Recent advances in our understanding of insulin signalling to the podocyte

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ABSTRACT

It is becoming increasingly clear that the insulin responses of a number of different cell types within the kidney are important in the maintenance of normal renal function. This review summarizes our current understanding of renal insulin signalling, with specific focus on the podocyte, presenting recent evidence that suggests these responses are altered in systemic insulin-resistant states and chronic kidney disease via a number of different mechanisms.

Keywords: AKT, diabetic nephropathy, insulin, podocyte

CELLULAR INSULIN SIGNALLING: AN OVERVIEW

To understand the recent advances in this field, it is first necessary to understand the cellular signalling pathways elicited by insulin.

Despite being a potent metabolic hormone, with the primary function the regulation of systemic glucose levels in the classical insulin responsive tissues such as skeletal muscle, liver and adipose tissue; insulin also has the capacity to exert an array of cellular responses in a variety of tissues [1]. The insulin signalling system is a complex network, involving multiple points of crosstalk and interaction with other cellular signalling networks, signal divergence and regulation, allowing for these cell type-dependent responses. An overview of cellular insulin signalling is presented in Figure 1.

The first point in this pathway is the activation of the insulin and insulin-like growth factor-I receptors (IR and IGF-IR, respectively). The IR exists in two isoforms, IR-A and IR-B, which have the ability to bind and form hybrid receptors with the IGF-IR. Differences in both ligand-binding affinities and downstream cellular outcomes following the activation of the homodimer/hybrid receptors have been reported [2]. Upon binding their respective ligands, receptor auto-phosphorylation on key tyrosine residues ensues, leading to the recruitment and activation of insulin receptor substrate (IRS) proteins, of which IRS1–4 are the best documented. Phosphorylation of the IRS proteins marks another key point of signal divergence and regulation, allowing for differential activation of phosphorylation cascades [3] (Figure 1).

One of the major networks activated following IRS phosphorylation is the phosphoinositide 3-kinase (PI3K) pathway. PI3K is recruited to the membrane via its regulatory subunit, p85, resulting in phosphatidylinositol-3,4,5-triphosphate (PIP3) production and phosphoinositide-dependent protein kinase 1 (PDK1) activation. Active PDK1 is thought to be responsible for activation of atypical protein kinase C (aPKC)λ/ζ and serum and glucocorticoid-inducible kinase (SGK) following insulin stimulation, which play roles in glucose and sodium transport, respectively. Importantly, Akt phosphorylation (at Thr308) also occurs by active PDK1. The mammalian target of rapamycin complex 2 (mTORC2) is thought to be responsible for Akt phosphorylation at Ser473 [1]. Three isoforms of Akt exist in vivo (Akt1-3); Akt2 in particular is important in metabolic insulin responses [4]. Recently, Akt2 has been found to be critical for podocyte survival in models of CKD [5], which will be discussed in more detail later in this article.

Downstream substrates of active Akt following insulin stimulation include: Akt substrate of 160 kDa (AS160), thought to be important in cellular glucose uptake [6]; glycogen synthase kinase 3 (GSK3) [7]; and a number of pro-survival signals including phosphorylation of the pro-apoptotic protein Bad and...
the forkhead box ‘Other’ (FoxO) family of transcription factors. Additionally, activation of mTORC1 occurs downstream of the PI3K/Akt pathway in response to insulin and IGF signalling and is central in the regulation of metabolism and cell growth, controlling multiple signals involved in nutrient uptake, ribosome production and translation initiation.
Tyrosine phosphorylation of c-Cbl has also been demonstrated following cellular insulin stimulation and is implicated in the cellular glucose uptake response, independent of PI3K activity [8], although the relevance of CAP/Cbl interactions in glucose transport *in vivo* remains controversial.

