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Up-Regulation of Renin-Angiotensin System in Diabetes and Hypertension: Implications on the Development of Diabetic Nephropathy

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1. Introduction

The growing worldwide epidemic of metabolic syndrome and other chronic degenerative diseases continues to expand, with a rapid decrease in the age at which they are being diagnosed (Guarnieri et al.; 2010; Hsueh & Wyne, 2011). Metabolic syndrome is a multi-factorial disorder, strongly influenced by several lifestyle factors, with symptoms clustering on abnormalities that include obesity, hypertension, dyslipidemia, glucose intolerance and insulin resistance (Guarnieri et al.; 2010; Tanaka et al.; 2006). The syndrome is also referred to as "Diabesity" highlighting the incidence of diabetes mellitus (DM) in combination with obesity as a result of changes in human behavior (Astrup & Finer, 2000; Farag & Gaballa, 2011; Hu, 2011).

Obesity is considered an independent predictor of the development of hypertension and it has been estimated that about half of individuals with essential hypertension are considered insulin resistant (Hall et al.; 2010; Kotsis et al.; 2010). Likewise, insulin resistance and hyperinsulinemia increase the risk of hypertension, and it usually accompanies DM, early in type 2 (DM2) and delayed in type 1 (DM1). Moreover, among patients being treated for hypertension, the risk of new-onset diabetes is doubled in those with uncontrolled blood pressure (BP) (Gress et al.; 2000; Gupta et al.; 2008; Izzo et al.; 2009). Although effective antihypertensive agents are available, achieving adequate BP control remains difficult in hypertensive patients, particularly in the context of concomitant diabetes.

It is widely known that individuals with DM and/or hypertension are prone to develop a broad range of long term complications, including cardiovascular disease and nephropathy (Farag & Gaballa, 2011; Guarnieri et al.; 2010; Houston et al.; 2005; Handelsman, 2011; Tanaka et al.; 2006), and it has already been shown that several modifiable risk factors are associated with poor renal and cardiovascular outcome, including BP, plasma glucose and lipid concentrations, smoking, and body weight (Miao et al.; 2011). It is important to

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highlight that both DM and hypertension exacerbate each other in terms of subsequent complications (Cooper & Johnston, 2000) increasing the burden of social dysfunction and high risk of premature death.

DM is a chronic metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Nowadays, diabetes afflicts around 6.6% of the global adult population, or approximately 285 million individuals, and this is projected to increase by more than 50% to a 7.8% worldwide prevalence in 20 years. Considering that DM is an important health problem and it has been recognized as a major risk factor for the development of complications in target organs, including retinopathy, neuropathy, nephropathy and cardiovascular disease, the comprehension of the mechanisms involved in the association among diabetes is the subject of many research groups (International Diabetes Federation, 2009).

Of these complications, diabetic nephropathy (DN), the most common etiology of chronic kidney disease (CKD) and common cause of end-stage renal disease (ESRD) in adults in the Western world (Choudhury et al.; 2010; Cooper, 1998; National Institute of Diabetes and Digestive and Kidney Diseases, 2010), is associated with the highest mortality (Cooper, 1998; Giacchetti et al.; 2005) making early diagnosis critical in preventing long term kidney loss. Approximately 30% of patients with either DM1 or DM2 develop DN (Dalla Vestra et al.; 2000), and in these patients, lowering of BP and of urinary albumin excretion significantly decrease the risk of progression to ESRD, myocardial infarction and stroke (Choudhury et al.; 2010; Cooper et al.; 2000; Gupta et al.; 2008; Handelsman, 2011; Keller et al.; 1996).

Approximately 80% of individuals with diabetic ESRD are affected by hypertension, which accelerates the progression rate of renal disease (Jandeleit-Dahm & Cooper, 2002). In DM1 the onset of hypertension appears to occur primarily as a consequence rather than as a primary cause of renal disease (Poulsen et al.; 1994). The link between glycemic control and the development of hypertension has been demonstrated in the follow-up of the landmark Diabetes Control and Complications Trial (DCCT), the Epidemiology of Diabetes Interventions and Complications (EDIC) study (Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group [EDIC], 2003). It demonstrated that hypertension was developed in 40% of the patients in the conventionally treated group compared with 30% in the group treated with an intensified insulin regimen in year 8 of the EDIC follow-up. These beneficial effects were seen in the context of reduced renal disease consistent with the view that hypertension in DM1 is primarily a manifestation of DN in these subjects. Therefore, it appears likely that hyperglycemia or insulin plays a role in influencing BP in DM1 (Elliott et al.; 2001). Regarding DM2, the combination with hypertension appears to cluster clinically as part of a syndrome involving not only these two conditions but also insulin resistance, dyslipidemia, central obesity, hyperuricemia, and accelerated atherosclerosis (Eckel et al.; 2005; Sowers et al.; 2001; Williams, 1994). The underlying explanation for this cluster of clinical features remains unexplained but insulin resistance has been postulated by many investigators as playing a pivotal role (Isomaa et al.; 2001; Sowers et al.; 2001; Williams, 1994).

Clinical progression of DN can be characterized into 5 phases: 1) hyperfiltration with renal hypertrophy, increased renal plasma flow and glomerular filtration; 2) normoalbuminuria with early renal parenchymal changes of basement membrane thickening and mesangial expansion; 3) microalbuminuria with early hypertension; 4)

overt proteinuria; and 5) ESRD (Mogensen, 1976). These factors collectively result in cell injury and apoptosis of podocytes, and an accumulation of extracellular matrix proteins in the glomerulus and in the tubule interstitium (Calcutt et al.; 2009; D'Agati & Schmidt, 2010; Decleves & Sharma, 2010; Ruggenenti et al.; 2010). In this process, the increasing severity of DN is rapid when there is progression from normoalbuminuria to macroalbuminuria, a transition which takes about ten years.

