Renin-angiotensin system within the diabetic podocyte

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Márquez E, Riera M, Pascual J, Soler MJ. Renin-angiotensin system within the diabetic podocyte. Am J Physiol Renal Physiol 308: F1–F10, 2015. First published October 22, 2014; doi:10.1152/ajprenal.00531.2013.—Diabetic kidney disease is the leading cause of end-stage renal disease. Podocytes are differentiated cells necessary for the development and maintenance of the glomerular basement membrane and the capillary tufts, as well as the function of the glomerular filtration barrier. The epithelial glomerular cells express a local renin-angiotensin system (RAS) that varies in different pathological situations such as hyperglycemia or mechanical stress. RAS components have been shown to be altered in diabetic podocytopathy, and their modulation may modify diabetic nephropathy progression. Podocytes are a direct target for angiotensin II-mediated injury by altered expression and distribution of podocyte proteins. Furthermore, angiotensin II promotes podocyte injury indirectly by inducing cellular hypertrophy, increased apoptosis, and changes in the anionic charge of the glomerular basement membrane, among other effects. RAS blockade has been shown to decrease the level of proteinuria and delay the progression of chronic kidney disease. This review summarizes the local intraglomerular RAS and its imbalance in diabetic podocytopathy. A better understanding of the intrapodocyte RAS might provide a new approach for diabetic kidney disease treatment.

ACE2; angiotensin II; podocytes; renin-angiotensin system

Functionally Local RAS in Podocytes: Enzymes, Peptides, and Receptors

A number of studies have described the RAS of mouse and human podocytes, reporting that they contain all RAS components required to generate ANG II and ANG-(1–7), the most active products of this system (Fig. 1). It has also been shown that the podocytes express both ANG II receptors, type 1 (AT1-R) and type 2 (AT2-R), but to our knowledge there are no studies showing the presence of the ANG-(1–7) receptor, called the Mas receptor (40, 86).

Enzymes. Renin catalyzes the first peptide transformation of RAS, angiotensinogen (Agt) into ANG I. The demonstration of physiological gene expression, protein expression, and enzymatic activity of renin within podocytes is relatively recent (40, 86). In the 1980s, renin was thought to be restricted to the juxtaglomerular apparatus (11). Subsequently, Sequeira López et al. (71) hypothesized that the ability of adult cells to synthesize renin depends on the cell lineages and the presence of a stimulus. Using reporter mice, they demonstrated that renin-expressing cells are widely distributed along the kidney during embryonic and fetal life. As maturation continues, the number of renin-expressing cells is restricted at the juxtaglomerular location, but a threat to homeostasis increases their number inside the glomerulus (71). Based on these experiments, Velez et al. (86) hypothesized that cultured podocytes can revert to a renin-expressing phenotype due to their immortalized status, and that diseases resulting in podocyte injury may lead to phenotype changes that elicit renin expression.

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The angiotensin-converting enzyme (ACE) is a membrane-bound zinc- and chloride-dependent peptidase whose main function is to catalyze the conversion of ANG I to ANG II. The presence or absence of ACE within podocytes is controversial. Although its presence has been demonstrated using both direct and indirect methods (40, 86, 98), Ye et al. (97) did not find ACE expression in podocytes using renal immunofluorescence and immunogold studies (97). Liebau et al. (40) also showed that podocyte incubation with captopril did not reduce ANG II concentration, suggesting the involvement of non-ACE pathways to generate ANG II in these cells. Angiotensin-converting enzyme 2 (ACE2) is the first active ACE human homolog that has been described (13, 81). Despite their homologous structure, these two enzymes have opposite actions: whereas ACE degrades ANG I to ANG II, ACE2 cleaves ANG II into ANG-(1–7) and ANG I into ANG-(1–9). In the kidney, ACE2 is highly expressed, and within the glomerulus it is mainly present in podocytes and mesangial cells (86, 97). The presence of ACE2 in cultured podocytes was demonstrated for the first time through ANG II incubation that resulted in DX-600-sensitive (an ACE2 inhibitor) ANG-(1–7) generation (86).

