Mechanisms of Hypoxia Responses in Renal Tissue

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ABSTRACT

pO2 in the kidney is maintained at relatively stable levels by a unique and complex functional interplay between renal blood flow, GFR, O2 consumption, and arteriovenous O2 shunting. The fragility of this interplay makes the kidney susceptible to hypoxic injury. Cells in the kidney utilize various molecular pathways that allow them to respond and adapt to changes in renal oxygenation. This review provides an integrative perspective on the role of molecular hypoxia responses in normal kidney physiology and pathophysiology, and discusses their therapeutic potential for the treatment of renal diseases.


Although 20% of total cardiac output is directed toward the kidneys, measured O2 tensions are surprisingly low, ranging from as low as 5 mmHg in the medulla to up to 50 mmHg in the cortex. Most of the kidney's O2 is utilized to fuel Na-K-ATPase, which drives tubular sodium reabsorption and other transport processes that move various solutes, glucose, and amino acids across cellular membranes. Because these transport processes are load dependent, they link renal O2 consumption (V\text{O}_2) to GFR. As GFR and renal blood flow (RBF) change in parallel under most conditions, an increase in RBF and thus arterial O2 delivery is largely offset by elevated V\text{O}_2, thus limiting the kidney's ability to raise pO2 by increasing RBF. As a result, regional pO2 pressures stay within a relatively narrow range. Recent physiologic studies indicate that in addition to V\text{O}_2 and O2 delivery in arterial blood, arteriovenous O2 shunting, which results from a counter-current exchange of O2 between arterial and venous vessels before arterial blood reaches the renal microcirculation, adds to the maintenance of renal pO2 at constant levels.

Because the kidney carries out its complex transport functions within a relatively narrow range of pO2, which is very low in the medulla, susceptibility to hypoxic injury is high. Renal cells have therefore evolved a variety of molecular mechanisms that allow them to respond and adapt to decreases in renal oxygenation. These mechanisms operate during development, under physiologic and under pathologic conditions, and have wide-ranging implications for the pathogenesis and treatment of renal diseases. Although several transcription factors are involved in control of hypoxia and oxidative stress responses, there has been much interest, from both the basic scientist and the clinician, in the hypoxia-inducible factor (HIF) pathway. This is because of its central role in cellular adaptation to hypoxia and its great potential for therapeutic exploitation in the areas of anemia treatment, cytoreduction, cancer, and wound healing. Here, I provide a focused perspective on key mechanisms that regulate and integrate this pathway with other hypoxia responses in the kidney and discuss its potential for renoprotection.

MOLECULAR O2 SENSORS IN THE KIDNEY: MORE THAN JUST HIF

Key components of cellular O2 sensing are Fe (II) and 2-oxoglutarate (2OG)–dependent oxygenases (Figure 1). These enzymes belong to a larger family of proteins; in humans, there are >60 members that couple the oxidative decarboxylation of 2OG to various chemical processes, which include collagen synthesis and fatty acid metabolism. In mammals, these reactions appear to be limited to hydroxylation and demethylation initiated by hydroxylation3 and produce succinate and CO2 (Figure 1). The 2OG oxygenases control hypoxic signaling by catalyzing the hydroxylation of specific proline residues within the oxygen-dependent degradation domain of HIF-\alpha under normoxia. HIFs are pleiotropic oxygen-sensitive, heterodimeric transcription factors that have key roles in the cellular adaptation to hypoxia, and regulate a multitude of biologic processes, which include erythropoiesis and iron metabolism, anaerobic glucose metabolism, angiogenesis, growth, and proliferation. Prolyl-hydroxylated HIF-\alpha is targeted for proteasomal degradation by the von Hippel-Lindau–E3 complexes.
ubiquitin ligase complex. HIF 2OG oxygenases function as O2 sensors because they require O2 for catalysis. Under hypoxia, hydroxylation is inhibited and HIF signaling is activated.4 In the thick ascending limb of Henle, this activation may be renoprotective for acute ischemic injury.5