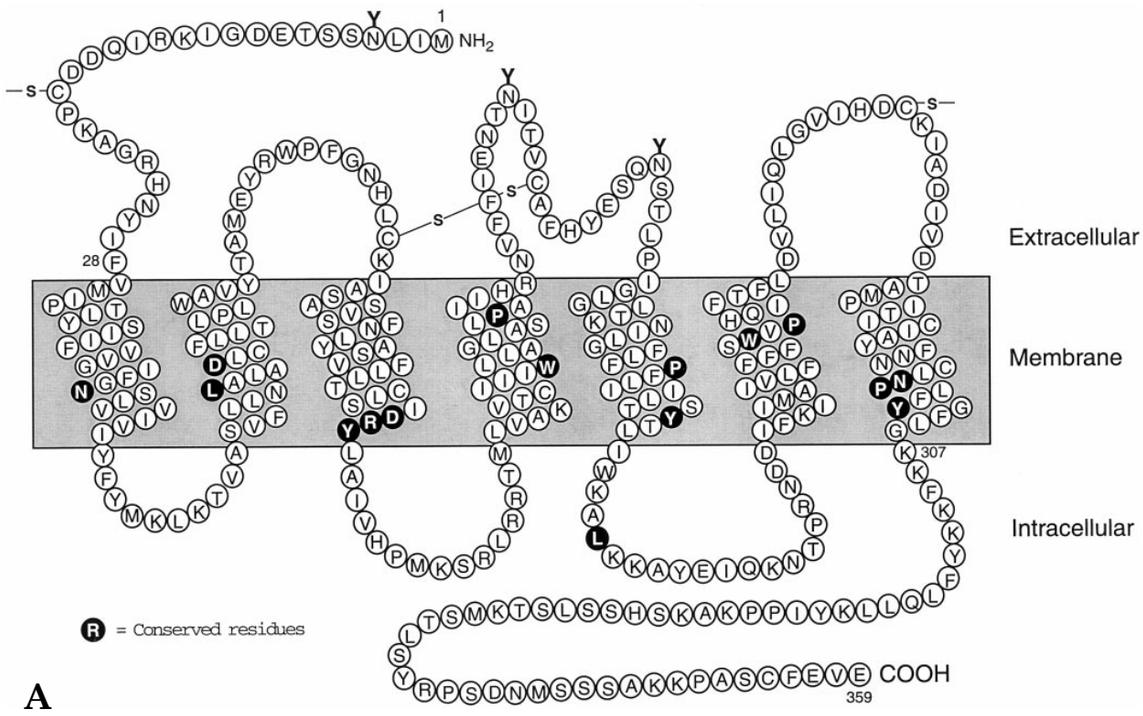
Pathogenesis of DN is strongly related to uncontrolled or chronic hyperglycemia, and various mechanisms that lead to pathological changes in the kidney, proteinuria, and decline in renal function seen in DN have been proposed (Calcutt et al.; 2009; Decleves & Sharma, 2010). Hyperglycemia can lead to the activation of oxidative stress and increased production of reactive oxygen species (ROS), increased formation of advanced glycation endproducts (AGEs), activation of the proinflammatory transcription factor NF-κB, activation of protein kinase C (PKC), transforming growth factor-β (TGF-β), and the renin angiotensin system (RAS) (Calcutt et al.; 2009; D'Agati & Schmidt, 2010; Decleves & Sharma, 2010; Ruggenenti et al.; 2010).

Apart from its importance in the regulation of arterial BP, salt balance and cardiovascular homeostasis, RAS is also involved in the control of almost every organ system and cell function. Recent advances in cellular and molecular biology, as well as cardiovascular and renal physiology, have provided a larger understanding of RAS involvement in many physiologic and pathophysiologic mechanisms and attesting to its importance in regulating the internal environment is the fact that overactivity of RAS can lead to arterial hypertension, congestive heart failure, and renal insufficiency (Kobori et al.; 2007; Navar et al.; 2011a; Navar et al.; 2011b; Ferrario, 2011; Unger et al.; 1998).

The RAS in diabetes has been studied in detail, including an assessment of the various components of this pathway in the kidney (Ferrario et al.; 2004; Ferrario & Varagic, 2010; Navar et al.; 2011a; Wehbi et al.; 2001; Zipelmann et al.; 2000). The system has been strongly implicated in the pathophysiology of diabetic renal disease on the basis of its ability to promote tissue remodeling (proliferation, hypertrophy and differentiation) and extracellular matrix remodeling repair and/or fibrosis (Hayden et al.; 2011) and of the therapeutic ability of angiotensin I-converting enzyme inhibitors (ACEi) and AT1 receptor blockers (ARB) to decrease microalbuminuria and the progression of DN to ESRD (Brenner et al.; 2001; Chan et al.; 2000; Heart Outcomes Prevention Evaluation [HOPE] Study Investigators; Lewis et al.; 2001; Parving et al.; 2001). Furthermore, it has been postulated that in diabetes there is a role for the RAS in mediating many of the functional effects, such as changes in intraglomerular hemodynamics as well as structural changes in the diabetic kidney at both glomerular and tubulointerstitial levels (Gilbert et al.; 1998). Based on these findings, pharmacologic interventions that inhibit production of angiotensin II (Ang II) or block angiotensin type-1 receptors (AT1R) that target the RAS are considered a cornerstone in the treatment of hypertension in patients with DN (Van Buren & Toto, 2011).

2. Circulanting and tissue renin-angiotensin systems

The RAS is a coordinated hormonal cascade initiated through biosynthesis of angiotensinogen (AGT), produced in the liver, that is cleaved by renin released from renal juxtaglomerular cells of the afferent arteriole. By this enzymatic cleavage, angiotensin I (Ang I)



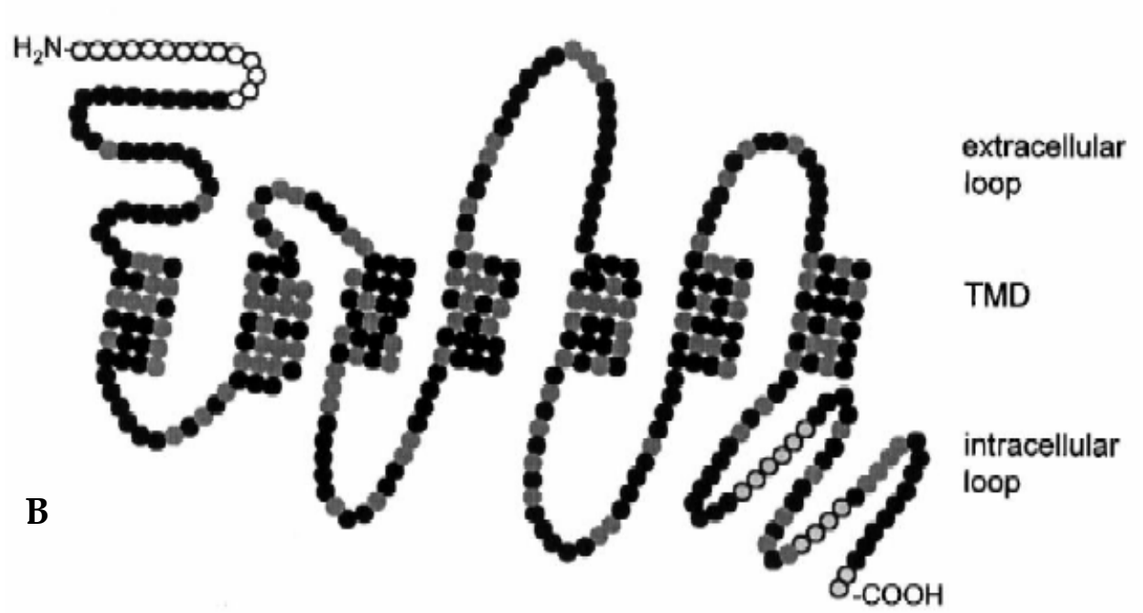


Fig. 1. (A) Secondary structure and consensus sequence of the mammalian angiotensin AT1 receptor. The amino acid residues that are highly conserved among G protein-coupled receptors are indicated in bold letters. The positions of the three extracellular carbohydrate chains, and of the two extracellular disulfide bonds, are also indicated (Adapted from de Gasparo et al.; 2000). (B) Comparison of the AT1 and AT2 receptors, sharing 33- 34% sequence homology. Grey circles indicate matching pairs of aminoacids. (TMD: transmembrane domain) (Adapted from de Gasparo & Siragy, 1999).

is generated, which, in turn is hydrolyzed by angiotensin I-converting enzyme (ACE) to produce Ang II. Over the last years, it has been established that most of the effects of Ang II are mediated through two distinct receptors, angiotensin type-1 receptors (AT1R) and angiotensin type-2 receptors (AT2R), acting antagonistically. AT2R shows only about 33–34% similarity to AT1R at the amino acid level (Figure 1A and 1B), which suggests that the two receptors derive from different ancestors (Mukoyama et al.; 1993; Kambayashi et al.; 1993; de Gasparo & Siragy, 1999; Unger & Sandmann, 2000; de Gasparo et al.; 2000).

