J Am Soc Nephrol 13: 1974–1976, 2002

# The Breathing Kidney

LEON G. FINE and JILL T. NORMAN
*Department of Medicine, Royal Free and University College Medical School, London, United Kingdom.*

For too long, the term ''hypoxia,'' when applied to biological systems, conjured up the image of cells and tissues being suffocated and then either surviving the insult or succumbing to it depending on the magnitude and duration of oxygen deprivation. Over the past decade, however, it has become apparent that evolution has built into all cells the means of responding to reduced oxygen tension by activating a hypoxia response pathway to upregulate specific genes, which act to facilitate adaptation to hypoxia-induced stress and survival under this adverse influence or, if such adaptation fails, to promote cell death by apoptosis (reviewed in references 1 and 2). In mammals, systems have evolved that enhance distribution of oxygen to all organs that may be subjected to sustained oxygen deprivation, and a hormone, erythropoietin (EPO), evolved to boost red cell production and hence the oxygen-carrying capacity of the blood (3). Why the interior of the kidney (4,5) and not some other organ or vascular structure is the source of this hormone in the adult remains a mystery. It may, however, have something to do with the fact that, whereas most tissues of the body have relatively uniform patterns of oxygen distribution, in the kidney, there is a graded fall in oxygen tension from the outer cortex to the inner medulla (6,7); the oxygen-sensing, erythropoietin-producing cells lie mainly in the inner cortical region (4,5), where $pO_2$ is not maximal, even for the kidney. Other systems also appear to have evolved to aid the process of tissue oxygenation when help is needed. Hypoxia-driven changes in ventilation, cardiac output, vascular tone, and neovascularization are all geared to assist the process of tissue oxygen distribution when and where it is being compromised.

Central to the hypoxia response are a family of hypoxia-inducible transcription factors (HIF), which are activated when oxygen concentrations fall below a critical level. To date, three members of the family have been identified. Each is composed of a hypoxia-regulated $\alpha$ subunit (HIF-1$\alpha$, HIF-2$\alpha$, and HIF-3$\alpha$) and an oxygen-insensitive ß subunit (HIF-1ß), which is also known as the arylhydrocarbon receptor nuclear translocator (ARNT) (reviewed in references 1, 2, and 8). Of the three members, HIF-1$\alpha$ has been most extensively studied (8), and an elegant series of experiments has shown that the $\alpha$ subunit is expressed under normoxic conditions but undergoes hydroxylation and is targeted for proteosomal degradation via the von Hippel Lindau ubiquitylation complex (9–11). Hypoxia blocks this posttranslational modification, allowing the protein to accumulate. Dimerization of HIF-1$\alpha$ with the ß subunit activates binding to hypoxia-response elements (HRE) in promoter, enhancer, and intronic sequences of target genes activating gene expression. Although recent studies have revealed the complexities of the molecular structure and regulation of HIF-1, there is still relatively little known about the role of the HIF *in vivo*.

The article by Rosenberger *et al.* (12) in this issue outlines the distribution of the hypoxia-inducible transcription factors HIF-1$\alpha$ and HIF-2$\alpha$ in hypoxic and ischemic kidneys and advances our understanding of how the kidney is able to ''take a deep breath'' (at least from a cellular perspective) when it is deprived of oxygen. This detailed anatomical localization of HIF expression provides some intriguing insights. First is the surprising observation that, by and large, tubular cells and vascular/perivascular cells upregulate different HIF$\alpha$ subunits in response to hypoxia and hence to activate hypoxia-inducible genes. Once again, an *in vivo* study has shown that overenthusiastic extrapolation from *in vitro* studies may be misleading. Whereas most cell lines exposed to hypoxia show induction of both HIF isoforms (13,14), in the kidney, HIF-1$\alpha$ seems to be relatively tubular cell–specific and HIF-2$\alpha$ finds its role in endothelial and peritubular cells. By inference, this implicates HIF-2$\alpha$ as a regulator of EPO expression.

A further insight is that the conventional understanding that oxygen tension declines linearly from the cortex to the inner medulla (6,7) is overly simplistic. Numerous studies have shown a gradient of hypoxia, but the findings of Rosenberger *et al.* (12) reveal that within a given region of the kidney (*e.g.*, inner cortex, outer medulla, etc.) the distance that a cell finds itself from a vascular bundle will determine whether or not it upregulates HIF expression in response to hypoxia. Those cells closest to the vascular bundles are not ''switched on,'' but those lying a short distance away are. Using HIF expression as a surrogate marker of local hypoxia, local oxygen gradients become apparent. This sort of information could never have come from inserting an oxygen-sensing electrode, however fine in diameter, into the kidney.

