

# Molecular Mechanisms Involved in Intrarenal Renin-Angiotensin and Alternative Pathways in Diabetic Nephropathy - A Review

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## ■ Abstract

Uncontrolled or chronic hyperglycemia causes kidney failure induced by the dysfunction of biomolecules and upregulation of inflammatory cytokines and growth factors. The renin-angiotensin system (RAS) is incorporated in the regulation of renal hemodynamics. In a healthy state, local RAS is independent of systemic RAS. However, in pathological conditions such as chronic hyperglycemia, angiotensin II (Ang II) increases locally and causes tissue damage, mainly through the induction of oxidative stress, inflammation, and upregulation of some growth factors and their receptors. Such tissue events may cause disruption of the glomerular filtration barrier, thickening and hypertrophy of the glomerular basement membrane, microvascular hyperpermeability, proteinuria, and finally decrease in the glomerular filtration rate (GFR). Reduced GFR causes the kidney to sense falsely a low blood pressure condition

and respond to it by stimulating systemic and local RAS. Therefore, patients with diabetic nephropathy (DN) suffer from chronic hypertension. In contrast to local RAS, there are alternative pathways in the kidney that act protectively by reducing tissue Ang II. Such autoregulatory and protective mechanisms are weakened in chronic kidney disease. Previously, it was presumed that systemic RAS inhibitors such as ACE inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) could prevent renal damage by controlling blood pressure and proteinuria. However, the progression of renal failure to end-stage renal disease (ESRD), despite such treatments, indicates the presence of factors other than Ang II. This review highlights the molecular mechanism in renal disease and discusses pharmaceutical and therapeutic approaches.

**Keywords:** nephropathy · vascular endothelial growth factor · glomerular filtration rate · angiotensin · angiotensin-converting enzyme · local renin-angiotensin system

## 1. Introduction

**D**iabetic nephropathy (DN) is a long-term kidney disease that occurs in people suffering from chronic diabetes mellitus [1]. As DN progresses, the most important structural changes occur in glomeruli. Glomerulonephritis (GN) is an inflammatory process in the glomeruli or small blood vessels in the kidney that is characterized by DN through diffuse or nodular sclerosing glomerulonephritis [2]. Glomerulosclerosis is associated with the thickening of the glomerular basement membrane (GBM) induced by an increase in the mesangial matrix that contains large amounts of collagen, especially types IV and VI, laminin, and fibronectin [3]. Mesangial expansion restricts the glomerular capillary surface area, disturbs

the filtering process, and allows the protein leaking from the blood into the urine [4]. Thus, the early stages of glomerulosclerosis are defined by proteinuria that is characterized by a positive microalbuminuria test caused by the progression of glomerular hypertrophy, GBM thickening, hyperperfusion, and hyperfiltration [5].

## 2. Uncontrolled hyperglycemia promotes vascular endothelial growth factor (VEGF) in the kidney

The abnormalities in the diabetic kidney begin with long-term, poorly controlled hyperglycemia. Uncontrolled or chronic hyperglycemia leads to the non-enzymatic formation of advanced glycation end

products (AGEs) through a variety of pathways, including the formation of a Schiff base, which alters the charge, solubility, and conformation of proteins [6]. AGE modification of proteins may result in abnormal molecular function, interaction, or signaling which contribute to renal expression of inflammatory cytokines and growth factors, like interleukin 1 (IL-1), interleukin 18 (IL-18), interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), transforming growth factor  $\beta$  (TGF- $\beta$ ), and insulin-like growth factor 1 (IGF-1) [7]. The production of reactive oxygen species (ROS) and oxidative stress coexist with inflammation and intensify mutual generation [8]. Releasing such factors from intrinsic renal cells or blood-borne cells plays an important role in renal fibrosis and mesangial expansion by the accumulation of extracellular matrix and interstitial proteins, especially collagen [9]. Increased ROS production and a shift towards fatty acid utilization enhance renal oxygen utilization and tissue hypoxia [10]. Hypoxia and oxidative stress conditions inhibit hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) from degradation and allow HIF-1 $\alpha$  to be bonded to HIF-1 $\beta$  (**Figure 1**) [11]. The stabilized heterodimer activates transcription of several genes involved in angiogenesis, cell proliferation, and anaerobic metabolism needed for adaptation in hypoxia and ischemia. Growth factor signaling pathways also upregulate HIF-1 $\alpha$  levels via oxygen-independent mechanisms [12].