**RENAL INSULIN SIGNALLING**

Although, as described, the primary targets of insulin action are considered to be skeletal muscle and liver; the kidney has long been regarded as an insulin-responsive organ. Insulin has been shown to bind both to the glomeruli and tubules and the importance of its action within this organ are increasingly being recognized. We have recently reviewed insulin responses in the kidney including tubules and the glomerulus [9]. Interestingly, Tiwari *et al.* have since demonstrated IR signalling in the proximal tubule to be important in the control of systemic glucose concentrations. The specific knockdown of the IR in proximal tubular cells results in a mild but significant hyperglycaemia compared with control animals. The rise in systemic glucose concentrations was not attributable to impaired glucose clearance, decreased insulin action or disrupted insulin secretion. The finding that both mRNA and activity of glucose-6-phosphatase were enhanced in kidneys from the proximal tubular IR-deficient mice, suggests renal gluconeogenesis, specifically in the proximal tubule, is enhanced following IR depletion, and the cause of increased plasma glucose levels, in these mice [10]. Considering the evidence of reduced renal IR expression in insulin-resistant rat models [11], this could imply that reduced proximal tubular insulin responses may contribute towards hyperglycaemia associated with systemic insulin resistance.

**GLOMERULAR INSULIN SIGNALLING**

A direct effect of insulin on glomerular cells was proposed in 1980 by Mogensen *et al.* when it was demonstrated that urinary albumin excretion doubled following intravenous insulin injection, with blood glucose concentrations remaining constant [12]. Specific insulin responses of glomerular endothelial cells, mesangial cells and podocytes have since been demonstrated [9].

**PODOCYTE INSULIN SIGNALLING**

Protein analysis has demonstrated that podocytes have the highest levels of both IR and IRS-1 expression when compared with endothelial and mesangial cells in primary culture [13]. We have previously shown that podocytes are able to respond to insulin *in vitro*, resulting in Akt and ERK1/2 phosphorylation, cytoskeletal rearrangement, increases in motility [14] and cellular glucose uptake, through both GLUT1 and GLUT4 glucose transporters [15]. The latter response is dependent on nephrin expression, potentially via its interaction with VAMP2 allowing GLUT4 membrane fusion [16]. Recently, we have shown insulin regulation of vascular endothelial growth factor-A (VEGF-A) production by podocytes, both *in vitro* and *in vivo* [17]. It is becoming increasingly clear that a tight control of VEGF-A production is critical for maintaining renal function, and the podocytes are the central source of this molecule within the glomerulus. The finding that insulin has the capacity to control VEGF-A production by the podocyte could highlight another critical role for this hormone within the kidney.

Podocyte-specific deletion of the IR *in vivo* highlights the importance of podocyte insulin signalling in the maintenance of a functional filtration barrier. Specifically, transgenic mice lacking the IR in podocytes develop albuminuria, podocyte foot process effacement and apoptosis. Furthermore, increased levels of glomerular matrix, glomerulosclerosis and thickening of the GBM are observed in these animals, yet blood glucose concentrations remain constant [14].

Other groups have suggested a role of insulin in the control of podocyte contractility, which may contribute to glomerular permeability [18,19]. Although podocytes are not excitable cells, it is suggested that such contractility is regulated by calcium ion influx via the coordinated action of large-conductance Ca\(^{2+}\)-activated K\(^+\) (BK) channels and the cation channel, TRPC6 [20,21]. Insulin has been shown not only to increase BK channel activity (via PI3K/Akt and ERK MAPK signalling pathways) [18], but to increase the surface expression of TRPC6 [19]. TRPC6 mobilization in response to insulin was demonstrated to be via a mechanism involving reactive oxygen species (ROS) production (specifically H\(_2\)O\(_2\)), and NADPH oxidase 4 (Nox4), the catalytic subunit of NADPH oxidase [19]. Recent work has expanded this knowledge, showing high concentrations of insulin to increase albumin permeability of both isolated rat glomeruli and podocyte monolayers in culture [22]. This study again demonstrated that insulin induces ROS production in podocytes, which can, at least in part, be attributed to an increase in Nox4 activation, and that this insulin-stimulated ROS generation in podocytes can result in cell membrane localization and dimerization of the cGMP-dependent protein kinase G type I\(z\) (PKG\(z\)). These observations, coupled with those of increased albumin permeability of glomeruli isolated from Zucker obese rats, in which both PKG\(z\) and Nox4 expression are increased, suggest a mechanism by which insulin may regulate filtration barrier permeability, which may be dysregulated in disease [22].