As expected, when exposed to a hypoxic or ischemic insult (anemia, carbon monoxide, cobalt chloride, or vascular ligation), the deeper the tubular cells lay within the kidney the more strongly they express HIF-1$\alpha$ in response to the different stimuli the investigators used to elicit this response. Interestingly, different stimuli have different potencies in this regard. Whether or not this is due to the fact that each discrete

Correspondence to Professor Leon G. Fine, Department of Medicine, Royal Free and University College Medical School, The Rayne Building, 5 University Street, London WC1E 6JJ, United Kingdom. Phone: 44-207-679-6186; Fax: 44-207-679-6211; E-mail: l.fine@ucl.ac.uk

1046-6673/1307-1974
Journal of the American Society of Nephrology


DOI: 10.1097/01.ASN.0000024380.91444.C8

hypoxia-mimicking stimulus acts via a different pathway or whether one stimulus (*e.g.*, carbon monoxide exposure) is more effective than another (*e.g.*, total ischemia) by virtue of accessing the cell more effectively in a quantitative sense is not clear, but there certainly seems to be a hierarchy of potencies.

One additional fascinating insight is provided. If, according to the common wisdom, the deep interior of the mammalian kidney lives on the brink of hypoxia (6,7) at an oxygen tension that is known to be low enough to activate the HIF response *in vitro* (15), why do these cells not express HIF constitutively? Is it because the HIF response has been eliminated in cells living at the top of the Andes or the tip of the papilla, depending on your point of view? The answer now appears to be clear. These cells under ''normal'' conditions do not show constitutive HIF expression, but they are the first to show it in response to a fall in oxygen tension. They are thus not quite at the summit and are breathing hard, and the last few steps are enough to bring out the replacement oxygen tanks, whereas their brethren living a slight distance away (nearer to the cortex) manage quite nicely at the ambient oxygen tension. Interestingly, differences in the pattern of induction of the individual HIF in different nephron segments in response to a particular stimulus suggest different thresholds of activation in different cell types and also differences in activation within the same cell type because not all cells within a particular nephron profile upregulate the protein. The factors or conditions that determine whether or not a cell or group of cells upregulates HIF remain to be established, but they raise the question of whether it is possible to increase or decrease the sensitivity of different cell types?

In addition to the insights provided by the article by Rosenberger *et al.* (12), two other points are worthy of consideration. First, it is simplistic to believe that the HIF transcription factors are uniquely responsible for mediating the entire, highly complex cellular response to hypoxia. Examples exist in which hypoxia-induced alterations in gene expression occur independently of HIF regulation, at least *in vitro* (16–19), and it is expected that a series of transcriptional regulatory factors responsive to hypoxia will emerge as the problem is probed more deeply. Second, what occurs acutely is likely to occur chronically when the stimulus (hypoxia) is persistent, even if it is at a lower level of intensity. Hypoxia plays a significant role in the pathogenesis of a variety of pathologic conditions (8). In the context of the progression of chronic renal disease, it is quite likely that tubulointerstitial hypoxia accompanied by microvascular obliteration plays an important role in the progressive scarring of the kidney (20,21). This area also awaits *in vivo* confirmation of the pro-fibrogenic effect of hypoxia that is seen in renal cells *in vitro* (22,23). The intact animal continues to surprise, *in vivo* studies will therefore remain the last port of call before a hypothesis becomes fact. The detailed study of the expression of HIF in the kidney not only provides new insights into function but may also open doors to pharmacologic intervention in kidney disease.