Among several angiogenic factors induced by HIFs, VEGF plays a critical role in minimizing hypoxia-induced events through neovascularization [13]. It is more intensely secreted from podocytes and the thick ascending limb and to a much lesser extent from the proximal and distal tubules [14]. However, VEGF receptors are mainly limited to endothelial cells in the glomerular capillary loops and peritubular capillaries [14, 15]. VEGF, along with other stimulators, including angiopoietins (ANGPTs), epidermal growth factor (EGF), semaphorin 3A (SEMA3A), TGF- $\beta$ , and C-X-C motif chemokine ligand 12 (CXCL12), signal in paracrine ways and regulate glomerular filtration barrier (GFB) function [16]. During the early stage of DN, VEGF levels and its receptors are upregulated in the glomerulus.

Several studies indicate that VEGF expression may be upregulated through various mechanisms. Heme oxygenase 1 (HO-1), overexpressed by ROS and nitric oxide (NO), enhances VEGF synthesis and is involved in angiogenesis [16]. VEGF also regulates HO-1 expression and activity through a positive-feedback loop [17]. In addition to sodium retention, aldosterone upregulates the expression of VEGF in the epithelial cells of the cortical collecting duct and stimulates inflammation and fibrosis by ROS generation and interaction with RAS [18]. Ang II has previously been reported to reinforce VEGF-mediated angiogenesis via the upregulation of VEGF receptors [19-21]. Ang II stimulates VEGF expression mainly through angiotensin II receptor 1 (AT1R) and increases the expression of VEGF-R2 mostly through angiotensin II receptor 2 (AT2R) [22]. Excess VEGF results in stimulating TGF- $\beta$  expression, enhancing IV and VI collagens, laminin and fibronectin synthesis, augmenting NO production, increasing endothelial

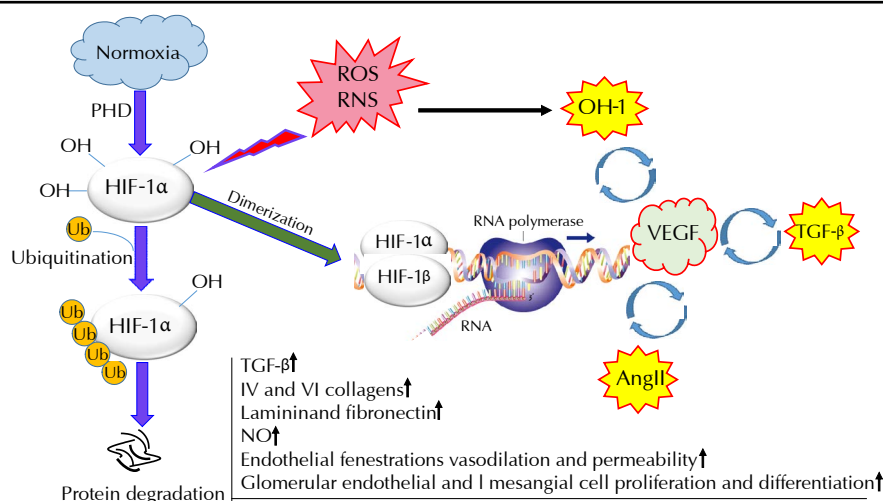
### Abbreviations:

ACE:	Angiotensin-converting enzyme
ACEIs:	ACE inhibitors
ADH:	antidiuretic hormone
AGEs:	advanced glycation end products
AMP:	alanyl aminopeptidase
Ang II:	angiotensin II
APA:	aminopeptidase A
AT1R:	angiotensin II receptor 1
AT2R:	angiotensin II receptor 2
DN:	diabetic nephropathy
ESRD:	end-stage renal disease
Flk-1:	fetal liver kinase 1
Flt-1:	Fms-like tyrosine kinase 1
GBM:	glomerular basement membrane
GFB:	glomerular filtration barrier
GFR:	glomerular filtration rate
GN:	glomerulonephritis
HIF-1 $\alpha$ :	hypoxia-inducible factor $\alpha$ subunit
HO-1:	heme oxygenase 1
ICAM-1:	intercellular adhesion molecule 1
IFN:	interferon
IL:	interleukin
NEP:	nepriylsin
NO:	nitric oxide
PAI:	plasminogen activator inhibitors
RAS:	renin-angiotensin system
PEP:	prolyl endopeptidase
PHD:	prolyl hydroxylase
Plt:	platelet
PRCP:	prolyl carboxypeptidase
RNS:	reactive nitrogen species
ROS:	reactive oxygen species
SMC:	smooth muscle cell
TGF- $\beta$ :	transcription growth factor
VCAM-1:	vascular cell adhesion molecule 1
VEGF:	vascular endothelial growth factor
VEGFR:	vascular endothelial growth factor receptor

fenestrations, vasodilation, and permeability, and inducing glomerular endothelial and mesangial cell proliferation and differentiation. These events result in the disruption of GFB, thickening and hypertrophy of GBM, microvascular hyperpermeability, proteinuria, and finally decrease in glomerular filtration rate (GFR) [15, 23].