Three main HIF prolyl hydroxylases have been identified, prolyl-4-hydroxylase domains (PHDs) 1, 2, and 3, which are also referred to as EGL nine homologs (EGLNs) 2, EGLN1, and EGLN3, respectively. PHD2 is the main enzyme that targets HIF for degradation under normoxia.4 All three PHDs are expressed in the kidney, where they control HIF activity.6 Based on immunohistochemical studies and RNA analysis, their expression levels vary between different renal cell types. Compared with PHD2, PHD1 and PHD3 are more abundant in glomeruli, whereas PHD1, PHD2, and PHD3 appear to be expressed at higher levels in the distal renal tubule compared with proximal tubular epithelium. A fourth potential HIF prolyl hydroxylase, P4H-TM, is localized in the endoplasmic reticulum membrane and has been shown to hydroxylate HIF-1α-derived peptides, but not type 1 collagen polypeptide in vitro. Although P4H-TM seems to be important for normal kidney function in zebra fish, where it regulates the integrity of the glomerular basement membrane, its role in hypoxic signaling in the mammalian kidney is unknown.7

The transcriptional activity of HIF is modulated by a second hypoxic switch, which operates within the carboxy-terminal transactivation domain of HIF-α. Factor inhibiting HIF (FIH) is a 2OG oxygenase that catalyzes the hydroxylation of an asparagine residue within the C-terminal transactivation domain of HIF-α, thereby inhibiting the binding of coactivators CREB-binding protein and p300 to the HIF transcriptional complex. Conversely, FIH inactivation facilitates CREB-binding protein/p300 recruitment and results in increased HIF target gene expression under hypoxia.4 In the kidney, FIH has been detected in podocytes and in the distal tubule.8 Although largely unexplored in the kidney, an additional level of complexity in the regulation of HIF-mediated hypoxia responses is added by oxygen-dependent microRNA expression.9

Although the role of PHDs and FIH in the regulation of HIF activity is well established, alternative hydroxylation targets have been identified that are likely to affect hypoxia responses in the kidney. PHD1, 2, and FIH, for example, catalyze the hydroxylation of components of the NF-κB pathway, linking O2 sensing to inflammatory responses,3,10 and PHD3 has been shown to interact with pyruvate kinase isoform 2 in the regulation of renal hypoxia mechanisms and selected 2OG oxygenase-regulated processes that affect renal physiology and pathophysiology. Renal oxygenation is regulated by arterial oxygen content, arteriovenous O2 shunting, and O2 consumption (\(\dot{V}O_2\)). Kidney injury results in structural and functional changes that negatively affect renal oxygenation. The 2OG-dependent oxygenases, which require molecular O2, 2OG, and ferrous iron for the hydroxylation of substrates, are inhibited under hypoxia. In addition to hypoxia, 2OG oxygenases are inhibited by structural 2OG analogs, ROS, NO, Krebs cycle metabolites succinate and fumarate, cobalt chloride and iron chelators such as desferrioxamine irrespective of O2 levels. HIF 2OG oxygenases either hydroxylate-specific proline residues located within the HIF-α oxygen-dependent degradation domain (PHD1, PHD2, and PHD3) or an asparagine within the C-terminal transactivation domain (FIH).

Figure 1. Overview of renal hypoxia mechanisms and selected 2OG oxygenase-regulated processes that affect renal physiology and pathophysiology. Renal oxygenation is regulated by arterial oxygen content, arteriovenous O2 shunting, and O2 consumption (\(\dot{V}O_2\)). Kidney injury results in structural and functional changes that negatively affect renal oxygenation. The 2OG-dependent oxygenases, which require molecular O2, 2OG, and ferrous iron for the hydroxylation of substrates, are inhibited under hypoxia. In addition to hypoxia, 2OG oxygenases are inhibited by structural 2OG analogs, ROS, NO, Krebs cycle metabolites succinate and fumarate, cobalt chloride and iron chelators such as desferrioxamine irrespective of O2 levels. HIF 2OG oxygenases either hydroxylate-specific proline residues located within the HIF-α oxygen-dependent degradation domain (PHD1, PHD2, and PHD3) or an asparagine within the C-terminal transactivation domain (FIH).
of glycolysis. Furthermore, it is likely that renal hypoxia responses are modulated by epigenetic changes that are carried out by other non-HIF 2OG oxygenases. While nothing is known about their role in kidney physiology and disease, 2OG oxygenases containing the jumonji domain catalyze the demethylation of methylated histones. Jumonji domain-containing oxygenases, some of which are also induced by hypoxia through HIF, are likely to provide additional functional links between acute and chronic alterations in pO2 levels, metabolism, and gene expression changes in the kidney.