Angiotensin actions via AT1R promotes vasoconstriction, inflammation, salt and water reabsorption and oxidative stress (Carey & Siragy, 2003). AT2R is generally associated with opposite actions to the AT1R, and it has already been shown that its activation induces bradykinin (BK) and nitric oxide formation, leading to natriuresis and vasodilatation. The AT2R is abundant in fetal tissue, decreasing after birth, with low amounts expressed in adult tissue such as kidney, adrenal and brain (Touys & Schiffrin, 2000; Carey & Padia, 2008; Rosivall, 2009) (Table 1A and 1B).

- Always expressed (Unger & Sandmann, 2000)
- Increased arterial pressure (Navar et al.; 2002)
- Aldosterone synthesis and secretion (Allen et al.; 2000; Navar et al.; 2002)
- Release of vasopressine (Unger & Sandmann, 2000)
- Decreased renal blood flow (Navar et al.; 2002)
- Renin secretion (Navar et al.; 2002)
- Cardiac contractility and hypertrophy (Allen et al.; 2000)
- Vascular smooth muscle cells proliferation (Touyz & Schiffrin, 2000)
- Mediates vasoconstriction, modulation of central sympathetic nervous system activity (Allen et al.; 2000; Unger & Sandmann, 2000)
- Mediates cell growth (Unger & Sandmann, 2000)
- Extracellular matrix formation (Touyz & Schiffrin, 2000)

Table 1A. Functions of AT1R

- Expressed during stress or injury (Unger & Sandmann, 2000)
- Fetal tissue development (Nakajima et al.; 1995; Stoll & Unger, 2001)
- Left ventricular hypertrophy (Senbonmatsu et al.; 2003)
- Mediates vasodilation (Unger & Sandmann, 2000)
- Neuronal regeneration (Stoll & Unger, 2001)
- Mediates cell differentiation (Unger & Sandmann, 2000)
- Inhibits cell growth (antiproliferation) (Unger & Sandmann, 2000)
- Cellular differentiation (Yamada et al.; 1999)
- Mediates tissue regeneration, apoptosis (Matsubara, 1998; Stoll & Unger, 2001; Unger & Sandmann, 2000)
- Modulation of extracellular matrix (Matsubara, 1998)

Table 1B. Functions of AT2R

The classical view of RAS cascade has been increasingly challenged with the discovery of new components such as the angiotensin converting enzyme 2 (ACE2). This enzyme with homology to ACE, is expressed in several tissues, including heart and kidney consistent with a

role for this enzyme in renal and cardiovascular physiology (Burrell et al.; 2004; Crackower et al.; 2002; Danilczyk et al.; 2003; Donoghue et al.; 2000; Harmer et al.; 2002; Tipnis et al.; 2000). Both isoforms of ACE are type-I transmembrane glycoproteins with an extracellular amino-terminal ectodomain and short intracellular cytoplasmic tail (Figure 2). This membrane localization is ideally positioned it to hydrolyse peptides in the extracellular milieu.

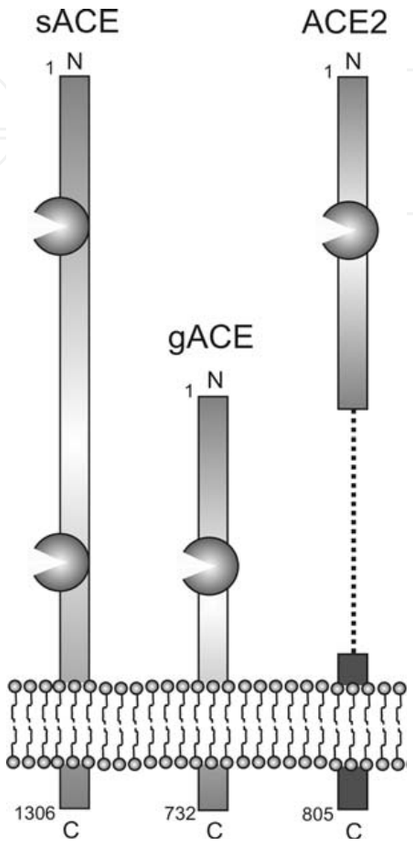


Fig. 2. Membrane topology and homology between ACE and ACE2. The ACE isoforms somatic ACE (sACE) and germinal ACE (gACE) and ACE2, are type I transmembrane proteins with an intracellular C-terminal domain and an extracellular N-terminal domain. In the case of the ACE isoforms and ACE2, the N-terminal extracellular domains contain HEMGH zinc-dependent catalytic domains (denoted as 'Pacman' symbols); two in ACE and one in both gACE and ACE2. Germinal ACE is entirely homologous to the C-terminal domain of sACE. ACE2 shares homology in its ectodomain with the N-terminal domain of sACE but has no homology with its C-terminal cytoplasmic domain (Adaptated from Lambert et al.; 2010).

ACE2 presents a single catalytic site and catalyzes the cleavage of Ang I to Ang 1-9, which can be further cleaved by ACE to Ang 1-7 (Burrell et al.; 2004; Donoghue et al.; 2000). Furthermore, Ang II can be converted directly by ACE2 to Ang 1-7. Ang 1-7 has been shown to exert vasodilatory properties and to antagonize the vasoconstriction mediated by Ang II, thereby contributing to the balance of vasodilators and vasoconstrictors generated by the various components of the RAS (Almeida et al.; 2000; Moriguchi et al.; 1995; Ferrario, 2006; Santos & Ferreira, 2007).

Another relevant change in our understanding of the classical endocrine RAS was the description of all components of the system in several tissues, including kidney, heart, brain, pancreas, adrenal, reproductive aparatus, retina, liver, gastrointestinal tract, lung and adipocytes, leading to the identification of new roles for angiotensins as paracrine and autocrine/intracrine function (Bataller et al.; 2003; Danser & Schalekamp, 1996; Lavoie & Sigmund, 2003; Navar et al.; 1994; Paul et al.; 2006; Ribeiro-Oliveira Jr et al.; 2008; Senanayake et al.; 2007; Tikellis et al.; 2003). RAS tissue appears to be regulated independently of the systemic one, and has been shown to contribute to a great number of homeostatic pathways, including cellular growth, vascular proliferation, extracellular formation and apoptosis (Paul et al.; 2006), via its specific receptors, such as AT1R, AT2R, prorenin/renin [(P)RR], Mas and also Ang III and IV receptors (Figure 3) (Nguyen et al. 2002; Santos et al.; 2003).