An advantage of a divergent signalling cascade as is found with insulin signalling is that it allows for redundancy and compensation. For the podocytes this is shown in the podocyte-specific Grb2 knockout mouse, which has no phenotype [23]. Grb2 is an important adaptor protein in a number of pathways including the insulin signalling pathway (Figure 1). However, it is obvious that its loss can be accommodated in the podocyte.

**INSULIN RESISTANCE AND THE PODOCYTE**

Insulin resistance, or a reduction in cellular responses following insulin stimulation, occurs as a consequence of complex genetic and environmental factors and is thought to play a
major role in the pathogenesis of type 2 diabetes [24] and the metabolic syndrome. The ‘metabolic syndrome’ is a commonly used term to describe a clustering of metabolic abnormalities, notably hypertension, hyperinsulinaemia, cellular insulin resistance and related obesity, associated with an increased risk of cardiovascular disease and diabetes [25]. Importantly, these metabolic abnormalities are also linked to the development of albuminuria. It is also recognized that insulin resistance occurs in, and contributes to the pathogenesis of, type 1 diabetes [9,26].

Cellular insulin signalling may be disrupted at any point within the pathway, from IR/IRS activation to factors involved in downstream cellular responses; decreases in glucose transporter levels, for example. Particularly, much evidence has recently pointed at numerous interactions between pathways involved in over-nutrition, inflammation and the development of cellular insulin resistance: a subject that is extensively reviewed in the literature [27,28].

Obesity has long been associated with insulin resistance, as adipose tissue can produce a number of mediators linked to decreased insulin sensitivity. For example, increased levels of free fatty-acid (FFA) within the circulation often arises in combination with obesity, and have been demonstrated to reduce insulin sensitivity through acting at a number of levels in the signalling cascade [29]. Additionally, chronic, low-grade inflammation, often characterized by increased cytokine production and inflammatory signalling, is associated with insulin resistance and related metabolic abnormalities. Prolonged metabolic disturbances, for example over nutrition and obesity, may contribute to states of chronic, low-grade inflammation and a number of inflammatory mediators are over-expressed in obesity [28].

EXTRINSIC FACTORS THAT MODULATE PODOCYTE INSULIN SENSITIVITY

Obesity related

We have previously demonstrated that podocyte insulin responses in vitro are disrupted following incubation with one of the predominant circulating fatty acids, palmitate [30], the levels of which are increased in conditions of insulin resistance and obesity. Further evidence for factors within the circulation influencing podocyte insulin signalling has been obtained from studies in the db/db mouse model of obesity-related type 2 diabetes. Podocytes isolated from these animals, with albuminuria and early glomerular disease, demonstrate reduced Akt phosphorylation in response to insulin, and a reduction in cell viability [31]. The adipokine adiponectin, levels of which are decreased in obesity and insulin resistant states, such as type-II diabetes, has been shown to reverse insulin resistance in mice [32]. Interestingly, whole-body adiponectin knockout mice develop severe glomerular disease as a consequence of podocyte dysfunction [33]. Furthermore, recent transgenic mouse work has revealed that renal damage associated with podocyte stress is exacerbated if adiponectin is lacking but relieved if it is overexpressed [34].

Upregulation of the lipid phosphatase SH2-domain-containing inositol polyphosphate 5-phosphatase 2 (SHIP2) has been demonstrated in the glomeruli of obese, diabetic rat models. This negative regulator of the insulin signalling pathway is thought to act at the level of IRS proteins and, following the overexpression of SHIP2 in podocytes in vitro, results in reduced insulin signalling at the level of Akt phosphorylation, potentially suggesting that suggest factors associated with obesity are directly responsible for disrupting podocyte insulin responses via SHIP2 upregulation [35].