### 3. Reduced GFR and constant hypertension in diabetes

The juxtaglomerular cells localized mainly in the walls of the afferent arterioles, and to a lesser extent in the wall of the efferent arterioles, release renin in response to a drop in blood pressure or when biochemically stimulated by macula densa cells [24]. The macula densa is an area at the point between the thick ascending limb of the Loop of Henle and the distal



**Figure 1. Degradation of HIF-1 $\alpha$  in normoxia, its dimerization with HIF-1 $\beta$  in oxidative conditions, and its adaption of the role of a transcription factor.** Induced VEGF stimulates the expression of TGF- $\beta$ , IV and VI collagens, laminin, and fibronectin, increases NO production, endothelial fenestration, vasodilation, and permeability, and induces glomerular endothelial and mesangial cell proliferation and differentiation. *Abbreviations:* Ang II - angiotensin II; NO - nitric oxide; PHD - prolyl hydroxylase; HO-1 - hemoxygenase-1; RNS - reactive nitrogen species; ROS - reactive oxygen species; TGF- $\beta$  - transcription growth factor; VEGF - vascular endothelial growth factor.

convoluted tubule. It consists of specialized cells, which are salt sensors and stimulate the juxtaglomerular apparatus by generating paracrine chemical signals [25]. A reduction in GFR during glomerulonephritis results in a decrease in the volume of filtered fluid and a reduction in the concentration of sodium reaching the macula densa [26]; both stimulate juxtaglomerular cells to release renin into the bloodstream. Following the response to blood renin by circulating RAS, blood pressure rises, i.e. the blood pressure regulatory system in the kidney falsely senses a low blood pressure condition and responds to it. Therefore, patients with diabetic nephropathy suffer from constant hypertension. Generally, proteinuria, increased blood pressure, and decreased GFR are the clinical manifestations of diabetic nephropathy, which gradually progresses to end-stage renal disease (ESRD) [27].

#### 4. Angiotensin II in systemic and local renal RAS

Renin hydrolyzes blood angiotensinogen to form Ang I which then is converted to Ang II by angiotensin-converting enzyme (ACE) present in many tissues, particularly in the pulmonary vascular endothelium [28]. Ang II increases blood pressure through acting on arteriolar smooth muscle to cause vascular constriction, on the zona glomerulosa of the adrenal cortex to stimulate aldosterone secretion and sodium reabsorption from the kidney, on the posterior pituitary gland to release vasopressin which elevates water resorption in the kidney, and on sympathetic fibers to release norepinephrine [29, 30]. In addition to systemic RAS, Ang II is also produced in local renal RAS [31, 32]. As is well known in both normal and pathological conditions, the role of local RAS in regulating renal hemodynamics is independent of that of systemic RAS. Besides tubular-glomerular feedback and myogenic responses to renal autoregulation, local Ang II also affects the tonicity of both afferent and efferent arterioles, which raises the systemic arterial blood pressure and decreases renal blood flow [33]. Despite this drop in renal blood flow, the

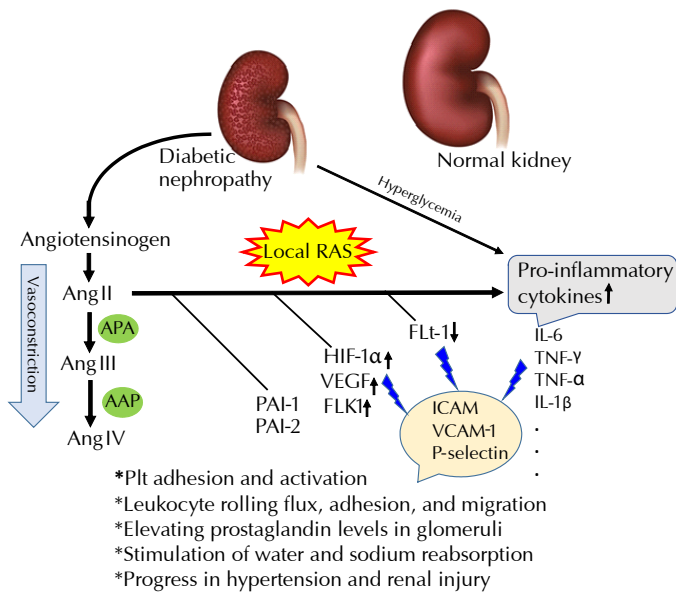
glomerular pressure should be maintained to continue blood filtration and maintain GFR [34].