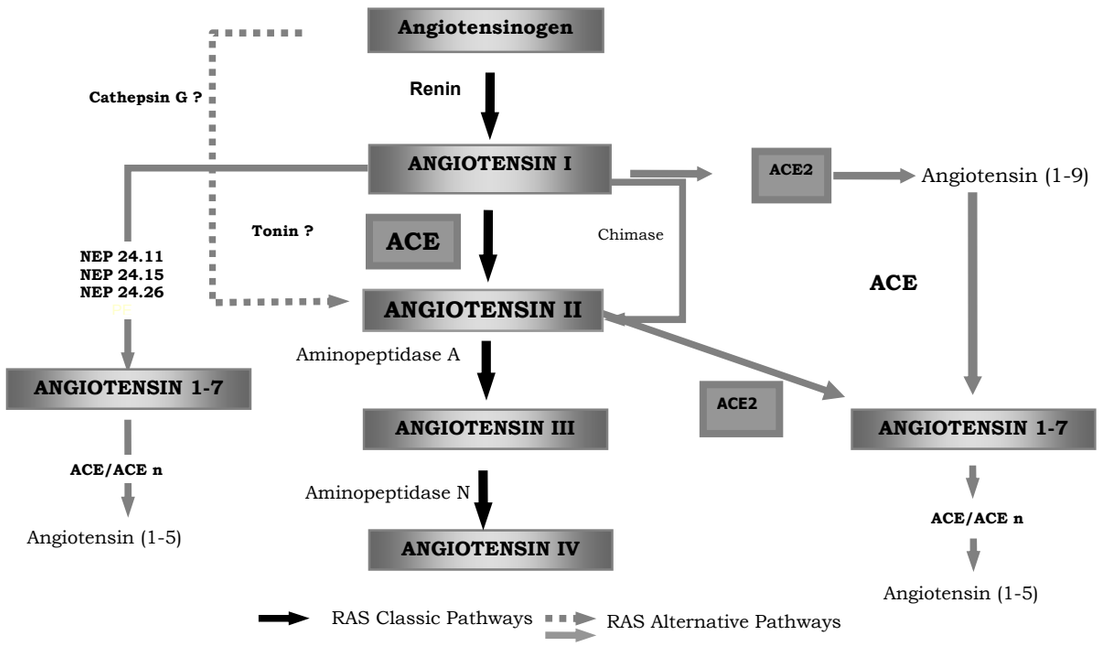


Fig. 3. Schematic representation of RAS. ACE, ACE 2, Neutral endopeptidase (NEP), N-domain ACE (ACEn).

3. The intrarenal RAS

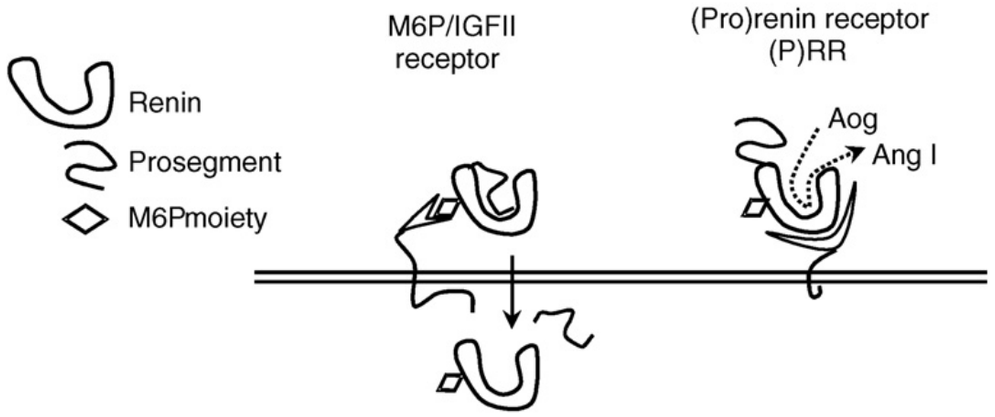
3.1 Angiotensinogen

AGT is a glycoprotein produced in the liver, kidney, heart, vessels and adipose tissue, which circulates as an inactive protein. AGT is hydrolyzed by renin to generate Ang I, and both the peptide and renin are considered the rate-limiting steps in the formation of Ang II. Studies with mice harboring the gene for human AGT fused to the kidney-specific androgen regulated protein promoter demonstrated that AGT mRNA and the protein were localized in the proximal tubule cells, and urinary AGT was described as a product secreted by the

proximal tubules and excreted in urine (Ding et al.; 1997; Kobori et al.; 2003). AGT synthesis is stimulated by inflammation, insulin, estrogen, glucorticoids (Kobori et al.; 2007; Prieto-Carrasquero et al.; 2004), and Kobori et al. (2001) described that Ang II can stimulate renal AGT mRNA and AGT protein synthesis, amplifying the activity of the intrarenal RAS (Kobori et al.; 2001).

3.2 Renin and prorenin

Renin is an aspartyl protease produced by the juxtaglomerular apparatus of the kidney. Its active form contains 339 amino acid residues after proteolytic cleavage at the N-terminus of prorenin, and in the circulation prorenin concentration is higher than that of renin. The activation of prorenin may occur by proteolytic or non proteolytic pathtways, both being able to generate Ang I from AGT. Circulating active renin and prorenin are originated mainly from the kidney, but other tissues are able to secrete both enzymes into the circulation, and therefore renin was also detected in urine suggesting its tubular formation, especially in the collecting duct (Prescott et al.; 2002; Prieto-Carrasquero et al.; 2004). As renin was also described in the collecting ducts, authors observed that Ang II is unable to inhibit renin secretion in this segment, the opposite to that which has been described in the juxtaglomerular apparatus (Kang et al.; 2008; Prieto-Carrasquero et al.; 2004; Rosivall, 2009).



Binding of renin and prorenin:	*via* M6P residues	Specific, K_D nM
Angiotensin I generation:	Intracellular	Cell surface
Activation of prorenin:	Proteolytic	Non-proteolytic
Signalling:	No, Clearance receptor	MEK1/2, ERK1/2 P38, HSP70
Cellular effects:	None	Up-regulation of TGFβ, PAI1, collagens, fibronectin expression, Mesangial cells proliferation

Fig. 4. Principal characteristics of the two receptors for renin and prorenin, the mannose-6-phosphate receptor and the (pro)renin receptor ((P)RR) (Adapted from Nguyen & Contrepas, 2008).

The specific receptor for renin and for its inactive proenzyme form, prorenin, was cloned in 2002 and called (P)RR for (pro)renin receptor. The PRR gene is named ATP6ap2/PRR because a truncated form of (P)RR was previously described to coprecipitate with the vacuolar H+-proton adenosine triphosphatase (V-ATPase) (Nguyen et al.; 2002). The (P)RR is a single trans-membrane domain receptor that acts as co-factor for renin and prorenin by increasing their enzymatic activity on the cell-surface and mediating an intracellular signaling. It activates the mitogen activated protein kinases ERK1/2 cascade leading to cell proliferation and to up-regulation of profibrotic gene expression (Nguyen, 2011).

Two (P)RRs have been characterized to date, the functional receptor specific for renin and prorenin (Nguyen et al.; 2002) and the ubiquitous mannose-6-phosphate receptor (M6P-R) which is admitted to be a clearance receptor (Saris et al.; 2001) (Figure 4). It is known that the binding of renin with (P)RR increases its catalytic efficiency upon its substrate, a phenomena that may be implicated in target-organ lesion in the kidney and the development of DN (Ichihara et al.; 2006; Nguyen et al.; 2002). On the other hand, increases in prorenin concentration may decrease the (P)RR expression that can act as a negative feedback (Ichihara et al.; 2006; Nguyen et al.; 2002; Staessen et al.; 2006). Moreover, studies in genetically modified animals overexpressing (P)RR a role for (P)RR cardiovascular and renal pathologies since rats overexpressing (P)RR in vascular smooth-muscle cells develop high BP and those with an ubiquitous overexpression of (P)RR have glomerulosclerosis and proteinuria (Nguyen & Contrepas, 2008).