Although oxygen-dependent, the catalytic activity of HIF hydroxylases is also modulated by multiple signaling molecules, such as reactive oxygen species (ROS) and nitric oxide (NO) (Figure 1), linking various intracellular signaling pathways, which include signaling through angiotensin II receptors or NO synthase, to renal O2 sensing. This has significant implications for CKD associated with diabetic nephropathy, the aging kidney, inflammatory renal diseases, and others. Additional insights into the complexity of molecular O2 sensing regulation and how it interplays with energy metabolism and other oxidative stress sensors come from the study of inherited renal tumor syndromes. Mutations in the Krebs cycle enzyme fumarate hydratase occur in hereditary leiomyomatosis and renal cell cancer syndrome, a rare form of familial renal cancer, and lead to the accumulation of fumarate. Fumarate competitively inhibits 2OG oxygenases as well as the degradation of NF (erythroid-derived 2)-like 2 (NRF2), a transcription factor that regulates cellular antioxidant responses. Although this is an active area of investigation in renal oncology, little is known about how links between O2 sensing, energy metabolism, and oxidative stress responses affect the pathogenesis of nonmalignant kidney disease.

THE KIDNEY IN CONTROL OF O₂ CARRYING CAPACITY

A classic systemic adaptation to hypoxia is the stimulation of red blood cell production through increased synthesis of erythropoietin (EPO). It was the interest in understanding the physiologic and molecular basis of this response that paved the way for the discovery of the PHD/HIF O2 sensing machinery. The kidney plays a key role in this response, because it is the main physiologic source of EPO in adults. Renal EPO synthesis is regulated by HIF-2, and not by HIF-1, as evidenced by several genetic and immunohistochemical studies and by mutational analysis of patients with congenital erythrocytosis. EPO-producing cells in the kidney are peritubular interstitial fibroblasts derived from neuronal lineages. The activity of HIF-2 in renal EPO-producing cells (REPCs) is controlled by PHD2. This is in contrast to nonrenal EPO-producing cell types; a role for PHD1 and PHD3 in REPC-based EPO synthesis is not apparent from genetic studies in mice. EPO transcription is highly oxygen responsive and represents one of the most sensitive hypoxia responses in the kidney. One may speculate that this response, which increases O2 carrying capacity, evolved in the kidney specifically because of its limited ability to augment O2 delivery through other regulatory mechanisms, such as increasing RBF.

In the kidney, EPO output is controlled by the number of REPCs and not by the incremental increase in cellular mRNA encoding EPO found in cell lines. In CKD, the loss of the kidney’s ability to produce adequate amounts of EPO in response to hypoxic stimuli results in anemia. Although not entirely clear, a potential mechanism underlying renal anemia may be the transition of REPCs to a myofibroblast phenotype, which would limit the number of cells that can be recruited to synthesize EPO when renal pO2 is low. Because HIF not only induces EPO synthesis, but also enhances intestinal iron uptake and utilization and promotes erythroid progenitor maturation in the bone marrow, clinical trials are currently underway to evaluate the efficacy of pharmacologic HIF stabilization using competitive PHD inhibitors (structural analogs of 2OG) for the treatment of renal anemia in CKD and dialysis patients.

O₂ AND METABOLIC REPROGRAMMING

The mitochondrial respiratory chain uses O2 to generate ATP, which fuels multiple cellular processes and is consumed mainly by Na-K-ATPase to transport solutes. Efficient metabolic adaptation to low pO2 is therefore imperative for the maintenance of renal transport functions and ultimately the promotion of cell survival. The PHD/HIF pathway has a central role in metabolic reprogramming under low pO2, because it regulates cellular energy and glucose metabolism at multiple levels. HIF shifts metabolism from oxidative phosphorylation to anaerobic glycolysis and suppresses mitochondrial respiration and ROS generation. It does this by increasing the expression of glycolytic enzymes, such as hexokinase, phosphofructokinase, aldolase, phosphoglycerate kinase 1, enolase, and lactate dehydrogenase, by blocking the conversion of pyruvate to acetyl CoA through transcriptional upregulation of pyruvate dehydrogenase kinase, and by regulating the expression of proteins that compose the mitochondrial respiratory chain. Because lactate production increases when glycolytic flux increases, HIF is also involved in the prevention of cellular acidification by regulating the expression of sodium/hydrogen exchanger 1 and monocarboxylic acid transporter-4, thus facilitating the excretion of protons and lactate. Moreover, HIF-1 activation in tumor cells keeps intracellular pH in a slightly alkaline range by increasing membrane-bound ectoenzyme carbonic anhydrase IX expression, which catalyzes the conversion of CO2 to bicarbonate. This effect of HIF activation may also be of relevance for renal adaptation to hypoxia.

