Mechanisms Leading to Differential Hypoxia-Inducible Factor Signaling in the Diabetic Kidney: Modulation by SGLT2 Inhibitors and Hypoxia Mimetics

Milton Packer

Sodium/glucose cotransporter 2 (SGLT2) inhibitors exert important renoprotective effects in the diabetic kidney, which cannot be readily explained by their actions to lower blood glucose, blood pressure, or glomerular filtration pressures. Their effects to promote erythrocytosis suggest that these drugs act on hypoxia-inducible factors (HIFs; specifically, HIF-1α and HIF-2α), which may underlie their ability to reduce the progression of nephropathy. Type 2 diabetes is characterized by renal hyperglycemia, oxidative and endoplasmic reticulum stress, and defective nutrient deprivation signaling, which (acting in concert) are poised to cause both activation of HIF-1α and suppression of HIF-2α. This shift in the balance of HIF-1α/HIF-2α activities promotes proinflammatory and profibrotic pathways in glomerular and renal tubular cells. SGLT2 inhibitors alleviate renal hyperglycemia and cellular stress and enhance nutrient deprivation signaling, which collectively may explain their actions to suppress HIF-1α and activate HIF-2α and thereby augment erythropoiesis, while muting organellar dysfunction, inflammation, and fibrosis. Cobalt chloride, a drug conventionally classified as a hypoxia mimic, has a profile of molecular and cellular actions in the kidney that is similar to those of SGLT2 inhibitors. Therefore, many renoprotective benefits of SGLT2 inhibitors may be related to their effect to promote oxygen deprivation signaling in the diabetic kidney.

Potential Renoprotective Actions of SGLT2 Inhibitors

The favorable effects of SGLT2 inhibitors on diabetic chronic kidney disease (CKD) cannot be explained by their action to lower blood glucose levels. The degree of renal protection is not closely associated with the anti-hyperglycemic effect in individual patients, and drugs that have greater glucose-lowering effects do not produce a rapid and marked reduction in adverse renal events. Similarly, the renal benefits of SGLT2 inhibitors cannot be ascribed to their modest action to lower blood pressure because even intensive control of blood pressure does not prevent serious renal events in type 2 diabetes.

SGLT2 inhibitors alleviate the increased intraglomerular filtration pressure that has been implicated in the genesis of diabetic nephropathy. Their inhibitory effects in the proximal renal tubule promote sodium delivery to the macula densa, which (through tubuloglomerular feedback) mitigates the afferent arteriolar vasodilatation produced by hyperglycemia. However, such an action has been disputed, and the reduction in glomerular hyperfiltration produced by experimental knockout of SGLT2 does not prevent kidney injury. Furthermore, SGLT2 inhibitors exert renal benefits in patients with glomerular filtration rates that are low enough to negate the ability of these drugs to cause glycosuria and decrease glomerular filtration pressures. Furthermore, SGLT2 inhibitors exert favorable effects in patients in whom glomerular hyperfiltration has already been meaningfully ameliorated with the use of inhibitors of the renin-angiotensin system. Collectively, these observations suggest that SGLT2 inhibitors exert renoprotective benefits by acting on non-hemodynamic pathogenic mechanisms within the kidney.

In clinical trials in type 2 diabetes, the pharmacologic profile of SGLT2 inhibitors is distinguished by 2 physiologic effects that are not seen with other antihyperglycemic drugs: ketogenesis and erythrocytosis. It is unlikely that the ketogenic action of SGLT2 inhibitors is responsible for the ability of these drugs to reduce the risk for serious renal events. Circulating levels of ketone bodies are already increased in patients with diabetes and the diabetic kidney is a ketogenic organ, in the absence of SGLT2 inhibition. Furthermore, ketonemia dilates afferent arterioles, thereby promoting glomerular hyperfiltration and its injurious effects and thus ketogenesis is likely to exacerbate (rather than ameliorate) diabetic nephropathy.

Additionally, SGLT2 inhibitors cause erythrocytosis by enhancing the production of erythropoietin, and some have proposed that an increase in oxygen-carrying capacity of the blood might improve kidney function. However, erythropoietin mimetics that yield increases in erythrocyte counts comparable to those seen with SGLT2 inhibitors do not favorably affect the course of kidney disease.