Although Ang II constricts both afferent and efferent arterioles, the resistance of efferent arterioles is greater than that of afferent arterioles because of their smaller diameter. Since AT<sub>2</sub>R is more abundantly present in the afferent arterioles than AT<sub>1</sub>R and because of the release of NO, the constriction of the afferent arterioles is stronger than that of the efferent arterioles [26]. These different tonicities in two vascular compartments force the blood to accumulate gradually in the glomerulus and increase the glomerular pressure. Also, Ang II can reduce the surface area available for filtration via elevating prostaglandin levels in glomeruli and constricting the glomerular mesangium [35]. This results in the maintenance of normal blood filtration by an increase in filtration fraction (i.e. the ratio of GFR to renal plasma flow). Following the stimulation of water and sodium reabsorption in the proximal tubule by local Ang II, the feedback mechanism is activated in macula densa, which reinforces the process. This plays an important role in the progression of hypertension and renal injury [35].

As shown in **Figure 2**, Ang II induces platelet adhesion and releases plasminogen activator inhibitors (PAI-1 and PAI-2), the potent coagulant/atherogenic factors [36]. Ang II becomes converted to Ang III (Ang (2-8)) by aminopeptidase A (APA) generally located in RBCs and the vascular surface of most tissues, mainly in endothelial and mesangial cells of the glomerulus and brush border compartments of the kidneys with high activity [37]. Ang III has 40% of the vasoconstriction properties and 100% of the aldosterone-producing activity of Ang II [38]. Ang IV (Ang (3-8)) generated by alanyl aminopeptidase (AMP) or arginine aminopeptidase has lesser activity than Ang III [39].

#### 5. Local RAS function in hyperglycemia

The hyperglycemic and inflammatory area of the diabetic kidney enhances the expression of local RAS components and the generation of local Ang II [40]. The autoregulatory mechanism of Ang II is disrupted



**Figure 2. Induction of local Ang II by the activation of local RAS which results in hypertension and endothelial and arterial injuries.** Ang II induces the stimulation of platelet adhesion and activation, leukocyte rolling flux, adhesion, and migration. It also causes the increase in prostaglandin levels in glomeruli, reabsorption of water and sodium, and progress in hypertension and renal injury. Conversion of Ang II to Ang III and then Ang IV by APA and AAP, respectively, reduces the destructive properties of Ang II and vascular contraction. *Abbreviations:* APA - aminopeptidase A; AAP - alanyl aminopeptidase; Flk-1 - fetal liver kinase 1 (also known as vascular endothelial growth factor receptor 2 (VEGFR-2)); Flt-1 - Fms-like tyrosine kinase 1 (also known as vascular endothelial growth factor receptor 1 (VEGFR-1)); HIF-1 $\alpha$  - hypoxia-inducible factor 1 alpha; ICAM-1 - intercellular adhesion molecule 1; IFN- $\gamma$  - interferon  $\gamma$ ; IL-6 - interleukin 6; PAI - plasminogen activator inhibitor; Plt - platelet; RAS - renin-angiotensin system; TNF- $\alpha$  - tumor necrosis factor  $\alpha$ ; VCAM-1 - vascular cell adhesion molecule 1; VEGF - vascular endothelial growth factor.

in chronic kidney disease, which is due to renal hypertrophy and increased intraglomerular pressure. Increased renovascular hypertension activates the intratubular RAS in the entire nephron population. Such local RAS hyperactivity plays a serious role in renal tissue injury via Ang II [41]. According to the results of several animal studies, elevated Ang II, in turn, alters local levels of pro-inflammatory cytokines such as IL-6, IFN- $\gamma$ , TNF- $\alpha$ , and IL-1 $\beta$  [42, 43], which are also stimulators of VEGF expression.

Liu *et al.* found an increase in the expression of VEGF, TGF- $\beta$ , VEGFR2 (Flk-1), and their levels of phosphorylation in cultured podocytes stimulated with Ang II, with a smaller effect on VEGFR1 (Flt-1) [44]. Sanchez-Lopez *et al.* reported that VEGF gene expression and promoter activation increased in cultured tubuloepithelial cells treated with Ang II. They also observed that Ang II induced HIF-1 $\alpha$  production and DNA-binding activity [45]. Ang II induces increased leukocyte rolling flux, adhesion, and migration via direct stimulation and augmentation of intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM-1), and P-selectin, independently of vasoconstrictor action (**Figure 2**) [42, 46]. This condition promotes the accumulation of macrophages in the kidney resulting in elevated local inflammatory cytokine levels and disease progression.

There are two main receptors for Ang II with opposite functions. The majority of biological actions and variety of pathophysiological effects of Ang II such as decreased renal blood flow, vasoconstriction, vascular smooth muscle cell proliferation, and renal tubular sodium reuptake are mediated by Ang II subtype 1 receptor (AT1R), while Ang II subtype 2 receptor (AT2R) counteracts the effects of AT1R and elicits protective effects via inhibiting cell proliferation and differentiation and promoting vasodilation and natriuresis [47]. Both receptors belong to G-protein-coupled receptors, but AT1R acts through Gq/11 [48] and subsequently phospholipase C and diacylglycerol. AT2R activates Gas [49] and the cAMP-dependent pathway. In renal tissue, AT1Rs are distributed throughout the renal compartments and located predominantly in the glomerulus, afferent and efferent arterioles, and renal tubules [50]. AT2R is highly expressed in fetal kidney where it is involved in cellular differentiation and tissue development and rapidly decreases after birth [51]. AT2R is found, to a lesser extent than AT1R, in the vessels, glomeruli, and tubules of the adult kidney [52]. It is involved in natriuresis via the bradykinin/NO/cGMP pathway that lowers blood pressure by increasing sodium excretion. However, the pathway is blocked by ACE via bradykinin degradation [53]. Interestingly, NO may have a positive feedback on AT2R upregulation [54].