THERAPEUTIC OPPORTUNITIES BEYOND RENAL ANEMIA

The effects of hypoxia and oxygen-dependent signaling on the kidney are
broad. Although acute changes in renal Po2 are frequently encountered in hospitalized patients and often result in AKI, not infrequently requiring renal replacement therapy, the cellular and molecular consequences of chronic or intermittent hypoxia on the kidney are less obvious. Some insights into the effects of subacute or chronic hypoxic signaling on healthy and diseased kidneys can be gained from studies of humans living at high altitude (see Arestegui for a review) and from patients with renal tumors caused by mutations in the O2 sensing machinery, such as patients with mutations in the von Hippel-Lindau tumor suppressor that result in constitutive HIF activation.

In the noncancerous kidney, HIF-α stabilization is found in both acute and chronic kidney diseases. In patients with diabetic nephropathy, for example, the degree of HIF activation correlates with severity of renal injury. HIF activation in the setting of CKD is due to chronic hypoxia or can result from oxygen-independent PHD inhibition. The causes of hypoxia in CKD are multifactorial and involve structural and functional changes that are commonly associated with fibrotic kidneys. These include abnormal renal perfusion from capillary rarefaction, glomerular injury, atherosclerosis, and altered vascular tone, as well as anemia and impaired O2 diffusion due to fibrosis. Increased V02 has been described in diabetic nephropathy and decreases renal Po2. This is partly due to mitochondrial dysfunction. Due to the unique features of renal O2 regulation, Po2 in the kidney is very sensitive to changes in intracellular O2 consumption caused by inefficient mitochondrial O2 utilization, as increased RBF, which raises GFR, is unlikely to compensate for increased O2 demand.

In the acute setting, HIF has been shown to mediate the effects of ischemic preconditioning, and pharmacologic HIF activation protects from ischemia-reperfusion injury in animal models of ARF. While the use of pharmacologic HIF activation in the prevention of acute renal injury is supported by preclinical studies, its role in CKD is debated; animal models of progressive kidney injury support both renoprotective and injury-promoting roles. Recent data using the remnant kidney model furthermore indicate that renoprotection is dependent on the timing of pharmacologic HIF activation.

While it has been suggested that HIF promotes progression of CKD by increasing the expression of profibrotic factors and by facilitating epithelial dedifferentiation, the mechanisms underlying renoprotection are likely to involve multiple signaling pathways and metabolic changes, which include HIF-induced expression of cytoprotective genes; reprogramming of glucose, energy, and adenosine metabolism; beneficial effects on mitochondrial O2 utilization, renal V02, and mitochondrial ROS production; enhanced ROS scavenging; suppression of renal inflammation; and maintenance of vascular health and integrity (Figure 1). Given that the regulation of the PHD/HIF axis and its downstream targets is cell type dependent and involves multiple feedback loops including changes in the epigenome, it is likely that the mechanisms of HIF-mediated cytoprotection differ between acute and chronic hypoxic conditions. This certainly poses a major challenge for the identification and validation of relevant molecular targets and cell types that mediate HIF-induced renoprotection.

A LOOK AHEAD IN RENAL HYPOXIA RESEARCH

In this review of renal hypoxia responses, I focused on selected aspects of molecular O2 sensing in the kidney and discussed its potential for therapeutic exploitation. Since the discovery of the PHD/HIF pathway, many new questions have emerged. Although the activity of renal HIF is controlled by specific HIF 2OG oxygenases, the significance of their re- action with non-HIF targets and the role of other non-HIF 2OG oxygenases in renal physiology and pathophysiology are still unknown. Fascinating are the therapeutic opportunities that the PHD/HIF pathway provides. Knowledge of the effects of pharmacologic HIF activation on human physiology and pathophysiology, however, is still limited. Controlled physiologic studies in humans, in concert with studies of patients that live at high altitude, are likely to advance our understanding of HIF responses in the kidney. This, together with animal and in vitro investigations, will hopefully lead to the development of new therapies that improve the life of patients with kidney diseases.

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DISCLOSURES

None.

REFERENCES