Neprilysin is a transmembrane zinc-dependent metallopeptidase widely expressed in tissues. Studies carried out in podocytes in culture have demonstrated that its role is to convert ANG I into ANG-(1–7) and indicated that neprilysin is probably the main source of this peptide in podocytes. Its levels were significantly decreased only by a specific neprilysin inhibitor (86, 87). Aminopeptidase A (APA) is a membrane-bound metalloproteinase with a widespread organ distribution. Its best-known function is to degrade ANG II into ANG III. Compared with other glomerular cells, coinubcation of epithelial glomerular cells with ANG II and amastatin (an aminopeptidase inhibitor) decreased ANG III formation, whereas in the absence of amastatin the degradation of ANG II to ANG III was rapidly detected (48, 86, 87). APA within the podocyte is also able to metabolize ANG I to ANG-(2–10) (86).

Besides the well-recognized degradation capacity of endopeptidases and aminopeptidases in podocytes, other RAS enzymes, such as prolylendopeptidase and prolylcarboxypeptidase, have also been found in these cells but with low activity (52, 70).

Peptides. Agt is the first peptide in the RAS pathway and the only known renin substrate. Its presence in podocytes has been demonstrated by detecting mRNA in mouse podocytes in culture (40).

ANG-(1–7) is the most abundant product of the RAS within the podocyte (86). ANG-(1–7), due to its opposite actions, may counteract the vasoconstrictor, growth-promoting, and profibrotic actions of intraglomerular ANG II (20). ANG-(1–7) is produced through two catalytic reactions: direct cleavage of ANG I by neprilysin and cleavage of ANG II by ACE2. A study in mice podocytes using specific inhibitors of both enzymes (thiorphan and DX-600, respectively) showed that neprilysin could play an important role in ANG-(1–7) generation in podocytes (86).

Podocytes have all the metabolic machinery necessary for autologous synthesis of ANG II, the most active peptide of the RAS. Formation of captopril-sensitive ANG II and involvement of non-ACE pathways in its synthesis have been demonstrated in podocytes (40, 86). It has also been shown that glomerular epithelial cells present a higher rate of disappearance of ANG II compared with glomerular endothelial cells (25).

Although RAS is one of the most studied systems involved in the physiopathology of DN, recently a new angiotensin-related peptide has been identified in the human kidney and urine. This peptide, named Big angiotensin-25 (Bang-25), is processed by renin into ANG I at a slow rate, but, remarkably, it is rapidly processed by chymase to produce ANG II. In the
kidney, Bang-25 seems to be mainly localized in podocytes; however, no specific studies or staining have been published (54). Although its physiological function remains unclear, Bang-25 might be an important renin-independent pathway for ANG II synthesis.

Receptors. The presence of the prorenin receptor (PRR) has been detected in podocytes (15, 29). The prorenin receptor binds renin with the same affinity as prorenin (a renin proenzyme precursor) and amplifies Agt conversion to ANG I. Moreover, it initiates signaling pathways that promote ANG II-independent fibrosis.

There is evidence of the presence (73, 89) and functionality (26, 66, 67) of AT1-R and AT2-R in the podocyte, AT1-R being the more important (40). They are localized on the cell surface, as intracellular clusters, and in the cell nucleus within the podocyte (40). Podocytes are a direct target for ANG II mainly through AT1-R. Rats overexpressing AT1-R in glomerular podocytes developed significant albuminuria associated with severe structural changes: formation of pseudocysts, foot process effacement, detachment, and loss of entire cells (26). Streptozotozin (STZ)-induced diabetic rats treated with AT1-R antagonists showed more podocytes per glomerulus than untreated animals, suggesting a protective effect of RAS blockade at this level in the podocyte (83). These findings provide evidence of the presence and functionality of AT1-R in podocytes that can lead to structural podocyte damage, protein leakage, and progression to glomerulosclerosis. The activity of AT2-R in podocytes has been demonstrated in several studies. Incubation of cultured podocytes with ANG II increased the advanced glycated end-products receptor (RAGE), which has a principal role in DN pathophysiology, through AT2-R (66). Administration of ANG II to AT2-R knockout (KO) mice caused significantly less RAGE expression than in the control group. These studies indicate that ANG II mediates RAGE induction in podocytes at least in part via AT2-R, emphasizing the relevance of these receptors in DN (67). Given the presence of both receptors on the podocyte surface, some authors have proposed the hypothesis that ANG II performs autocrine effects in these cells via receptor-mediated endocytosis (40). More studies are needed to elucidate its role within podocytes.