Previous studies have suggested that Ang III is the regulating ligand in renal sodium excretion and mediates the AT2R-dependent natriuretic response [53, 55]. Although Ang III is the preferred AT2R agonist, both Ang II and Ang III stimulate vasodilation and decrease vascular resistance and natriuresis via activation of AT2R in the proximal tubules through the renal bradykinin/NO/cGMP signaling pathway [56]. However, studies have shown that Ang II must be converted to Ang III to induce AT2R-dependent responses [53, 57]. AT2R also lowers blood pressure by inhibiting renin synthesis and prevents atherosclerosis via inhibition of cellular proliferation and hypertrophy [58]. Generally, the characteristics attributed to AT2R include anti-inflammation, decreased sympathetic activity, anti-apoptosis, anti-fibrosis, and anti-growth [53, 59].

The AT4 receptor (AT4R), another angiotensin receptor, has been defined as the specific binding site for Ang IV. It has a general distribution in some tissues, including the kidney, adrenal gland, heart, and lung. AT4R is expressed in several renal cells with a mediatory role in the expression of PAI-1. However, its function in the kidney needs to be investigated [60]. The other local RAS components that counteract with Ang II signals are ACE2, Ang (1-7) and Mas receptors which are upregulated by AT2R.

## 6. Inhibition of RAS in diabetic nephropathy

The deterioration in kidney function may be significantly attenuated by effective control of blood pressure and reducing proteinuria [61]. As mentioned above, hyperglycemia activates local RAS by upregulating intracellular RAS components including renin, ACE, and chymase (an Ang II-generating enzyme) in mesangial cells, AT1R, AT2R, and renin in endothelial cells, and renin and AT1R in podocytes

[62]. Some contradictory results have been reported on the effects of hyperglycemia on AT1R levels, but most of them have shown an increase in AT1R density [62, 63]. Hyperglycemia also increases angiotensinogen production in glomerular mesangial cells, podocytes, and endothelial cells. Ang II, in turn, stimulates the proximal tubule to express angiotensinogen and release it into the tubular fluid [62]. Therefore, urinary concentration of angiotensinogen may be a signal for kidney damage [64].

At first, it was presumed that RAS blockades such as ACE inhibitors (ACEIs) or that angiotensin receptor blockers (ARBs) could prevent renal damage by controlling blood pressure and proteinuria, but the progression of renal failure, despite such considerations, indicated the interference of factors other than Ang II [64]. In addition to the ACE pathway, hyperglycemia increases Ang II generation via non-ACE pathways, including chymase, kallikrein, cathepsin G, and elastase-2 responsible for Ang II formation in human tissues [65].

Chymase is a chymotrypsin-like serine protease which is mainly secreted by mast cells and to a lesser extent by cardiac fibroblasts and vascular endothelial cells and localized in tissue bound to heparin proteoglycans; such bonding protects the activity of chymase for several weeks [66]. Chymase is an important multifunctional enzyme in tissue remodeling and a potent pro-inflammatory agent with a role in converting Ang I to Ang II more effectively than ACE [67]. It is poorly expressed in glomeruli and other parts of the normal kidney, but significantly upregulated in diabetic kidneys because of inflammatory tissue conditions. Chymase promotes glomerulosclerosis and tubulointerstitial fibrosis via Ang II, and induces TGF- $\beta$  formation directly from pro-TGF- $\beta$  and indirectly through Ang II stimulation [66].