Hyperglycaemia

Hyperglycaemic is a consequence of type-I and type-II diabetes. Recently, it has been demonstrated that podocytes of the type-1 diabetic (Akita) mouse model have reduced insulin responses, with respect to Akt and Erk MAPK phosphorylation. This study by Drapeau et al. demonstrates the expression of the protein phosphatase Src homology-2 domain-containing phosphatase-1 (SHP-1) to increase both in podocytes of Akita mice in vivo and in podocytes in vitro following high glucose (HG) exposure. They also demonstrate that this phosphatase interacts with the IRβ subunit, impeding insulin signalling [36].

A study by Mima et al. [13] demonstrated reduced insulin signalling within the glomeruli of type-1 and type-II diabetic rodent models, where insulin responses in the tubular compartment remain unchanged. Their data demonstrates that this can at least in part be attributed to high glucose levels leading to an increase in polyubiquitination of IRS-1.

Inflammation

Glomerular inflammation is apparent in a number of glomerular diseases, including diabetic kidney disease. The importance of inflammatory responses in renal pathology in the context of diabetes and insulin resistance is increasingly being recognized [37,38]. Given the links between chronic inflammation and insulin resistance, it stands to reason that factors released in glomerular inflammation may disrupt insulin responses of intrinsic renal cells, thereby contributing to disease progression.

Recent work has linked glomerular inflammation with podocyte insulin resistance early in the development of Diabetic Nephropathy (DN). Nucleotide-binding oligomerization domain-containing 2 (NOD2) is an intracellular pattern recognition receptor (PRR), responsible for immune activation following recognition of the bacterial cell wall component muramyl dipeptide (MDP). Although PRRs function as part of innate immunity, recognizing pathogen-associated molecular patterns, many PRRs can be activated by endogenous danger signals. Recently, NOD2 levels in patients with a range of inflammation-associated renal diseases have been shown to negatively correlate with estimated glomerular filtration rate. Of particular interest was the observed increase in NOD2 levels in patients with DN and glomeruli of high fat diet (HFD)/streptozotocin (STZ) murine models. In vitro, NOD2 activation in podocytes, following MDP treatment, reduced insulin-stimulated glucose uptake, disrupting GLUT4 translocation, p85/IRS-1 interactions and inducing inhibitory serine
Interestingly, elevated urea levels as a consequence of chronic kidney disease (CKD) have been implicated in the development of systemic insulin resistance: both in vivo, through the observation that urea infusion into mice results in insulin resistance and increased adipokines within the circulation; and in vitro, following urea treatment of adipocytes. The mechanism was suggested here to be a result of increased ROS production [45].

Podocytes are terminally differentiated cells and their survival is key to maintaining integrity of the glomerular filtration barrier. It has also been speculated that loss of podocyte function is crucial in accelerating the progression of CKD. A recent paper by Canaud et al. [45] has shown that Akt2 may be a key molecule in podocyte survival and protect against the progression of renal disease in the setting of nephron loss. As described, Akt2 is the major isoform of Akt activated following cellular insulin stimulation [46] (Figure 1).

Cauuard et al. demonstrated that Akt2 is particularly abundant in the podocyte in the glomerulus, where it is activated following glomerular stress both in mice and in patients with a variety of glomerular diseases. The podocyte-specific deletion of Akt2 results in a more rapid disease progression in mouse models of glomerular disease, indicating podocyte Akt2 expression and activation is essential in podocyte function and survival. Clinically, this study showed that the mTOR inhibitor, sirolimus can directly inhibit Akt2 activation and this may account for its proteinuric effects when given in situations of glomerular stress and CKD.