To our knowledge, there is no published evidence of the presence of the Mas receptor, the ANG-(1–7) receptor, in podocytes. The lack of its expression would contribute to the accumulation of ANG-(1–7), the most abundant peptide in podocytes, which would not exert the antiproliferative effects it has when interacting with the Mas receptor. However, the conversion of ANG II to ANG-(1–7) would also be important to prevent ANG II accumulation.

RAS Imbalance in Diabetic Podocytopathy

Metabolic factors such as hyperglycemia and hemodynamic alterations are main features of DN pathophysiology that modify RAS balance in the podocyte. Studies by Yoo et al. (98) and Deb et al. (10) have shown that high glucose levels increased Agt gene and protein expression in cultured podocytes, leading to higher ANG II levels. Both in vitro and in vivo studies have shown that hyperglycemic conditions increase Agt expression (10, 98) and renin expression and activity (15, 98). A marked increase in prorenin was detected within the glomerulus of hyperglycemic rats, even before the development of overt nephropathy (15). These findings suggest an important role of renin and prorenin starting at the earliest stages of DN, probably not limited to Agt cleavage.

ANG-(1–7) and its receptor are decreased in the kidneys of several diabetic animal models (43, 79). It exerts a renoprotective role in DN, modulating inflammation, oxidative stress (OS) (2, 22, 51), and regulating part of the process of renal fibrosis (74). Nonetheless, not all studies supported this assertion, and the protective role of this enzyme within the diabetic kidney is not clear. For instance, Shao et al. (72) reported that infusion of ANG-(1–7) in STZ-induced diabetic rats significantly increased urinary protein excretion compared with untreated diabetic rats. At high concentrations, ANG-(1–7) binds to the AT1-R, suggesting that the observed adverse effects may be ascribed to activation of the AT1-R signaling pathway (72). Despite the potentially interesting role of ANG-(1–7) in the diabetic podocyte, to our knowledge there are no specific studies focused on clarifying this topic.

The effect of pathological diabetic conditions such as mechanical stress or high glucose media on neprilysin has not been investigated in cultured podocytes, and studies in animal models showed contradictory results. Tschope et al. (82) found decreased levels of neprilysin in the kidney cortex from STZ-induced diabetic rats compared with the control group. In contrast, Fredersof et al. (18) found attenuated albuminuria levels and a decrease in glomerular desmin expression, as a marker of podocyte damage, in diabetic rats that received a neprilysin inhibitor.

ACE2 is decreased in glomeruli from diabetic mice (97), and pharmacological or genetic downregulation of ACE2 worsens albuminuria and glomerular lesions in experimental models of diabetic kidney disease (76). In a recent study, STZ-induced diabetes in transgenic mice that selectively overexpressed ACE2 in podocytes provided evidence of the role of ACE2 within the diabetic podocyte. The transgenic mice showed decreased albuminuria levels, less increase in mesangial area, decreased glomerular area, a significant increase in nephrin expression, and a decrease in podocyte loss compared with nontransgenic diabetic mice (53). These studies suggest that in the podocyte there is a RAS balance that is lost in DN. ACE2 deficiency in podocytes leads to ANG II accumulation and subsequently worsened DN, namely, albuminuria and glomerular lesions: an increase in ACE2 within podocytes favors ANG II degradation and subsequently protects against DN injury.