## References


1. Bunn HF, Poynton RO: Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* 76: 839–885, 1996
2. Wenger RH: Mammalian oxygen sensing, signalling and gene regulation. *J Exp Biol* 203: 1253–1263, 2000
3. Jelkmann W: Erythropoietin: Structure, control of production and function. *Physiol Rev* 72: 449–489, 1992
4. Bachmann S, Le Hir M, Eckhardt K-U: Colocalisation of erythropoietin mRNA and ecto-5'-nucleotidase immunoreactivity in peritubular cells of rat renal cortex indicates that fibroblasts produce erythropoietin. *J Histochem Cytochem* 41: 335–341, 1993
5. Maxwell PH, Ferguson DJP, Nicholls LG, Iredale JP, Pugh CW, Johnson MH, Ratcliffe PJ: Sites of erythropoietin production. *Kidney Int* 51: 393–401, 1997
6. Epstein FH, Agmon Y, Brezis M: Physiology of renal hypoxia. *Ann NY Acad Sci* 718: 72–81, 1994
7. Epstein F: Oxygen and renal metabolism. *Kidney Int* 51: 381–385, 1997
8. Semenza GL: HIF-1: Mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol* 88: 1474–1480, 2000
9. Maxwell PH, Pugh CW, Ratcliffe PJ: Insights into the role of the von Hippel-Lindau gene product. A key player in hypoxic regulation. *Exp Nephrol* 9: 235–240, 2000
10. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Slaic A, Asara JM, Lane WS, Kaelin Jr WG: HIF $\alpha$ targeted for VHL-mediated destruction by proline hydroxylation: Implications for $O_2$ sensing. *Science* 292: 464–468, 2001
11. Jaakola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kreigsheim AA, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ: Targeting of the HIF-$\alpha$ to the von Hippel-Lindau ubiquitinylation complex by $O_2$-regulated prolyl hydroxylation. *Science* 292: 468–472, 2001
12. Rosenberger C, Mandriota S, Jürgensen JS, Weisener MH, Hörstrup JH, Frei U, Ratcliffe PJ, Maxwell PH, Bachmann S, Eckardt K-U: Expression of hypoxia-inducible factor-1$\alpha$ and -2$\alpha$ in hypoxic and ischemic kidneys. *J Am Soc Nephrol* 13: 1721–1732, 2002
13. Maxwell PH, Pugh CW, Ratcliffe PJ: Inducible operation of the erythropoietin 3' enhancer in multiple cell lines: Evidence for a widespread oxygen sensing mechanism. *Proc Natl Acad Sci USA* 90: 2423–2427, 1993
14. Wiesener MS, Turley H, Allen WE, William C, Eckardt K-U, Talk KL, Wood SM, Gatter KC, Harris AL, Pugh CW, Ratcliffe PJ, Maxwell PH: Induction of endothelial PAS domain protein-1 by hypoxia: Characterisation and comparison with hypoxia-inducible factor-1 $\alpha$. *Blood* 92: 2260–2268, 1998
15. Jiang BH, Semenza GL, Bauer C, Marti HH: Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of $O_2$ tension. *Am J Physiol* 271: C1172–C1180, 1996
16. Yan SF, Zou YS, Mendelsohn M, Gao Y, Naka Y, Du Yan S, Pinsky D, Stern D: Nuclear factor interleukin 6 motifs mediate tissue specific gene transcription in hypoxia. *J Biol Chem* 272: 4287–4294, 1997
17. Discher DJ, Bishopric NH, Wu X, Peterson CA, Webster KA: Hypoxia regulates ß-enolase and pyruvate kinase-M promoters by modulating Sp1/Sp3 binding to a conserved GC element. *J Biol Chem* 273: 26087–26093, 1998
18. Xu Q, Ji Y-S, Schmedtje JF: Sp1 increases expression of cyclooxygenase-1 in hypoxic vascular endothelium. *J Biol Chem* 275: 24583–24589, 2000
19. Nishi H, Nishi KH, Johnson AC: Early growth response-1 gene mediates up-regulation of epidermal growth factor receptor expression during hypoxia. *Cancer Res* 62: 827–834, 2002

20. Fine LG, Orphanides C, Norman JT: Progressive renal disease: The chronic hypoxia hypothesis. *Kidney Int* 65: S74–S78, 1998
21. Kang D-H, Kanellis J, Hugo C, Truong L, Anderson S, Kerjaschki D, Schreiner G, Johnson RJ: The role of the microvascular endothelium in progressive renal disease. *J Am Soc Nephrol* 13: 806–816, 2002
22. Orphanides C, Fine LG, Norman JT: Hypoxia stimulates proximal tubular cell matrix production via a TGFß1- independent mechanism. *Kidney Int* 52: 637–647, 1997
23. Norman JT, Clark IM, Garcia PL: Hypoxia promotes fibrogenesis in human renal fibroblasts. *Kidney Int* 58: 2351–2366, 2000

See related article, "Expression of Hypoxia-Inducible Factor-1$\alpha$ and 2$\alpha$ in Hypoxic and Ischemic Rat Kidneys," on pages 1721–1732.