models of non-diabetic CKD have yielded controversial results. With respect to glomerular disease, in male rats with experimental glomerulonephritis induced by anti-Thy-1 antibody, treatment with Ang-(1–7) (576 μg/kg of body weight per day) for 5 days caused a reduction in proteinuria, decreased glomerular mRNA expression of pro-fibrotic proteins including fibronectin and TGF-β1 and decreased glomerulosclerosis and inflammatory cell infiltration [50]. In contrast, in male rats with glomerular injury induced by adriamycin, a model associated with a favourable response to RAS blockade, Ang-(1–7) (576 μg/kg of body weight per day) had no effect on proteinuria and did not affect BP [77]. In that study, plasma levels of Ang-(1–7) were elevated after administration, confirming that delivery of the peptide was not a limitation. Whether these contrasting effects of Ang-(1–7) can be explained by differences in the state of activation of the RAS or distinct cell targets that might dictate responses to Ang-(1–7) (e.g. mesangial cells compared with podocytes or endothelial cells) requires further study.

Beneficial effects of exogenous Ang-(1–7) have been observed in the mouse remnant kidney model of CKD induced by 5/6 Nx (nephrectomy). In male C57Bl/6J mice with 5/6 Nx, treatment with Ang-(1–7) (300 μg/kg of body weight per day) for 12 weeks attenuated elevations in plasma urea and creatinine, and preserved cardiac function compared with untreated mice or mice treated with the antihypertensive agent hydralazine [78]. Interestingly, Ang-(1–7) was associated with a similar degree of BP lowering as hydralazine in that study, suggesting that its protective effects are not mediated by a reduction in BP alone [78]. In male FVB/N mice with 5/6 Nx, a shorter course of Ang-(1–7) (576 μg/kg of body weight per day for 4 weeks) significantly increased plasma and renal levels of Ang-(1–7), but did not alter GFR (glomerular filtration rate) and induced only a non-significant decline in albuminuria [79]. Ang-(1–7) treatment significantly increased relative mesangial area in that study. Finally, and unexpectedly, in male rats subjected to 5/6 Nx for 10 days, Ang-(1–7) (576 μg/kg of body weight per day) significantly increased systolic BP, proteinuria, and plasma urea and creatinine compared with vehicle-treated 5/6 Nx rats [80]. However, elevations in plasma Ang-(1–7) were not detected after Ang-(1–7) administration in that study, an effect attributed to
increased cardiac ACE activity [80]. Although the effects of Ang-(1–7) in the 5/6 Nx model of CKD remain uncertain from these studies, we propose that differences in species, mouse strain, and the dose and duration of Ang-(1–7) treatment may be contributing factors. In this regard, in rats, Ang-(1–7) accumulates in plasma over time when given by subcutaneous injection [81], suggesting that the duration of administration may be a critical factor in these models.

Genetic deletion of the Mas receptor [Mas-KO (Mas receptor knockout)] has served as a useful tool to study the role of Ang-(1–7) in mouse models of CKD. C57Bl/6 Mas-KO mice have reduced urine volume and fractional sodium excretion [82]. Although they exhibit normal BP, Mas-KO mice have elevated GFR, increased proteinuria and increased renal expression of TGF-β1 mRNA and extracellular matrix proteins [82]. These findings suggest that the Mas receptor mediates anti-fibrotic responses in the mouse kidney, although Mas-KO mice had increased renal AT1 receptor mRNA expression in this study [82]. Thus the potential contribution of AT1 receptors to the renal fibrogenic response requires further investigation.

Esteban et al. [83] studied the role of the Mas receptor in renal fibrosis induced by UUO (unilateral ureteral obstruction). Mas-KO mice (on the C57Bl/6 background) with UUO are surprisingly protected from renal injury compared with wild-type mice. Thus, 5–7 days after UUO, obstructed kidneys from Mas-KO mice demonstrate reduced intrarenal levels of NF-κB and decreased matrix deposition, apoptosis and inflammatory cell infiltration compared with obstructed kidneys from wild-type mice with UUO. Furthermore, wild-type mice with UUO that received infusions of Ang-(1–7) had more severe renal injury and fibrotic responses compared with untreated mice. These adverse effects did not appear to be due to interaction of Ang-(1–7) with AngII receptors, since infusion of Ang-(1–7) into mice with genetic deletion of AT1a, AT1b and AT2 receptors produced similar responses to those seen in wild-type mice. How can these results be explained in the context of the protective effects of Ang-(1–7) in hypertensive or diabetic nephropathy? The answer is far from evident, although we speculate that exogenous Ang-(1–7) excretion increases markedly throughout pregnancy, suggesting that Ang-(1–7) may serve a vasodilatory role [89]. Interestingly, in women with pre-eclampsia, plasma levels of Ang-(1–7) are also elevated [89]. Despite RAS activation, women typically remain normotensive, suggesting the presence of a counter-regulatory mechanism to oppose AngII-stimulated vasoconstriction and sodium retention.