In 2005, Xu et al. (95) described for the first time that high glucose significantly increased ANG II levels both in cell lysates and in media from podocytes in culture. Multiple studies have demonstrated that a diabetic environment leads to activation of the local RAS in podocytes by increasing ANG II through ACE-dependent and -independent pathways, as well as increasing AT1-R density (14, 15, 49, 98). Durvasula et al. (15) demonstrated that the exposure of podocytes in culture to high glucose increased ANG II production in a dose-dependent fashion. Whereas preincubation with the ACE inhibitor captopril failed to abrogate the glucose-induced increase in ANG II, a nonselective chymostatin restored ANG II levels to baseline, thus enforcing the importance of non-ACE pathways in the local generation of ANG II in podocytes.

In 2001, Endlich et al. (17) provided the first evidence that podocytes are mechanosensitive. Mechanical stretch caused...
changes in podocyte shape and profound alterations in the actin cytoskeleton compared with control cells (17). Under stretch conditions, there was a significant increase in podocyte ANG II levels at 24 h with involvement of non-ACE pathways in its generation. In addition, mechanical strain increased AT1-R in podocytes in culture and in an experimental model of glomerular capillary hypertension (14).

Effects of ANG II on Podocytes in DN

High ANG II levels have been found as a consequence of hyperglycemia, advanced glycation end products, and mechanical stress in DN (7, 19, 94, 102). ANG II has been associated with deleterious effects on podocyte structure, apoptosis, and possibly epithelial-mesenchymal transition (EMT). Consequently, there is progressive injury, detachment, and finally a decrease in the number of podocytes, clinically reflected as the appearance of proteinuria. RAS blockade decreases proteinuria and delays the progression of DN by blocking ANG II that has hemodynamic and nonhemodynamic effects (37, 63). These known benefits are partially achieved through direct effects on podocytes.

Slit diaphragm and cytoskeleton. Through the AT1-R, ANG II leads to the reorganization of the actin cytoskeleton with a redistribution and decrease of slit diaphragm (SD) proteins such as nephrin and zonula occludens (ZO)-1 (28, 44, 49). Several mechanisms are involved in this process. AT1-R-Scr tyrosine kinase interaction activates phospholipase PLCγ1, which in turn activates Rac1, a member of the Rho family of GTPases. This pathway leads to a reduction of 4-α-actinin and ezrin/radixin/moesin (ERM) phosphorylation. The main function of 4-α-actinin and ERM is to regulate the attachment of membrane proteins to the actin cytoskeleton; therefore, those changes lead to cytoskeletal rearrangement, retraction of foot processes, and SD protein redistribution (28, 44). Moreover, Rac1 activation increases reactive oxygen species (ROS) production, which causes a decrease in RhoA, another member of the Rho family of GTPases, leading to a migratory phenotype that contributes to chronic podocyte loss (28).

Recently, c-maf-inducing protein (c-mip) has been described to play a significant role in the regulation of a number of signaling transduction pathways, such as the p85 subunit of phosphatidylinositol 3-kinase, Erk, Akt, NF-κB, and N-Wasp. It has been isolated from lymphocytes of patients with nephrotic syndrome of different etiologies, although not specifically in DN. Activation of c-mip by ANG II reduces nephrin phosphorylation and produces cytoskeletal reorganization (99). Other pathways are activated by ANG II. There is evidence that Notch1/Snail activation by ANG II results in nephrin phosphorylation and produces cytoskeletal reorganization (99). Other pathways are activated by ANG II. There is evidence that Notch1/Snail activation by ANG II results in nephrin phosphorylation and produces cytoskeletal reorganization (99).

Functionally, these changes result in cells with less resistance (68) and more stiffness (16), making them unable to adapt their shape in the face of mechanical stress. They also have a migratory phenotype (28) that leads to barrier dysfunc-

Fig. 2. Schematic overview of ANG II’s effects on podocytes in a diabetic milieu. Main molecular mechanisms are involved in podocyte phenotypic changes, slit diaphragms (SD), and cytoskeleton rearrangements, all of them leading to the development of proteinuria.