Nevertheless, the parallel effect of SGLT2 inhibitors to promote the production of erythropoietin and ameliorate
the decline in glomerular function suggests that these 2 effects may have a shared underlying mechanism. It is therefore noteworthy that hypoxia-inducible factors (HIFs) are the primary driver of erythropoietin synthesis, and additionally, derangements in HIF signaling have been implicated in the pathogenesis of diabetic CKD. Is it possible that an effect of SGLT2 inhibitors on HIFs contributes to the renoprotective actions of these drugs?

**HIF Signaling in the Kidney**

HIFs regulate red blood cell synthesis by transactivating the gene for erythropoietin. Two inducible isoforms, HIF-1α and HIF-2α, are upregulated by hypoxia or by drugs that mimic hypoxia under normoxic conditions (eg, cobalt chloride). These 2 isoforms coordinately regulate the expression of numerous genes and proteins that play a critical role in promoting oxygen delivery (by stimulating erythropoietin synthesis and angiogenesis) and reducing oxygen use (by inhibiting metabolic pathways and intracellular organelles that consume oxygen; Fig 1).

**HIFs and Erythropoiesis**

HIF-2α is the isoform that is responsible for erythropoietin synthesis. This action is related to the expression of HIF-2α in specialized peritubular interstitial cells in the kidney, although HIF-2α also mediates erythropoietin synthesis in the liver. In contrast, although HIF-1α can influence erythropoiesis if HIF-2α signaling is impaired, HIF-1α primarily activates the transcription of genes encoding for metabolic enzymes, transporters, and mitochondrial proteins that decrease oxygen use, and it is expressed in a broad range of cell types in the kidney.

Both HIF-1α and HIF-2α are degraded by prolyl hydroxylases, whose inhibition potentiates the actions of 1 or both HIF isoforms. Prolyl hydroxylase inhibition (eg, with roxadustat) boosts erythropoietin synthesis and has been shown to effectively treat the anemia of CKD in clinical trials. However, the erythrocytosis produced by these drugs is related to potentiation of the actions of HIF-2α, and not HIF-1α; this HIF-2α upregulation may occur in both the kidney and liver. Cobalt chloride is a hypoxia mimetic that acts by inhibiting prolyl hydroxylase. Although it can augment the activity of both HIF-1α and HIF-2α, it promotes erythrocytosis primarily by binding directly to and signaling through HIF-2α. The deficiency of erythropoietin and efficacy of prolyl hydroxylase inhibitors in CKD suggest that HIF-2α is downregulated in these patients, a hypothesis that has been confirmed in the experimental setting. In light of these observations, it seems likely that other drugs that promote erythropoietin production and erythropoiesis in kidneys under stress (eg, SGLT2 inhibitors) also act to promote HIF-2α signaling.

**HIF: Organelles and Autophagy**

In addition to their actions on erythropoietin, HIFs are important regulators of genes and are posttranslational modifiers of cytosolic and mitochondrial proteins that serve to decrease oxygen consumption. Specifically, HIF-1α inhibits the biogenesis and oxidative functions of mitochondria and promotes the autophagic clearance of damaged mitochondria (mitophagy). whereas HIF-2α stimulates the autophagic disposal of injured peroxisomes (pexophagy). Both mitophagy and pexophagy act to suppress the activity of the cell’s most important oxygen-consuming organelles, an effect consistent with the paradigm that HIFs serve to preserve limited supplies of intracellular oxygen. Mitophagy and pexophagy also dampen the major organelar sources of reactive oxygen species, thus explaining why HIF-1α and HIF-2α signaling has a powerful effect to mute oxidative stress in glomerular and renal tubular cells. The suppression of organellar activity by HIFs provides an important negative feedback loop because the presence of functional mitochondria that generate reactive oxygen species is necessary for hypoxia-induced activation of HIF-1α. Theoretically, the effect of both HIF-1α and HIF-2α to promote autophagic flux can also mitigate other forms of cellular stress that are triggered by states of low oxygen tension (eg, endoplasmic reticulum stress), thereby enhancing the cellular adaptation to hypoxia and mitigating the consequences of ischemic injury. Therefore, the actions of HIF-1α and HIF-2α to activate mitophagy and pexophagy may play a crucial role in the cellular adaptation to both oxygen deprivation and oxidative stress.