3.3 Angiotensin I-converting enzyme (ACE)

ACE is an ectoenzyme located in many vascular beds and also on cell surface of mesangial, proximal and collecting duct cells in the kidney and was described as a dipeptidyl carboxypeptidase (Camargo de Andrade et al.; 2006; Redublo Quinto et al.; 2008). It catalyzes the conversion of the decapeptide Ang I to the octapeptide Ang II, which is a potent vasoconstrictor, and in addition inactivates the vasodilator BK (Erdos, 1976).

The ACE gene encodes two enzymes: a somatic isozyme (150–180 kDa) and a germinal or testicular isozyme isozyme (90–100 kDa) identical to the C-terminal portion of endothelial ACE, only expressed in sperm (Hall, 2003; Lattion et al.; 1989). A soluble isoform of ACE, which is derived from the membrane bound isoform by the action of secretases, is also present in serum and other body fluids such as urine (Casarini et al.; 1995; Casarini et al.; 2001; Xiao et al.; 2004). ACE homologs have also been found in other animal species, including chimpanzee, cow, rabbit, mouse, chicken, goldfish, electric eel, house fly, mosquito, horn fly, silk worm, Drosophila melanogaster and Caenorhabditis elegans, and in the bacteria Xanthomonas spp. and Shewanella oneidensis (Corvol & Williams, 1998; Riordan, 2003). The cDNA of one form of D. melanogaster ACE (termed AnCE) encodes a protein of 615 amino acids that have a high degree of similarity to both domains of human sACE, indicating that the D. melanogaster protein is a single-domain enzyme (Williams et al.; 1996; Riordan, 2003) (Figure 5A). It contains a signal peptide but no carboxy-terminal membrane-anchoring hydrophobic sequence. A second ACE-related gene product, termed Acer, has also been identified in D. melanogaster. Selective inhibition by phosphinic peptides (containing -PO2-CH2- links instead of -CO-NH- links) indicates that Acer has active site features characteristic of the N - domain of sACE (Riordan, 2003).

ACE presents two distinct catalytic domains, called N- and C-terminus (Wei et al.; 1991) (Figure 5 A and B), and both sites hydrolyze Ang I. However, the N-domain has two specific physiological substratum, Ang 1-7 and N-acetyl-Seryl-Aspartyl-Lysyl-Proline, a hematopoietic peptide (Jaspard et al.; 1993; Rousseau et al.; 1995). ACE is distributed along human and rat kidney, and has already been described in glomeruli, mesangial cells and also in proximal and collecting duct cells (Camargo de Andrade et al.; 2006; Redublo Quinto et al.; 2008). Casarini et al. (1995 and 2001) observed two N-domain ACE isoforms (nACE)

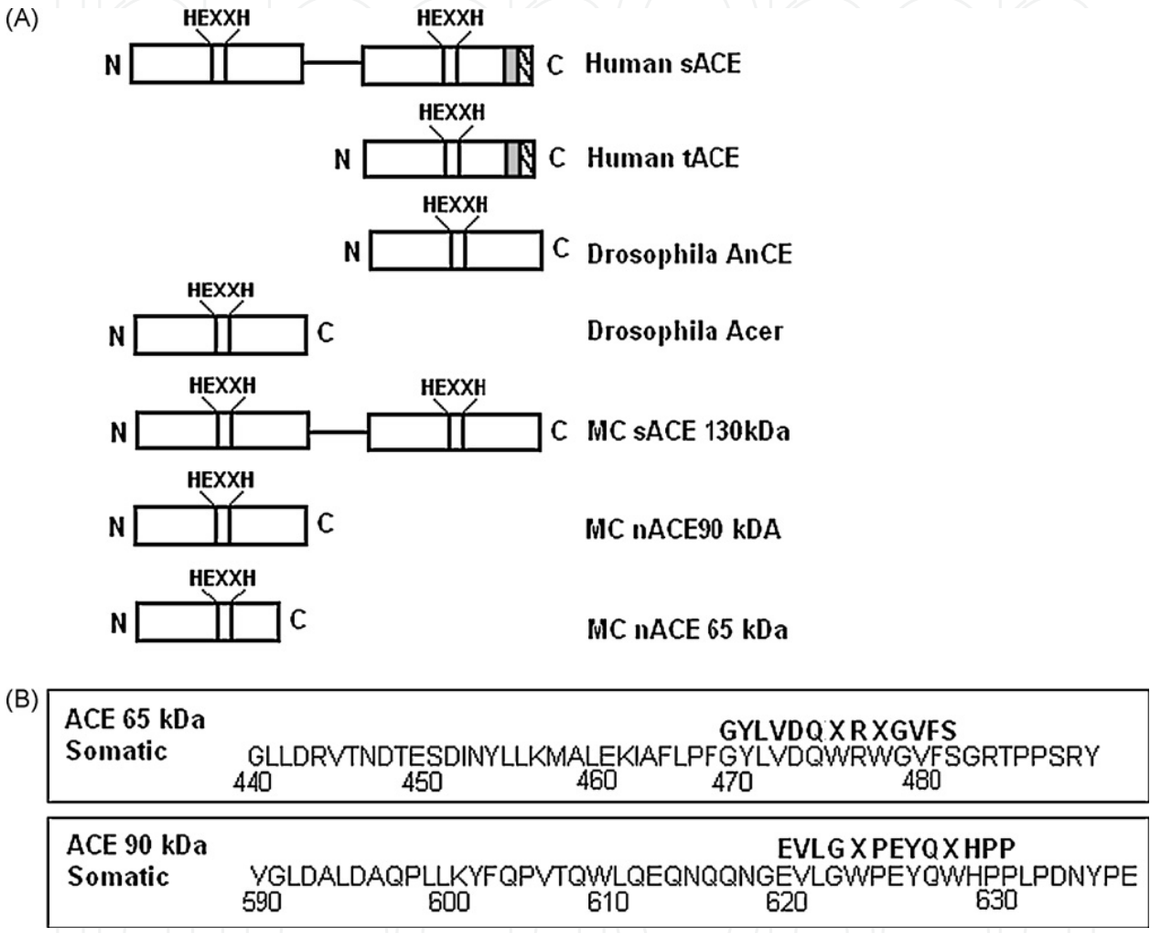


Fig. 5. Schematic representation of primary structure of several members of the ACE protein family. (A) Location of the active-site-zinc-binding motifs are indicated by HEXXH; transmembrane domains are in black. The sequence of testicular ACE (tACE) is identical to that of the C-domain of the sACE, except for its first 36 amino acids. Human tACE and sACE have the same carboxyl-terminal transmembrane and cytosolic sequence. Drosophila ACEs, cDNA of one form of D. melanogaster ACE (termed AnCE) and a second ACE-related gene product (termed Acer) lack a membrane-anchoring sequence. Dimensions are not to scale. N, amino terminus; C, carboxyl terminus (Adapted from Riordan, 2003). (B) The C-terminal alignment of 65 kDa nACE with rat ACE ended at Ser482. The same analysis for 90 kDa nACE evidenciated that the enzyme finished at Pro629 amino acid after their alignment with rat ACE. Both structures are similar for urine, tissue and mesangial cells (Adapted from de Andrade et al.; 2010).