Apoptosis. It has been demonstrated that ANG II leads to podocyte apoptosis in both in vivo and in vitro models and by direct and indirect methods [exposure to high concentration of ANG II and to ACE inhibitors (ACEi)/ANG II receptor blockers (ARB), respectively] (12, 19, 31, 49, 80). Several signaling pathways involved in ANG II-induced apoptosis appear to be mediated by both AT1- and AT2-R (12, 80) (Fig. 3). The MAP kinase signaling cascade, specifically ERK 1/2, is one of the main mechanisms involved in ANG II-induced apoptosis (1, 42, 100). ERK 1/2 pathway activation and consequently translocation of NF-κB induce podocyte apoptosis by intracellular calcium overload due to an increase in TRCP6 calcium channel synthesis (1, 100). ROS are crucial to the ANG II transduction cascade and therefore to ANG II-dependent apoptosis. ANG II decreases peroxiredoxin, a peroxidase, increasing ROS and leading to apoptosis (27). Furthermore, this increase in ROS is essential for TRCP6 overexpression (1, 100).

The presence of high levels of ANG II leads to the caspase 3/9 activation by various mechanisms: decreases in phosphor-
A study in type 2 diabetes mellitus (DM) patients showed that the majority of urinary cells characterized as podocytes expressed fibroblast-specific protein 1 (FSP1; expressed in the cytoplasm of epithelial cells converted into fibroblasts) (96). In an analysis of kidney biopsies of these patients, the intraglomerular expression of FSP1 was almost exclusively on podocytes and it correlated with the severity of the glomerular lesions. Those cells selectively expressed Snail and another EMT trigger, integrin-linked kinase 1 (96).

**Other effects of ANG II on podocytes.** The anionic charge on the podocyte surface is partially responsible for the permselectivity of the glomerular filtration barrier. In culture, ANG II stimulation of podocytes resulted in a relative decrease in the proteoglycan (4, 84). In several kidney diseases, such as DN, there is a decrease in heparan sulfate in the glomerular filtration barrier secondary to an increase in heparanase expression. Glomerular heparanase expression in proteinuric models is mainly localized in podocytes, suggesting that these cells are responsible for its regulation. van den Hoven et al. (84) demonstrated that ANG II can directly induce heparanase expression in vitro. Stimulation of podocytes with ANG II resulted in the induction of heparanase, which could be inhibited by ARB, but not by AT2-R blockade. This demonstrates that ANG II-induced heparanase expression by podocytes is mediated via the AT1-R, which corresponds to the reported ANG II-induced heparan sulfate proteoglycan reduction in human podocytes (84).

Podocytes react in front of an injury, such as a diabetic milieu, undergoing cell hypertrophy that plays a critical role in early functional and structural changes of diabetic kidney disease. Podocytes cultured in high-glucose media show an ANG II-dependent increase in p27Kip1 expression, a cyclin that mediates cell cycle arrest and hypertrophy (95). ANG II elicits a robust direct activation of the ERK 1/2 pathway and Akt signaling that induces protein synthesis and subsequently hypertrophy. Furthermore, high glucose and ANG II have additive responses to this activation (33).

**Podocytes as a Therapeutic Target**

**Relevance of podocyte injury to DN progression.** Studies in diabetic patients, in addition to studies developed in animal models, highlighted the importance of podocyte injury and loss in DN progression. The analysis of kidney biopsies from type 1 and type 2 diabetic patients showed an association between broadening podocyte foot processes and reduction in estimated filtration slit length density, and in podocyte number per glomerulus in patients with clinical nephropathy, and even in their urine sediment were not apoptotic (96). These facts confirmed that apoptosis is not the only mechanism that leads to podocyte detachment (36, 62).