**HIFs and Renal Inflammation**

Although HIF-1α and HIF-2α signaling has concordant effects to downregulate the function of oxygen-consuming organelles, these 2 isoforms have opposite effects on proinflammatory and profibrotic pathways, both in the kidney and other tissues. A key action of HIFs in states of renal ischemia is to increase oxygen delivery by promoting angiogenesis, an action that is mediated by HIF-1α but not by HIF-2α. However, any effect of HIF-1α to enhance...
vascularization is necessarily accompanied by activation of proinflammatory cytokine production, inflammatory cell signaling, and profibrotic mechanisms, which act collectively to promote the development of nephropathy. Accordingly, HIF-1α expression is accompanied by renal inflammation and fibrosis (particularly in diabetes), and augmentation of HIF-1α signaling potentiates the proinflammatory and profibrotic actions of endogenous mediators that can cause kidney injury. Conversely, experimental knockout or inhibition of HIF-1α attenuates mesangial matrix expansion, extracellular matrix accumulation, and glomerulosclerosis and alleviates tubulointerstitial inflammation and fibrosis.

In striking contrast to the proinflammatory effects of HIF-1α, activation of HIF-2α mutes inflammation and reduces injury in CKD (especially when the disease process is well established), explaining why decreased HIF-2α expression (and impaired erythropoietin synthesis) are associated with increased expression of inflammatory and angiogenic markers.

HIFs and Hypoxia Mimetics: Effect of SGLT2 Inhibitors, Cobalt Chloride, and Novel Prolyl Hydroxylase Inhibitors

The diabetic kidney is characterized by abnormalities in HIF-1α and HIF-2α signaling, and the interplay of the deranged actions of these isoforms appears to play an important role in the pathogenesis of nephropathy; Figure 2.

HIF-1α Upregulation and HIF-2α Downregulation in the Diabetic Kidney

Oxygen consumption by the kidney is principally driven by the metabolic requirements imposed by renal tubular sodium reabsorption. In type 2 diabetes, proximal sodium reabsorption is markedly increased, and the resulting increase in oxygen use predisposes absorptive segments of the kidney to hypoxia. In experimental studies, renal hypoxia is an early finding in diabetes and it may precede evidence of kidney injury or albuminuria and occurs independently of hyperglycemia. Clinical studies using blood oxygen level–dependent magnetic resonance imaging have confirmed the presence of renal hypoxia in patients with diabetes. Importantly, heightened sodium transport in the diabetic kidney is confined to specific segments involved in sodium reabsorption, and the resulting impairment in renal oxygenation is regional. Thus, the hypoxia-triggered enhancement of HIF-1α expression and its associated inflammatory and fibrotic response are often localized.

Additionally, the diabetic kidney is characterized by the accumulation of glucose and lipid intermediates that are an important cause of oxidative and endoplasmic reticulum stress. Advanced glycation end products and free fatty acids can elicit a maladaptive unfolded protein response and promote mitochondrial and peroxisomal dysfunction leading to the excessive generation of reactive oxygen species. These cellular stresses would normally be mitigated by the ability of cells to enhance autophagy, a lysosome-dependent degradative process that mutes cytosolic stress and maintains cellular homeostasis. Healthy podocytes and renal tubules maintain high levels of autophagy to sustain their structural and functional integrity. However, autophagic flux is impaired in the kidney in type 2 diabetes and thus it cannot function to adequately constrain cellular stress. Exaggerated oxidative stress has been directly implicated in the dysfunction and demise of both podocytes and renal tubular cells, as well as the stimulation of proinflammatory pathways, thus contributing to the pathogenesis of diabetic CKD. Importantly, reactive oxygen species are an important stimulus to HIF-1α activation, possibly due to their action to impair oxygen sensing by mitochondria and peroxisomes. Additionally,
hyperglycemia and advanced glycation end products can directly promote HIF-1α transcription in both glomerular mesangial and renal tubular cells.