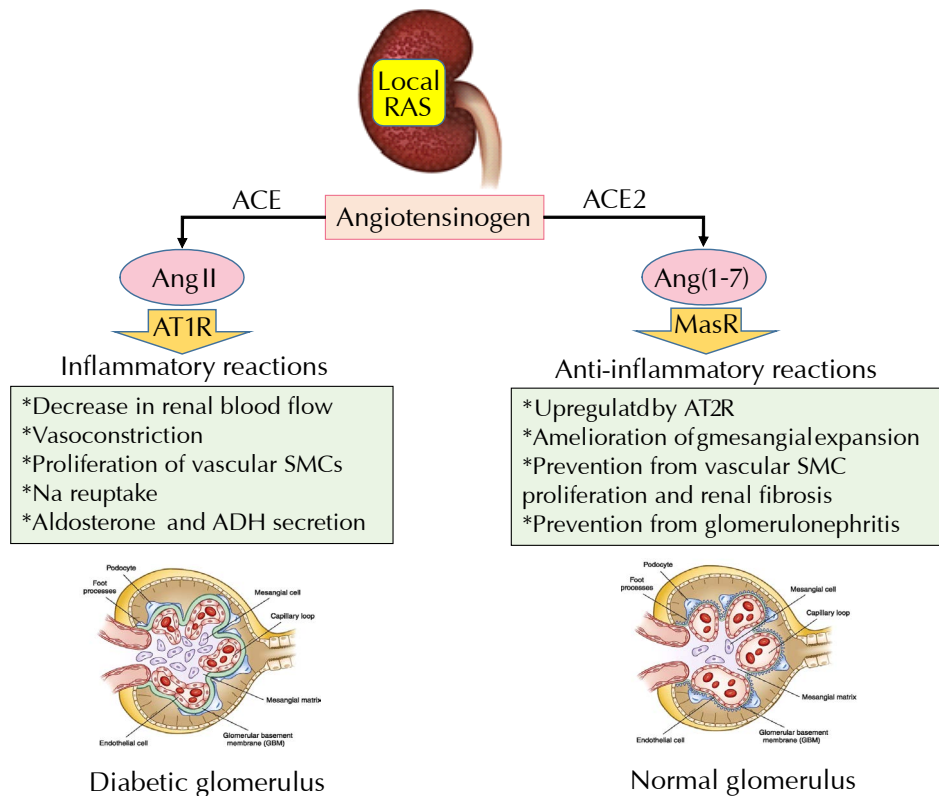
Kallikreins are a subgroup of serine proteases with various physiological functions, one type of which exists in the plasma with about fifteen closely related types in various tissues [67, 68]. Bradykinin and kallidin are the most important mediators in the kallikrein-kinin system and participate in blood pressure regulation as vasodilators. ACE possesses a strong affinity to degrade bradykinin. ACE inhibitors increase bradykinin levels in tissues which, in turn, cause mast cell degranulation and chymase release (tricking ACE inhibitors) [69, 70]. The kallikrein-kinin system, which is in counteraction with RAS, inhibits apoptosis, inflammation, and fibrosis via suppression of oxidative stress, TGF- $\beta$ 1 expression, and MAPK activation; it also has a protective effect against developing microalbuminuria [71, 72]. Therefore, independent of the vasodilatory effect, the system is capable of protecting the kidney from tubular damage, glomerulosclerosis, and perivascular remodeling. Clinical studies have shown that the excretion of kallikrein in the urine of diabetic patients with nephropathy is significantly lower than in diabetic patients without nephropathy and in healthy individuals [73].

Cathepsin G, a serine proteinase involved in the inflammatory responses, exists in high concentrations in the azurophil granules of neutrophils and peroxidase-positive granules of monocytes and macrophages and to lesser amounts in the blood stream and various tissues [74]. It was also capable of converting Ang I to Ang II and even of generating Ang II directly from angiotensinogen [75]. The half-life of free circulating cathepsin G is short, about 4-5 minutes, but binding to its native inhibitors, such as  $\alpha$ 1-antichymotrypsin in plasma or secretory leukocyte proteinase inhibitor in the kidney, as sources of active cathepsin G, increases its stability and can be reactivated by partial proteolysis of the inhibitor [76, 77]. However, little research has been done on the role of cathepsin G in kidney diseases until today.

Elastase-2, a serine protease elastase family member 2A, is widely found in several organs [78]. In addition to breaking down elastin and collagen, destroying bacteria by neutrophils, and playing a role in immunological balance, it participates in an alternative pathway for Ang II generation, especially in the presence of ACE inhibitors which increase elastase-2 contribution to Ang II generation [79].