Another recent study has investigated the role of TLR inhibition in the db/db mouse model of diabetic renal disease. Following administration of GIT27, a selective TLR2/4/6 inhibitor, db/db mice displayed evidence of improved insulin sensitivity, and reduced kidney and adipose tissue mass. Interestingly, albuminuria and urinary nephrin levels were significantly reduced in db/db mice treated with GIT27, suggesting a reduction in podocyte damage, and of the renal cells examined TLR4 expression was found to be highest in podocytes. Reduction in glomerular macrophage infiltration and urinary TNF-α and IL-2 concentrations, indicative of reduced glomerular inflammation, was also observed in GIT27-treated db/db mice [44]. Although, again, the renoprotective effects of TLR inhibition seen in the model could be attributed to the inhibition of TLRs on circulating immune cells, in vitro conditions of FFA and HG increased TLR4 expression in podocytes, which was responsible for an increase in pro-inflammatory cytokine synthesis by podocytes under these conditions. Nox4 expression was also blocked in podocytes treated with GIT27 in vitro [44]. Given the recent links between Nox4 activation and insulin stimulation in podocytes [22] and the association between GIT27 treatment and improved systemic insulin sensitivity [44], it may be interesting to specifically investigate how glomerular insulin responses are altered following TLR activation and inhibition.

**EVIDENCE THAT AUGMENTING PODOCYTE INSULIN RESPONSES MAY BE BENEFICIAL IN GLOMERULAR DISEASE MANAGEMENT**

There is evidence that strategies that enhance cellular insulin sensitivity, including peroxisome proliferator-activated receptor gamma agonists, such as rosiglitazone and pioglitazone, are beneficial in preventing kidney damage in animal models of diabetic nephropathy in both type-I [47] and type-II disease [48, 49], as well as other non-diabetic chronic kidney diseases [50, 51].

Interestingly, in human studies, there is also evidence that these agents may be beneficial in treating early microalbuminuric diabetic renal disease [52–54]. It is possible that these drugs are exerting part of their beneficial effects by directly enhancing insulin sensitivity of the podocyte. In line with this hypothesis, we have previously shown rosiglitazone to directly augment insulin signalling in human immortalized podocytes in vitro [55].

**CONCLUDING REMARKS**

There is accumulating evidence that the insulin signalling pathway in the podocyte is crucial for normal renal function. It is now becoming clear that a variety of stimuli can inhibit this pathway at different levels in both diabetic and non-diabetic renal disease. A summary of our current understanding of these factors known to directly interfere with podocyte insulin responses is displayed in Figure 2. Augmenting this pathway may have therapeutic potential in the management of glomerular disease in the context of insulin resistance.
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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES


FIGURE 2: A summary of proposed mechanisms involved in the development of podocyte insulin resistance. In situations associated with metabolic dysfunction (examples here being obesity, inflammation and hyperglycaemia), a number of interacting networks and pathways are likely to enhance and augment each other. (a) Chronic/low-grade inflammation is associated with an increase in pro-inflammatory cytokines, e.g. TNF-α. Both obesity and hyperglycaemia may indirectly contribute to inflammation [27]; (b) palmitate is a circulating fatty acid, increased in situations of obesity and has been shown to directly disrupt podocyte insulin responses [30]; (c) overexpression of SHIP-2 reduces podocyte insulin responses in vitro and this phosphatase is upregulated in glomeruli of obese Zucker rats [35]; (d) NOD2 expression is increased in podocytes following exposure to high glucose, TNF-α and in HFD/STZ mice, and is associated with reduced insulin signalling and glucose uptake. Increased NOD2 levels may also exacerbate inflammation [39]; (e) increased TLR4 expression in podocytes is observed following high glucose/FFA exposure, this increase is associated with an increase in pro-inflammatory cytokine synthesis by podocytes. Blockade of Nox4 signalling following TLR4 inhibition may imply that increased TLR4 expression has a role in disrupting podocyte insulin responses [44]; (f) increased SHP-1 expression following high glucose exposure is associated with increased IRβ interactions and reduced insulin signalling [36] and (g) AKT2 may be a crucial link between CKD and insulin responsiveness in the podocyte [5].


Coward RJM, Welsh GI, Kozziell A et al. Neprin is critical for the action of insulin on human glomerular podocytes. Diabetes 2007; 56: 1127–1135


Kim EY, Dryer SE. Effects of insulin and high glucose on mobilization of sIiB1A channels in podocytes. J Cell Physiol 2011; 226: 2307–2315


Griffin ME, Marcucci MJ, Cline GW et al. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes 1999; 48: 1270–1274


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