Finally, HIF-1α and HIF-2α activation in the kidney is also regulated by nutrient deprivation sensors; specifically, sirtuin1 (SIRT1) and adenosine monophosphate-activated protein kinase (AMPK). Diabetes is perceived as a state of energy overabundance and thus SIRT1 and AMPK signaling is markedly impaired in glomeruli and renal tubules in the diabetic kidney. The suppression of SIRT1/AMPK activation can directly promote mitochondrial dysfunction, oxidative stress, and proinflammatory pathways, and downregulation of SIRT1/AMPK also eliminates a major stimulus to autophagic flux. For these reasons, suppression of SIRT1/AMPK signaling and autophagy appear to contribute importantly to the pathogenesis of the glomerular and tubular lesions in diabetic nephropathy, whereas AMPK/SIRT1 activation and promotion of autophagy leads to favorable effects on the development of CKD.

It is therefore noteworthy that even in the presence of hypoxia, activation of both AMPK and SIRT1 inhibits HIF-1α (although studies in the kidney are limited). However, in striking contrast, SIRT1 activates HIF-2α. Therefore, the SIRT1/AMPK signaling deficiency that is seen in the renal parenchyma in type 2 diabetes can be expected to activate HIF-1α and suppress HIF-2α.

As a result of the interplay of renal hypoxia, cellular stress, and impaired nutrient deprivation signaling, it is not surprising that experimental and clinical diabetic CKD is accompanied by decreased HIF-2α expression but increased HIF-1α expression. HIF-1α activation and HIF-2α suppression promote the inflammatory and fibrotic responses that are essential to diabetic kidney injury. Conversely, the suppression of HIF-1α and the hypoxia mimetic activation of HIF-2α ameliorates the development of diabetic nephropathy.

Effect of SGLT2 Inhibitors on HIF Signaling in the Diabetic Kidney

Hyperglycemia and hyperinsulinemia cause SGLT2 upregulation in the proximal renal tubules, explaining the SGLT2 activation in the diabetic kidney. Enhancement of the actions of SGLT2 has been directly linked to enhanced oxidative stress, mitochondrial dysfunction, inflammation, and cellular demise, independent of hyperglycemia. These deleterious effects on cellular homeostasis are mitigated by SGLT2 inhibitors in experimental models of diabetic kidney injury.

The role played by renal hypoxia in the interplay between SGLT2 and kidney disease has not been clearly defined (Fig 2). In the diabetic kidney, sodium reabsorption is enhanced in the proximal renal tubules, which is expected to increase oxygen consumption and predispose to hypoxia in the renal cortex. Presumably as a compensatory mechanism, hypoxia decreases SGLT2 expression, suggesting that oxygen tension in the kidney is not only a determinant of HIF signaling but also of renal tubular sodium transport. When SGLT2 is upregulated (as in type 2 diabetes), SGLT2 inhibitors block proximal tubular sodium reabsorption and thereby reduce oxygen consumption and alleviate renal hypoxia. As a result, these drugs may reduce the stimulus for HIF-1α production. The HIF-1α suppression by SGLT2 inhibitors has been proposed to underlie the renoprotective benefits of these drugs. Additionally, in type 2 diabetes, SGLT2 inhibitors act to enhance nutrient deprivation signaling as a result of their effect to promote the loss of calories in urine. In doing so, SGLT2 inhibitors upregulate both AMPK and SIRT1, which (in turn) act to suppress HIF-1α and activate HIF-2α.