## 7. Intrarenal RAS in diabetic nephropathy

Diabetic nephropathy starts with a significant stimulation of juxtaglomerular cells to release renin [1, 20]. Both circulatory and tissue RAS appear to be overactivated in the development of glomerulosclerosis and renal fibrosis [4, 32]. Thus, early intervention via RAS inhibitors in diabetic patients with normal albuminuria seems rational. However, what are the natural pathways in the body that reduce or regulate the local RAS system? The components of the alternative RAS pathway including ACE2, Ang (1-7), and Mas receptors with proven cardio- and the reno-protective roles are upregulated by AT2R (**Figure 3**) [80]. ACE and ACE2 with 40-42% homology traverse the plasma membrane and also exist in soluble forms in the bloodstream when dissociated from the cell membrane [81]. Both co-localize in the brush border of mouse proximal tubules and different cell types of glomeruli [82]. In the glomerulus, ACE2 is present in epithelial and mesangial cells and ACE is mainly expressed in endothelial cells. In contrast to the fibrogenic, proinflammatory, and proliferative actions of the ACE/Ang II/AT1 pathway, it has been demonstrated that the ACE2/Ang (1-7)/Mas axis has anti-inflammatory, anti-fibrogenic, and anti-proliferative properties and protects against glomerulosclerosis and Ang II-induced tubulointerstitial fibrosis [81, 82]. Activating Mas-related G-protein-coupled receptor antagonizes AT1R, reduces ROS and inflammation, and thus ameliorates renal injury. Animal studies have demonstrated a downregulation in kidney ACE2 in nephropathic kidney, possibly because of enhanced Ang II-mediated TGF- $\beta$ /Smad and NF- $\kappa$ B signaling pathways. Also, inhibition of ACE2 with an inhibitor like MLN-4760 accelerates albuminuria [81, 83]. Oudit *et al.* administered recombinant ACE2 to mice treated with Ang II and observed an attenuation of increased blood pressure



**Figure 3. Comparison of ACE and ACE2 and the function of their products.** The ACE pathway is an inflammatory process, and the ACE2 pathway, as an alternative pathway, is an anti-inflammatory process. The balance between the two pathways regulates glomerular function and renal homeostasis, which is disrupted in diabetic nephropathy. *Abbreviations:* ACE - angiotensin-converting enzyme; ADH - antidiuretic hormone; Ang (1-7) - angiotensin 1-7; Ang II - angiotensin II; AT1R and AT2R - angiotensin II receptor 1 and 2; MasR – Mas receptor (also known as G protein-coupled receptor); RAS - renin-angiotensin system; SMC - smooth muscle cell.

and a decrease in oxidative stress, renal Ang II levels, and fibronectin levels as a marker of fibrosis [84]. It can be concluded that ACE and ACE2 collaborate to regulate the intrinsic renal homeostasis by balancing the expression of Ang II and Ang (1-7).

Several small peptides have been recognized to originate from Ang II degradation with local physiological effects in the kidney, including Ang (1-7), Ang (2-8), Ang (3-8), Ang (3-4), Ang (1-5), and Ang (1-4) [85]. Among them, the protective role of Ang (1-7) in kidney complications, which is produced by ACE2 from the hydrolysis of Ang II and has an affinity for Mas receptor, has been studied in detail [86]. Although there are conflicting results for the effect of Ang (1-7) on the development of chronic kidney disease (CKD), most of them emphasize its protective role. According to several reports, the renoprotective effects of Ang (1-7) exerted by the molecular mechanisms include the inhibition of mechanisms induced by AGR [87] and the MAPK/ERK1/2 pathway [88], a reduction in the expression of collagen IV, VEGF, TGF- $\beta$ , NOX4, p47 phox, PKC $\beta$ 1, and PKC $\alpha$ , and the phosphorylation of Smad3 [88, 89]. Therefore, Ang (1-7) antagonize the effects of Ang II on tissue inflammation and fibrosis and on lipid accumulation by upregulation of the LDL receptor.

Although ACE2 is the main producer of Ang (1-7), there are other enzymes such as neprilysin (NEP) [90], prolyl-endo-peptidase (PEP) [91], and prolyl-

carboxy-peptidase (PRCP) [92] that can convert Ang I or Ang (1-9) to Ang (1-7). Among the aforementioned enzymes, NEP, a membrane-bound, zinc-dependent metalloendopeptidase (MME) with widespread tissue distribution has been abundantly detected in the kidney, mainly in the brush border of proximal tubular cells [90]. In addition to forming Ang (1-7) from Ang I cleavage, it degrades natriuretic and other vasoactive peptides including substance P, bradykinin, endothelin, and Ang II. Although some of the NEP activities like Ang (1-7) formation and Ang II and endothelin degradation help to prevent kidney failure, lowering the levels of natriuretics and other vasoactive peptides has unfavorable effects which exacerbate CKD [93]. Thus, targeting NEP may have potential benefits and adverse consequences. For example, NEP inhibition decreases blood pressure by increasing natriuresis and diuresis and by inhibiting sympathetic stimulation, but, on the other hand, it induces inflammation and fibrosis in the kidney by upregulation of Ang II and reduction of Ang (1-7) [93, 94]. Therefore, to reduce intraglomerular pressure and proteinuria, NEP inhibitors are used in combination with an ACE or AT1R blocker to diminish the production or the effect of excess Ang II [95]. The use of an ACE inhibitor may be more effective than an AT1R blocker because of a shifting of Ang I to ACE2 or the NEP/Ang (1-7)/Mas axis.