Altered regulation of the RAS has been implicated in the pathophysiology of pre-eclampsia. In normal human pregnancy, the RAS is activated early with pro-renin levels peaking 20 days after conception and remaining high thereafter. Plasma levels of angiotensinogen, renin and AngII are also elevated [89]. Despite RAS activation, women typically remain normotensive, suggesting the presence of a counter-regulatory mechanism to oppose AngII-stimulated vasoconstriction and sodium retention. In this regard, plasma levels of Ang-(1–7) are elevated in women with normal pregnancy and urinary Ang-(1–7) excretion increases markedly throughout pregnancy, suggesting that Ang-(1–7) may serve a vasodilatory role [89]. Interestingly, in women with pre-eclampsia, plasma levels of Ang-(1–7) are significantly reduced compared with women with normal pregnancy of the same gestational age [90]. In cultured placental explants from women with pre-eclampsia, secretion of sFlt1...
and sEngI increases to a greater extent compared with placental explants from women with a normal pregnancy [88]. Furthermore, incubation with either AngII or Ang-(1–7) inhibits secretion of these proteins in placentas from women with a normal pregnancy, but not in placentas from women with pre-eclampsia [88]. These observations strongly suggest that Ang-(1–7) deficiency disrupts placental angiogenesis and thereby promotes pre-eclampsia.

Studies in animal models have uncovered additional insights into the potential role of Ang-(1–7) in pregnancy and pre-eclampsia. ACE2-KO mice have increased BP during pregnancy, experience reduced weight gain and give birth to smaller pups [91]. The increase in BP occurs in the absence of changes in plasma or renal levels of AngII. By contrast, placental AngII levels increase in ACE2-KO mice compared with levels in wild-type mice. Moreover, plasma Ang-(1–7) levels are significantly decreased in pregnant ACE2-KO mice compared to virgin ACE2-KO mice, and placental and renal Ang-(1–7) levels do not increase [91]. Thus, in the pregnant ACE2-KO mouse placenta, the vasoconstrictive actions of AngII may be relatively unopposed due to an increase in the ratio of AngII/Ang-(1–7), leading to increased placental vascular resistance and systemic hypertension. Indeed, findings from Brosnihan et al. [89] support a vasodilatory role for Ang-(1–7) in normal pregnancy. Isolated mesenteric arteries from pregnant rats demonstrate a concentration-dependent vasodilatory response to Ang-(1–7) that is absent in vessels from virgin rats [89]. Taken together, these studies indicate an important role for ACE2 and Ang-(1–7) in the maintenance of normal BP and vascular responsiveness during pregnancy.

In the RUPP (reduced uterine perfusion of pre-eclampsia) model in rats, characterized by hypertension and proteinuria, renal levels of Ang-(1–7) are decreased compared with rats with normal pregnancy [92]. This response is associated with decreased proximal and distal tubular immunostaining for Ang-(1–7). By contrast, renal AngII levels are elevated in both pregnant and RUPP rats compared with virgin rats [92]. Accordingly, a relative deficiency of renal Ang-(1–7) in the RUPP model could contribute to pregnancy-induced hypertension and altered renal function. However, whether a decrease in renal Ang-(1–7) levels translates into diminished plasma levels of Ang-(1–7) in the RUPP model, as observed in human pre-eclampsia, remains unknown, and a role for correcting Ang-(1–7) deficiency in this condition as a therapeutic strategy requires further study.