Effect of Hypoxia Mimetics and Novel Prolyl Hydroxylase Inhibitors on HIF Signaling in the Diabetic Kidney

Hypoxia mimetics enhance HIF-1α and/or HIF-2α activity, generally by inhibiting 1 or several of the 3 prolyl hydroxylases that are responsible for degradation of the isoforms. Prolyl hydroxylases differ with respect to their expression profiles and effects on HIF-1α or HIF-2α. For example, inhibition of prolyl hydroxylase 2 boosts the activity of HIF-1α whereas prolyl hydroxylase 3 acts preferentially on HIF-2α. The classic hypoxia mimetic cobalt chloride typically activates both HIF-1α and HIF-2α in the absence of diabetes. However, in the diabetic kidney, the drug acts to normalize renal oxygen consumption, explaining its action to reduce diabetes-related activation of HIF-1α. However, cobalt chloride directly binds to and activates HIF-2α which underlies its action to promote the production of erythropoietin, despite the relief of hypoxia (Fig 2). The effect to augment HIF-2α may be potentiated by a simultaneous action of cobalt chloride to activate SIRT1. These mutually reinforcing actions to promote signaling through HIF-2α and SIRT1 may explain why cobalt chloride ameliorates proinflammatory and profibrotic pathways and prevents injury in the diabetic kidney, an effect that is accompanied by erythropoiesis. Therefore, the pattern of HIF-1α and HIF-2α signaling, erythropoietin production, and renoprotection with cobalt chloride is strikingly similar to that seen with SGLT2 inhibitors, and this parallelism is further underscored by the finding that cobalt chloride downregulates SGLT2 in renal tubules. Interestingly, HIF-2α activation by both SGLT2 inhibitors and cobalt chloride may contribute to the ability of these drugs to improve glucose tolerance. Further studies are needed to determine whether this mechanistic overlap between cobalt chloride and SGLT2 inhibitors is seen in the clinical setting, that is, in patients with or at risk for diabetic nephropathy.

Drugs that have been specifically designed to inhibit prolyl hydroxylases have been developed to treat the
anemia of CKD and their actions to modulate HIF signaling might influence the course of diabetic CKD. In 2 studies, prolyl hydroxylase inhibition was reported to ameliorate several metabolic abnormalities (while augmenting HIF-1α in experimental models of the diabetic kidney). More importantly, these drugs enhance the activity of both HIF-1α and HIF-2α, and it is their effect on HIF-2α (and not HIF-1α) that is responsible for their action to promote the synthesis of erythropoietin. The possibility that novel prolyl hydroxylase inhibitors have renoprotective effects primarily because of their actions to activate HIF-2α has not been explored to date.

Conclusions

The diabetic kidney is characterized by increased activation of HIF-1α but suppression of HIF-2α. These actions appear to result from the combined effects of renal hypoxia, oxidative and endoplasmic reticulum stress, and suppressed SIRT1/AMPK activity and may contribute importantly to glomerular and renal tubular dysfunction, renal inflammation and fibrosis, and the impairment in erythropoiesis production seen in diabetic CKD. SGLT2 inhibitors appear to reduce HIF-1α and promote HIF-2α signaling in the diabetic kidney, potentially by their actions to alleviate renal hypoxia and cellular stress and enhance nutrient deprivation signaling. These effects may contribute to the ability of these drugs to augment erythropoiesis while muting organellar dysfunction and proinflammatory and profibrotic pathways, thereby ameliorating the progression of diabetic nephropathy. Cobalt chloride, a drug conventionally regarded as a hypoxia-mimetic, has a profile of molecular and cellular actions in the kidney that is similar to those of SGLT2 inhibitors. Therefore, many renoprotective benefits of SGLT2 inhibitors may be related to their effect to promote oxygen deprivation signaling.

References


Article Information

Author’s Affiliation: Baylor Heart and Vascular Institute, Baylor University Medical Center, Dallas, TX; and Imperial College, London, United Kingdom.

Address for Correspondence: Milton Packer, MD, Baylor Heart and Vascular Institute, 621 N Hall St, Dallas, TX 75226. E-mail: milton.packer@baylorhealth.edu

Support: None.

Financial Disclosure: Dr Packer has recently consulted for Abbvie, Actavis, Akcea, Amgen, AstraZeneca, Boehringer Ingelheim, Cardiorentis, Daiichi Sankyo, Johnson & Johnson, NovoNordisk, ParatusRx, Pfizer, Sanofi, Synthetic Biologics, and Theravance.

Peer Review: Received February 25, 2020. Evaluated by 2 external peer reviewers, with direct editorial input from an Associate Editor and a Deputy Editor. Accepted in revised form April 26, 2020.