with molecular weight of 190 and 65 kDa in the urine of healthy subjects, and two isoforms of 90 and 65 kDa, both nACE, in the urine of hypertensive patients (Casarini et al.; 1995, 2001). The same nACE enzymes were obtained by Marques et al. (2003) in the urine of Wistar–Kyoto and Spontaneously Hypertensive rats (SHR), and by Ronchi et al. (2005) in different tissues of SHR, suggesting that the 90/80 kDa ACE could be a possible biological marker of hypertension (Marques et al.; 2003; Ronchi et al.; 2005). Moreover, Deddish et al. (1994) described an active soluble form of nACE in human ileal fluid, with a molecular mass of 108 kDa, thereby differing from the enzymes described in human urine (Deddish et al.; 1994).

Apart from the classic actions of ACE, several groups have recently demonstrated that ACE presents novel actions, mainly related to cell signaling. As demonstrated by Kolstedt et al (2004), ACE also functions as a signal transduction molecule and binding of ACE substrates or inhibitors to the enzyme initiates a cascade of events, including the phosphorylation of its Ser1270 residue, increasing ACE and COX2 synthesis. Moreover, using *in vitro* models such as Chinese hamster ovary and melanoma cells, it was demonstrated that Ang II can also interact with ACE evoking calcium signaling and promoting an increase in the generation of ROS (Guimaraes et al.; 2011; Kohlstedt et al.; 2004).

3.4 ACE2

ACE2 is a new member of RAS, homologue of ACE, which acts as a monocarboxipeptidase. The enzyme consists of 805 amino acids and is a type I transmembrane glycoprotein with a single extracellular catalytic domain (Donoghue et al.; 2000; Tipnis et al.; 2000). Unlike somatic ACE, ACE2 removes a single C-terminal Leu residue from Ang I to generate Ang 1-9, a peptide with unknown function. Although ACE2 was described originally for its ability to generate Ang 1-9 from Ang I (Donoghue et al.; 2000), it also degrades Ang II to the biologically active peptide Ang 1-7 (Burrell L et al, ,2004; Vickers et al.; 2002). *In vitro* studies showed that the catalytic efficiency of ACE2 for Ang II is 400-fold greater than for Ang I (Vickers et al.; 2002), indicating that the major role for ACE2 is the convertion of Ang II to Ang 1-7.

The human ACE2 gene has been cloned and mapped to the X chromosome (Crackower et al.; 2002). This enzyme exists as a membrane-bound protein in the lungs, stomach, spleen, intestine, bone-marrow, kidney, liver, brain (Gembardt et al.; 2005) and the heart and is not inhibited by ACE inhibitors (Ribeiro-Oliveira Jr et al.; 2008). ACE2 is abundantly expressed in renal epithelial cells including proximal tubular cells (Danilczyk & Penninger, 2006; Donoghue et al.; 2000; Shaltout, et al.; 2007), and in the pancreas, ACE2 was found to be localized to acini and islets following a similar distribution to that of ACE (Tikellis et al.; 2004).

Several studies support a counter-regulatory role for Ang 1-7 by opposing many AT1R-mediated actions, especially regarding vasoconstriction and cellular proliferation (Ferrario, 2006, Santos et al.; 2005). Thus, Ang 1-7 has become a key component of the RAS system due to its beneficial effects in the cardiovascular system. Although the pathophysiological significance of ACE2 in renal injury remains to be established, emerging evidence suggests that ACE2 deficiency leads to increases in intrarenal Ang II levels (Ribeiro Oliveira Jr et al.; 2008; Ferrario, 2006; Oudit et al.; 2010; Wolf & Ritz, 2005; Ye et al.; 2006). Thus, recently ACE2 has also been proposed as an acute biomarker of renal disease, considering that upregulation of ACE2, and the subsequent increase in Ang 1-7 levels, may be a compensatory response to

protect against tissue injury. In fact, in response to chronic injury, ACE2 protein levels are significantly downregulated in the kidneys of hypertensive (Crackower et al.; 2002), diabetic (Tikellis et al.; 2003) and pregnant rats (Brosnihan et al.; 2004; Brosnihan et al.; 2003) suggesting the potential role of the enzyme as a kidney disease biomarker.

3.5 Angiotensins and receptors

BP is modulated by changes in plasma concentrations of Ang II, due to an increase in total peripheral resistance to maintain arterial BP in face of an acute hypontesive modification as blood loss and/or vasodilation. Ang II causes a slow pressor response to stabilize the arterial BP mediated by a renal response, through mechanisms that include a direct effect to increase sodium reabsortion in proximal tubules, release of aldosterone from adrenal and altered renal hemodynamics (Carey et al.; 2000), including increased capillary glomerular pressure, hyperfiltration and proteinuria (Navar & Harrison-Bernard, 2000). Ang II also has important effects on cardiovascular system, stimulating migration, proliferation, hypertrophy, increased production of growth factors and extracellular matrix proteins such as collagen, fibronectin (Carey et al.; 2000).

Angiotensins have their actions exerted through AT1R and AT2R interaction, and Ang II, but not Ang I, has affinity to both of them. The actions of AT1R include vasoconstriction, aldosterone secretion, tubular sodium retention, release of vasopressin, increased sympathetic nervous activity and increased thirst. In the long term, actions of AT1R also include cell growth, organ hypertrophy, inflammation, remodeling and erythropoietic stimulation. On the other hand, AT2R mediates effects that are opposed to the actions of AT1R, and it has already been shown that AT2R is upregulated in response to tissue injury, suggesting its important role in the pathophysiology of several diseases (Hunyady & Catt, 2006).

Several studies demonstrated AT1R and AT2R expression in renal tissue, and their role in the development of renal disease. A study with SHR after 32 weeks of STZ-induced DM, suggested that hypertension, increased albuminuria and renal injury were resulted from the reduction of expression of enconding genes for AT1R, and treatment with ibersatan prevented the down regulation of the AT1R receptor, with no effect on AT2R expression (Bonnet et al.; 2002). Moreover, Velloso et al. (2006) also demonstrated an interaction between RAS and the insulin signaling pathways, through AT1R as a result of treatment with ARB (Velloso et al.; 2006).

Changes in the population of renal ATR can be involved in DN. Diabetes reduced gene and protein expression of AT1R but not AT2R in the kidneys of SHR rats, without changes in Wistar-Kyoto (WKY) strain. This reduction is supposed to be a protective mechanism against the intrarenal RAS activation by diabetes, and this effect was cancelled by the ARB ibersatan (Bonnet et al., 2002). Also, the cross-talk between AT1R and insulin receptor signaling pathways is related to the association between diabetes and hypertension, and may contribute to tissue damage (Velloso et al. 2006) induced by these pathologies.