Prolyl carboxypeptidase (PRCP) [92] and prolyl endopeptidase (PEP) [95] are lysosomal and cytosolic peptidase, respectively; they have been detected as the main enzymes in circulation (in white blood cells and plasma). Both enzymes are involved in the maturation and degradation of a variety of peptides and the conversion of Ang II to Ang (1-7), which may be considered as their protective role against Ang II-induced hypertension [92, 95, 96]. Previous studies demonstrated that PRCP participates in the processing of Ang II in human kidney extracts and cultured human glomerular endothelial cells [97]. However, more studies are needed to reveal their roles in kidney disease.

## 8. RAS therapeutic interventions used in diabetic nephropathy

DN, characterized by persistent proteinuria and a decline in GFR, is the leading cause of ESRD [1, 3]. In addition to controlling blood glucose, major therapeutic interventions include antihypertensive treatment and restriction of dietary proteins. Drug classes employed in DN include ACE inhibitors, angiotensin receptor blockers (ARB), beta-adrenergic blocking agents, calcium channel blockers, and diuretics [98]. The findings of clinical and animal studies suggest that ACE inhibitor users show a lower risk of ESRD compared with those on ARB [98, 99]. A meta-analysis of 119 randomized controlled trials (n = 64,768) concluded that ACE inhibitors provide more renal benefits and safety than ARBs [100]. ACE inhibitors, including benazepril, zofenopril, perindopril, trandolapril, captopril, enalapril, lisinopril, and ramipril lower blood pressure by causing relaxation of blood vessels via inhibition of ACE to produce Ang II and hydrolyze bradykinin [101]. In addition to being beneficial in cardiovascular diseases such as acute myocardial infarction and heart failure, they help reduce renal complications of DN by lowering blood pressure and strengthening perfusion in the glomerular artery [102]. ACE inhibitors are frequently the first choice of medication in DN [100]. All have common mechanisms, but according to the pharmacological reports, ACE inhibitors may differ in their affinity for tissue ACE [103]. One of the benefits of this treatment is the increase in Ang I due to the prevention of Ang II formation, which can be used to tread the alternative route of RAS by ACE2 to convert to Ang (1-7).

Ang (1-7) prevents the progression of nephropathy by ameliorating mesangial expansion, reducing oxidative stress and inflammation, and suppressing vascular smooth muscle cell proliferation and renal fibrosis. Ang (1-7) acts through downregulating TGF- $\beta$ 1/Smad signaling, blocking the activation of MAPKs and VEGF-mediated pathways, suppressing AT1R expression, and inducing ACE2 expression as a positive feedback mechanism via the Mas receptor [104]. ACE2 is not inhibited by ACE inhibitors and does not produce Ang II or metabolize bradykinin, thereby causing intrarenal

vasodilation via stimulation of NO and vasoactive prostanoid production, which protects against a decrease in renal perfusion and GFR [105]. However, prior studies have demonstrated that patients treated with these inhibitors alone, even with effective doses, do not show suppression of Ang II formation [106]. As discussed above, Ang II may also be produced by ACE-independent Ang II formation pathways in human tissues that are insensitive to ACE inhibitors [92, 95]. Interestingly, ACE inhibitors increase bradykinin levels in tissues, which in turn cause mast cell degranulation, chymase release, and Ang II production. Therefore, in the presence of ACE inhibitors, Ang II can still be produced and maintained at a normal level [107]. For this reason, combination therapy is commonly used by some physicians to control blood pressure.

ARBs, including azilsartan, candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, and valsartan, are Ang II antagonists that bind to and inhibit AT1R [108]. Although the blockers suppress the AT1R pathway, in this condition, Ang II is free to stimulate further AT2R, which causes vasodilation and normalizes blood pressure. Both ARB and ACE inhibitors suppress RAS and slow the progression of DN, but increase blood potassium levels in diabetic patients with renal failure, which may be exacerbated by diabetic acidosis.

## 9. Conclusions

Patients with DN suffer from chronic hypertension because of decreased GFR and renal response through systemic RAS activation and elevated blood Ang II. In such conditions, in addition to precise control of blood sugar, the main treatment is to reduce RAS activity by ACE inhibitors or ARBs, use diuretics, and limit salt and protein intake to prevent hypertension and albuminuria.

In chronic hyperglycemia, the production of Ang II is also locally increased in the kidney, causing tissue damage, mainly through oxidative stress, inflammation, and the upregulation of certain growth factors and their receptors. There are alternative pathways in the kidney against local RAS which have been demonstrated to be protective in that they reduce tissue concentration of Ang II. Such autoregulatory pathways are disrupted in chronic kidney diseases. Thus, given the increased activity of local RAS, controlling blood pressure by inhibiting systemic RAS via ACE inhibitors or ARBs may not be sufficient to prevent the progression of renal failure. Further research with pharmaceutical and therapeutic approaches should be designed to strengthen the alternative pathways through upregulation of ACE2, neprilysin, and Ang (1-7), and interrupt other cell pathways related to local Ang II production.

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