**TGF-β** is one of the most important signaling pathways mediating EMT processes. In podocytes, TGF-β with its key mediator, Snail, reduce the expression of P-cadherin, nephrin (21, 39), and ZO-1 and induces desmin expression and interstitial matrix components such as fibronectin and collagen I. All these changes are consistent with loss of the epithelial features (21, 39). As ANG II in the DN rises, the levels of TGF-β increase, mainly through the OS pathway (14, 36, 78, 102); EMT could be a possible mechanism of podocyte injury by ANG II and thus be susceptible to therapeutic manipulation.

A study in type 2 diabetes mellitus (DM) patients showed that the majority of urinary cells characterized as podocytes expressed fibroblast-specific protein 1 (FSP1; expressed in the cytoplasm of epithelial cells converted into fibroblasts) (96). In an analysis of kidney biopsies of these patients, the intraglomerular expression of FSP1 was almost exclusively on podocytes and it correlated with the severity of the glomerular lesions. Those cells selectively expressed Snail and another EMT trigger, integrin-linked kinase 1 (96).

**Proapoptotic effects of ANG II are mediated by other mechanisms such as stimulation of TGF-β production by podocytes.** (12). Recently a new mechanism involving c-Abl, a kinase with a role in the regulation of the cell cycle in a p53-dependent manner, has been described. ANG II increases c-Abl, inducing podocyte apoptosis (6). Apoptosis regulation seems to be fundamental to avoid the ANG II effects. Beyond RAS blockade, the effect of other drugs with antiapoptotic effects and synergism with ACEi or ARB on podocytes, such as vitamin D or its analogs, should be evaluated (10, 90, 101).

**PPAR-γ agonists have demonstrated a protective role in podocytes** by preventing apoptosis via RAS-dependent mechanisms (30, 49). All these experimental evidences indicate that ANG II-mediated apoptosis is a target mechanism that could interfere with the effects of ANG II.

**EMT.** EMT is a process by which fully differentiated epithelial cells undergo transition to a fibroblast phenotype with changes in differentiation markers: from cell-cell junction proteins and cytokeratin intermediate filaments to vimentin filaments and fibronectin. These modifications lead to functional changes associated with the conversion of stationary cells to motile cells that can invade the extracellular matrix. These modifications could be considered the functional hallmark of the EMT (62).

Depletion of glomerular podocytes is an important feature in the progression of DN. Although the main cause for this podocyte depletion is detachment from the GBM after cellular apoptosis, the EMT could be a mechanism for losing podocyte adhesion, leading to detachment and finally decrease in podocyte number in DN. Podocytes obtained from urine of diabetic rats were viable in culture for up to 72 h (60). In humans, a study in type 2 DM patients showed that 95% of podocytes in
diabetic patients independently of kidney function (9, 58, 77). Studies developed in animals showed similar results (75). Urinary podocyte loss has not been observed in normal subjects, whereas podocyuria has been found in diabetic patients as an indirect measure of podocyte detachment from glomeruli (55). Nephrin and podocalyxin, specific podocyte markers, were found in urine of type 1 and type 2 diabetic patients regardless of the presence or absence of proteinuria (25, 59).

Podocyte loss, detected by podocyuria or by podocyte markers in urine, could also work as a strong predictor of renal disease progression in type 1 and type 2 DM patients (77, 92, 93). Recently, Weil et al. (91) published a comprehensive transmission electron microscopy study analyzing podocytes in type 2 DM patients. Podocyte detachment correlated negatively with podocyte number and positively with increasing albuminuria. Podocyte number did not correlate with albuminuria, suggesting a role for podocyte injury, not simply podocyte loss, as a main structural factor responsible for altered permselectivity in DN (91). Although these studies are limited by small sample size or by their descriptive nature, their results indicate early podocyte damage in the glomerular filtration barrier in DM. Thus strategies aimed at preventing podocyte damage before albuminuria development, such as RAS blockade or other modulation pathways involved in DN progression, are of great interest.