4. RAS and diabetes

The activation of renal RAS, and the subsequent generation of Ang II, is the primary etiologic event in the development of hypertension in people with DM. Subsequently, the

increase of Ang II is responsible for the development of DN, a major cause of ESRD, via several hemodynamic, tubular and growth-promoting actions, as evidenced by the fact that blockade of this system has a beneficial effect on the kidney (Lewis et al.; 1993, 2001).

RAS inhibition is important to prevent renal and cardiovascular complications of both DM1 and DM2, through mechanisms that include improvement in endothelial fuction (Mukai et al.; 2002), decrease in inflammatory response (Mervaala et al.; 1999), increase in BK and Ang 1-7 levels (Maia et al.; 2004). The initial studies with RAS inhibition in people with DN demonstrated that there was an effect beyond BP lowering. When compared with conventional antihypertensive therapy, those who received RAS blockade consistently had greater improvement in DN despite presenting similar BP control, through effects of RAS blockade on insulin resistance and glucose homeostasis (Gillespie et al.; 2005; Lewis et al.; 1993; Ravid et al.; 1998). Thus, it was suggested a role for ACE in mediating renal injury by increasing local Ang II formation, prevented by both ACEi and ARB in the kidney. ACEi reduce the production of Ang II, and decrease degradation of endothelial BK, resulting in vasodilatation by stimulating nitric oxide and prostacyclin production and BP reduction. Moreover, ACEi have been shown to decrease the rate of progression of diabetic and non-diabetic nephropathies, and improve insulin sensitivity, allowing better insulin action in patients with DM2 (Lewis et al.; 1993; Yusuf et al.; 2000). On the other hand, ARB have also been shown to decrease the risk of stroke in patients with hypertension and reduce the rate of progression of DN (Lewis et al.; 1993). ABR prevent the binding of Ang II to AT1R, leading to accumulation of Ang II, which in turn is converted to Ang 1-7 and increases the levels of this vasodilator peptide (Barra et al.; 2009; Ferrari, 2005; Maia et al.; 2004).

Several studies have demonstrated that activity of circulating (systemic) RAS is normal or suppressed in DM, as reflected by measurements of plasma renin activity and Ang II concentrations, while local renal tissue RAS (tRAS) has already been shown to be activated on cell culture, in response to high glucose exposure, and also on spontaneously or induced diabetic animals (Carey & Siragy.; 2003b).

During the activation of tRAS in DM, Ang II activates NADPH oxidase enzyme which contributes to the generation of ROS. This process may result from over production of precursors to reactive oxygen radicals and or decreased efficiency of inhibitory and scavenger systems. In DM, the additional AT1R activation results in a vicious cycle of ROS production which contributes to organ damage (Hayden et al.; 2011). The mechanisms that contribute to increased oxidative stress in diabetes may include not only increased non enzymatic glycosylation (glycation) and autoxidative glycosylation (Baynes, 1991), but it is also related to several abnormalities, including hyperglycemia, insulin resistance, hyperinsulinemia and dyslipidemia, each of which contributes to mitochondrial superoxide overproduction in endothelial cells in large and small vessels as well as the myocardium.

The pathophysiological mechanism that underlies diabetic complications could be explained by increased production of ROS via the polyol pathway flux, increased formation of advanced glycation end products, increased expression of the receptor for AGEs, activation of protein kinase C isoforms and overactivity of the hexosamine pathway. Furthermore, the effects of oxidative stress in individuals with DM2 are compounded by the inactivation of two critical anti-atherosclerotic enzymes: endothelial nitric oxide synthase and prostacyclin synthase (Folli et al.; 2011).

Increased AGT expression, in response to high glucose exposure, was also described to be involved in the development of DN, *in vitro (*Hsieh et al, 2003) and *in vivo.* Using an *in vitro* model, Vidotti et al. (2004) demonstrated that high glucose exposure increased Ang II generation, decreased prorenin secretion and induced an increase in intracellular renin activity of mesangial cells. In response to 72h of high glucose exposure, there was an increase in mRNA levels for AGT and ACE, while 24h of the stimulus increased mRNA levels of ACE, prorenin and cathepsin B. In this study, increased generation of Ang II, induced by high glucose exposure, was shown to be dependent on at least three factors: a time-dependent stimulation of (pro)renin gene transcription, a reduction in prorenin enzyme secretion, and an increased rate of conversion of prorenin to active renin, probably mediated by cathepsin B. Moreover, the consistent upregulation of ACE mRNA suggests that, along with renin, ACE is directly involved in the increased mesangial Ang II generation induced by high glucose (Vidotti et al.; 2004).

In the kidney of streptozotocin (STZ)-induced diabetic animals, an increase in intrarenal AGT mRNA is attributed to the proximal tubule, and it seems to be mediated by glucose response element located in the AGT promoter (Zimpelman et al.; 2000). Studies with Zucker obese rat, a model of DM2 with nephropathy and hypertension, is also associated with increased activation of RAS, as demonstrated by an increase in intrarenal Ang II generation, which was prevented by treatment with ACEi (Sharma et al.; 2006). Using Non-obese diabetic model (NOD) (Makino et al.; 1980), our group demonstrated that diabetes onset increases ACE activity and expression and decreases ACE2 expression in kidney, suggesting that the higher renal ACE/ACE2 ratio may contribute to renal injury leading to overt nephropathy (Colucci et al.; 2011).

Ronchi et al. (2007) studied the association between sACE with 136 kDa and nACE with 69 kDa from Wistar (W) rat tissue with DM. The authors analysed three groups: control (CT), insulin treated diabetic (DT) and untreated (D). In D group, urine ACE activity increased for both substrates, Hippuryl-His-Leu and Z-Phe-His-Leu, that distinguished nACE from somatic ACE when compared with CT and DT, despite the decreased activity in renal tissues. Immunostaining of renal tissue demonstrated that ACE is more strongly expressed in the proximal tubule of D than in the same nephron portion in the other groups. Ang I increased in the renal tissue of D and DT groups, but Ang II levels decreased in the D and DT groups when compared to the control. Ang 1-7 was detected in all studied groups with low levels in DT. These findings indicate that Ang I increase and Ang II decrease, as a result of renin and NEP simultaneous activation, increasing Ang 1-7. Since Ang 1-7 can counterbalance Ang II effects, this modulation of angiotensin peptides has a protective role against renal damage in DM (Ronchi et al., 2007).

Few studies were described using animal models with genetic alterations in the RAS in DN. Studies have suggested associations between incidence of DN and a variety of genetic polymorphisms. An association was identified between nephropathy in DM1 and the D allele of an insertion/deletion (I/D) polymorphism in intron 16 of ACE gene. Huang et al.; (2001) described that the induction of diabetes by SZT was not affected by ACE gene copy number. The authors compared the changes with the time of BP of one, two and three–copy mice with the pressures of untreated controls. The BPs of untreated mice were not affected by ACE gene copy, however the BP of the three-copy diabetic mice with genetically higher ACE activity increased with time, and 12 weeks after induction of diabetes were 10-20 mmHg higher than the BPs of the one and two copy diabetic mice (Huang et al.; 2001).