**ACUTE KIDNEY INJURY**

AKI (acute kidney injury) is characterized by a sudden loss of kidney function, usually within hours to days, and often occurs in the setting of renal ischaemia due to extracellular fluid volume depletion, blood loss or sepsis. The kidneys are particularly sensitive to hypoxic injury, due to their prominent blood supply and oxygen utilization, especially within cortical tubules. Thus, in AKI, histological injury is characterized by acute tubular necrosis, with a secondary loss of glomerular filtration [93]. The incidence of AKI in hospitalized patients ranges from 2 to 7%, but climbs to greater than 10% in patients admitted to critical care areas. The mortality rate associated with AKI remains alarmingly high, ranging between 30 and 70% in most studies. Furthermore, patients who survive AKI are at significantly increased risk of developing CKD [94]. Accordingly, AKI represents an important area for research focused on prevention and treatment.

The vasodilatory properties of Ang-(1–7) and its ability to antagonize AngII under certain conditions position it as an attractive therapeutic agent in AKI. In rats, renal ischaemia/reperfusion injury is associated with a pronounced reduction in renal levels of Ang-(1–7), which is accompanied by a significant increase in renal expression of the Mas receptor and a decrease in AT1 receptors [21]. Thus the ischaemic kidney may be primed to respond to Mas receptor activation. In C57Bl/6 mice subjected to bilateral renal ischaemia and reperfusion, administration of the non-peptide Ang-(1–7) agonist AVE0991 induced significant renoprotection, with diminished serum creatinine, decreased histological injury and reduced renal and pulmonary leucocyte infiltration [95]. Mas-KO mice experienced a similar degree of renal ischaemic injury as wild-type mice, suggesting that endogenous activation of the Mas receptor does not contribute to protection from ischaemic injury. In the context of human AKI, these promising results indicate that exogenous activation of the Mas receptor may protect against renal ischaemic injury by reducing inflammatory cell infiltration.

However, that study stands in stark contrast with the results of Esteban et al. [83], who reported that C57Bl/6 Mas-KO mice with ischaemia/reperfusion injury experienced less renal damage compared with wild-type mice. Furthermore, infusion of Ang-(1–7) (576 μg/kg of body weight per day) to wild-type mice exacerbated renal ischaemic injury, which was associated with increased numbers of infiltrating leucocytes and activation of NF-κB [83]. It is difficult to reconcile these contrasting data sets, although differences in experimental protocols might be responsible. As emphasized earlier, the dose of Ang-(1–7) or degree of Mas receptor activation may be an important factor that determines whether inflammatory responses are activated or inhibited in this setting.

In severe human AKI, current therapy is supportive, consisting of renal replacement with dialysis until tubular and vascular cell regeneration can restore adequate
renal function. In recent years, research has focused on strategies to enhance regenerative capacity in the kidney using bone-marrow-derived progenitor cells or other cell-based therapies [96]. Studies by Heringer-Walther et al. [97] revealed that Ang-(1–7) potently stimulated the proliferation and differentiation of human cord-blood-derived CD34+ cells and mononuclear cells in vitro. In immunodeficient mice, injections of Ang-(1–7) markedly increased the engraftment of human CD34+ progenitor cells in bone marrow and spleen (up to a 600-fold increase). These exciting initial findings therefore suggest that Ang-(1–7) may have therapeutic potential as a regenerative compound in conditions such as AKI.

CONCLUSIONS

Ang-(1–7) is generated in the kidney at relatively high levels via several enzymatic pathways, including the actions of ACE2. The biological effects of Ang-(1–7) are primarily mediated by interaction with the receptor Mas. However, other complex effects have been described that may involve receptor–receptor interactions with AT1 or AT2 receptors, as well as nuclear receptor binding. In several kidney diseases, including hypertensive and diabetic nephropathy, glomerulonephritis, tubulointerstitial fibrosis, pre-eclampsia and AKI, a growing body of evidence supports a role for endogenous or exogenous Ang-(1–7) as a protector against nephron injury and as an antagonist of signalling mediated by AT1 receptors. However, in certain experimental protocols, Ang-(1–7) paradoxically accelerates renal injury. We have summarized the potential mechanisms for the protective and deleterious effects of Ang-(1–7) in kidney disease in Table 1. Further research should explore the precise mechanisms for the adverse effects if Ang-(1–7) is to realize its potential as a therapeutic agent in humans with kidney disease.

FUNDING

Our own work was supported by the Canadian Institutes of Health Research [grant number MOP-115036] and the Kidney Foundation of Canada [grant number KFOC100005].

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Angiotensin-(1–7) in kidney disease