Effect of RAS blockade on podocytes in DN. RAS blockade decreases proteinuria and delays the progression of chronic kidney disease by blocking ANG II (37, 38, 46). Given the evidence found in the literature about the major role of podocytes in DN progression, these cells are one of its most important targets (34, 47). ACEi or ARB treatments, or their combination, have been shown to improve podocyte injury at different levels in several diabetic animal models, with attenuation of nephrin loss (3, 32) and a decrease in desmin staining (8, 57). These changes are accompanied by attenuation in foot process broadening, irregular foot process interdigitation, and a reduction in podocyte loss (50). These effects are reflected in a decrease in proteinuria (3, 32, 57).

Renal biopsies were examined from type 2 diabetic patients who had been randomized to receive treatment with an ACEi or placebo for the preceding 2 yr. Glomeruli from placebo-treated patients with DN showed a reduction in nephrin ex-
pression compared with control subjects. In contrast, nephrin RNA in glomeruli from perindopril-treated patients was similar to that in the nondiabetic control group (35). Moreover, patients on treatment with ACEi alone or ACEi and ARB in combination showed a decrease in podocyturia (56) or podocyte urinary markers (synaptopodin, nephrin, and podocin) (88). However, the protective effect of modulating other elements of the RAS within podocyte should not be underestimated (65). A previously mentioned study also demonstrated that the amplification of ACE2 within the podocyte reestablished podocyte number in diabetic rats (53).

Although the studies cited above show that RAS blockade attenuated podocyte damage in diabetes, this treatment strategy only partially prevented podocyte loss and renal injury. In contrast, combined treatment using an ARB and the antioxidant nitro-oleic acid significantly attenuated renal damage and prevented podocyte loss compared with ARB treatment (41). Similarly, dual RAS blockade and statin treatment normalized podocyte number and reduced proteinuria (103). Identical effects were observed with the combination of ACEi and an endothelin-1 inhibitor (20). Recently, a study developed in the leptin-deficient BTBR ob/ob mouse reaffirmed this concept. These mice presented podocyte loss early in DN progress, and podocyte density was restored only in leptin-treated mice. In contrast, treatment with ARB or ACEi did not significantly alter podocyte density (61). In agreement with this finding, our group recently demonstrated that insulin administration prevented kidney damage such as podocyte decrease and albuminuria in NOD diabetic mice (65). All these studies suggest that a multiple approach is needed to prevent renal lesions in diabetes, including RAS modulation, antioxidant therapies, statins, and metabolic control.

Effect of insulin on podocyte RAS. The effect of insulin on podocyte RAS has not been widely studied. Marquez et al. (45) recently demonstrated that insulin increases ACE2 gene transcription, protein expression, and enzymatic activity in podocytes. The effects of insulin appeared early and were maintained in long-term incubation. However, we did not find any effect on ACE2 expression after the incubation with albumin, but its presence blocked insulin’s effects. In addition, we found a marked colocalization of ACE2 and endoplasmic reticulum (ER) in the group coincubated with insulin and albumin. This finding seems to reflect the increase in the synthesized proteins that need to be processed (ACE2 in the presence of insulin), along with a decrease in ER capacity to manage this overcharge in the presence of albumin (Fig. 4). Together, these results suggest that insulin may exert a renoprotective effect within the podocyte by increasing ACE2 protein expression, decreasing the ACE/ACE2 gene expression index, decreasing fibronectin gene expression as a marker of fibrosis, and decreasing podocyte apoptosis. This effect disappears in the presence of albumin (45).

Concluding Remarks

Podocytes are highly specialized epithelial cells that play a major role in the maintenance and modulation of the glomerular filtration barrier. It has been demonstrated that podocyte injury leads to development of albuminuria. These cells have their own RAS that is activated during DN. Part of the beneficial effects of RAS blockade may be ascribed to its direct effects on podocytes. TGF-β, MAPK (mainly ERK 1/2), and the Rho family of GTPases seem to be the main intracellular signaling pathways activated by ANG II during DN in podocytes. Nonetheless, ANG II intracellular signaling is not completely understood. It is essential to delve into the exploration of these pathways to understand the pathophysiology of DN podocytopathy, define specific therapeutic targets, and finally design more specific drugs to slow its progression.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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