Regarding ACE2, differences in renal enzyme levels have been detected in hypertensive humans when compared with controls (Van Buren & Toto, 2011). Wong et al. (2002) have shown that pharmacological inhibition of ACE2 and genetic ablation in different rodent of models of diabetes, increased albuminuria and glomerular lesions. Furthermore, animals with STZ-induced DN have decreased renal expression of ACE2 (Tikellis et al.; 2003). In humans, biopsies from patients with DN showed a decrease in glomerular expression of ACE2, suggesting that a therapy increasing the activity of this enzyme can help in the future in the treatment of diabetic kidney disease (Wong et al.; 2007).

Aldosterone has been implicated in DM complications. Sato et al (2003) in a study in patients with DM2 described that patients with aldosterone escape of 40% have higher albumin excretion than those without. Treating these patients with spironolactone associated to ACEi, the authors detected reduction in urinary albumin excretion over a 24-week period. They conclude that RAS activation in DN could be related to an aldosterone-mediated increase in disease progression. (Sato et al.; 2003).

5. Conclusion

We reviewed the physiology of the RAS in DM and hypertension, highlighting the importance of this system in diabetic nephropathy. The RAS is up or down regulated in the kidney and Table 2 summarizes the role of components of the RAS in diabetic nephropathy. Figure 6 presents our understanding of the intrarenal RAS in diabetic nephropathy. Increased Ang II is responsible for both intrarenal insulin resistence and renal injury, as well as, decrease AT2R expression might contribute to accelerated renal injury (Carey and Siragy, 2003b). In addition to controling blood pressure, we evidenciated the importance of ACEi and ARB in protecting the kidney against injury. The newly discovered components of RAS, such as renin receptor, ACE2, Ang IV and also aliskeren, the renin inhibitor, represent that

Description	References
RAS up regulation	
• Renal Ang II increase • AT1R activation - organ damage • Increased AGT expression – diabetic nephropathy • Increased mRNA levels of ACE, prorenin and cathepsin B. • Ang I increase and renin/neutral endopeptidase activation	Lewis et al., 1993, 2001; Zimpelman et al., 2000 Hayden et al.., 2011. Hsieh et al, 2003. Vidotti et al., 2004. Ronchi et al., 2007.
RAS down regulation	
• ACE inhibition – increase in BK and Ang 1-7, control of blood pressure • ACE inhibition, improve insulin sensitivity • ACE2 decrease • ACE2 inhibition - increased albuminuria and glomerular lesions	Maia et al., 2004. Lewis et al., 1993; Yusuf et al., 2000. Colucci et al., 2011; Wong et al., 2007 Wong et al.,2002.

Table 2. RAS is up- or down-regulated in diabetic kidneys

research is open in this field. New pathways and signaling compounds can be discovered explaining the modulation of the RAS resulting in expression of other genes.

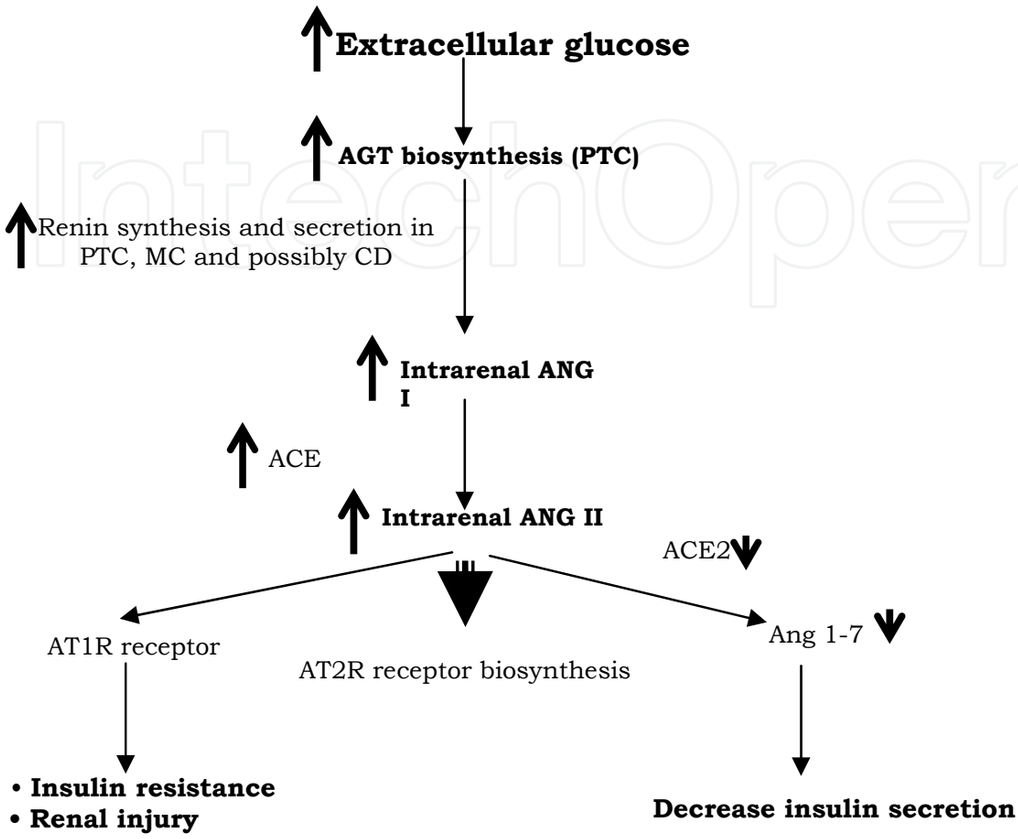


Fig. 6. Pathways through which the intrarenal renin–angiotensin system contributes to diabetic nephropathy based on current evidence. Solid arrows represents biochemical pathways or ligand–receptor interactions. The broken arrow indicates decreased ligand–receptor interaction owing to decreased AT2R synthesis. Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme2; AGT, angiotensinogen; ANG, angiotensin; AT1R, angiotensin II receptor; AT2R angiotensin II receptor; MC, glomerular mesangial cell; PTC, proximal tubule cell. Adapted from Carey and Siragy, 2003b.

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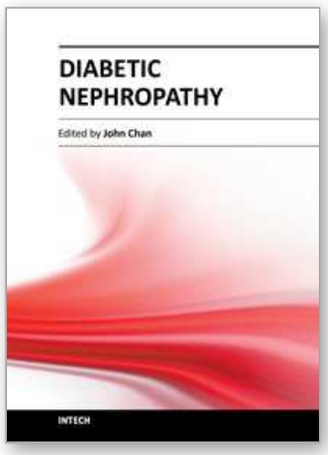

Diabetic Nephropathy
Edited by Dr. John Chan

ISBN 978-953-51-0543-5
Hard cover, 166 pages
Publisher InTech
Published online 20, April, 2012
Published in print edition April, 2012

Internationally renowned experts have provided data on their own studies, and discuss the relative usefulness of their work in relation to diabetic nephropathy. The first section describes the novel role of intrarenal renin-angiotensin-aldosterone system (RAAS) and oxidative stress in the development of diabetic nephropathy and discusses the current and novel pharmacological interventions in the treatment of diabetic nephropathy. The second section discusses other important contributors outside of the RAAS in the pathogenesis of diabetic nephropathy including AGE/RAGE, epithelial-mesenchymal-transition (EMT) and immune cytokines. Features: Provides novel information on various pathophysiological determinants in the development of diabetic nephropathy Provides novel information on various pharmacological interventions of diabetic nephropathy

How to reference
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