

Perspectives on Oxygen Sensing

Minireview

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Physiological Perspective

Regardless of whether bacteria, yeast, invertebrates, or vertebrates are analyzed, every organism has the ability to sense and respond to reduced O₂ availability (hypoxia). As with other physiologic stimuli, a hypoxic stimulus/response can be categorized as either acute or chronic (Table 1). Acute responses occur over a time scale of seconds to minutes and involve the posttranslational modification of proteins or other macromolecules through reduction–oxidation reactions, phosphorylation–dephosphorylation, etc. Chronic responses occur over a time scale of minutes to hours and involve changes in gene expression.

In mammals, O₂ sensing was originally attributed solely to specialized chemoreceptor cells in the carotid and neuroepithelial bodies that sense O₂ concentrations in the bloodstream and airways, respectively, and rapidly depolarize as a result of hypoxia-induced K⁺ channel inhibition, resulting in the activation of neural circuits that lead to alterations in cardiovascular and respiratory output, respectively. It is now appreciated that all mammalian cells can sense changes in O₂ concentration. A universal response to reduced O₂ availability involves the expression of hypoxia-inducible factor 1 (HIF-1), a transcriptional activator of genes encoding erythropoietin (EPO), which stimulates red blood cell production, and vascular endothelial growth factor, which stimulates angiogenesis, both serving to increase O₂ delivery; and glucose transporters and glycolytic enzymes, which facilitate ATP production in the absence of O₂ (reviewed by Semenza, 1998). This modulation of target gene transcriptional activity is achieved via O₂-dependent regulation of the HIF-1 α subunit at multiple levels, including mRNA expression, nuclear localization, transactivation domain function, and protein stability via modulation of ubiquitination/proteasomal degradation.

Medical Perspective

Not only does hypoxia represent a fundamental physiologic response in all organisms, but hypoxia is also critical to the pathogenesis of major causes of mortality, including myocardial ischemia, stroke, cancer, and chronic lung disease. Although these issues are beyond the scope of the present discussion, genetic and pharmacologic strategies designed to amplify adaptive responses to hypoxia in ischemic tissues and to inhibit these responses in cancer cells hold great promise as novel and effective therapies for these fatal disorders.

Biochemical Perspective

Despite progress in identifying cellular and systemic responses to hypoxia, the precise mechanism(s) by which O₂ concentrations are sensed by multicellular organisms remains undetermined. Indeed it is unknown

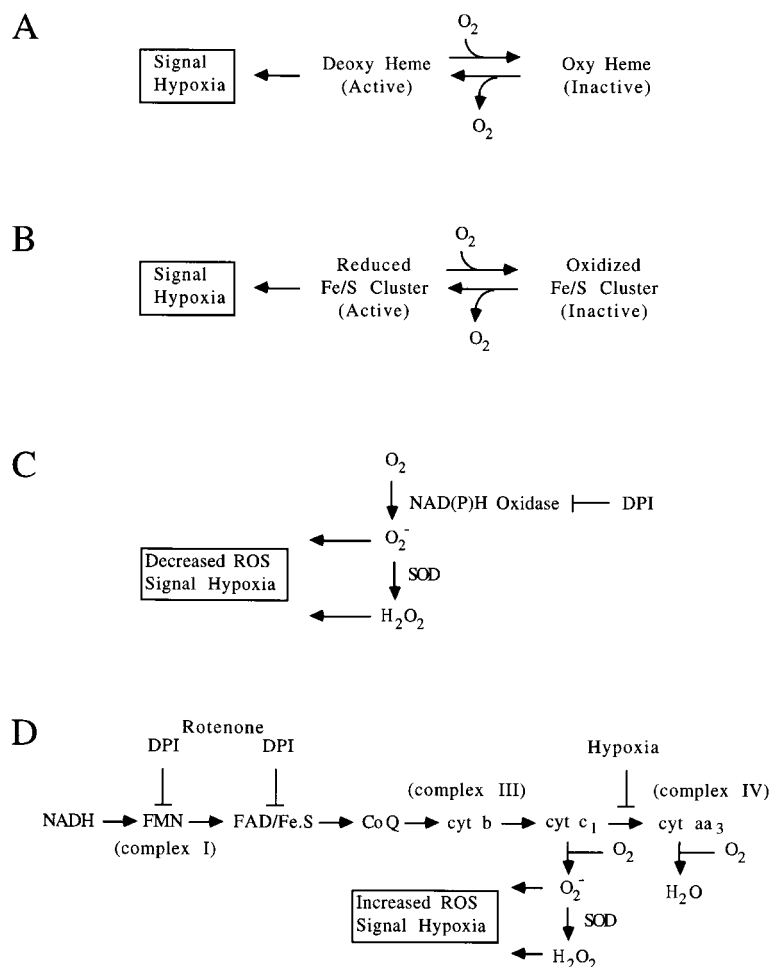
whether similar or different O₂ sensing mechanisms are utilized when one compares acute and chronic responses or responses in depolarizable and nondepolarizable cells. Progress in this field has been slow because of the limitations inherent to the use of pharmacologic tools to dissect complex pathways of fundamental physiologic importance, and genetic approaches to the problem have been lacking.

A priori, one could postulate a variety of mechanisms by which cells might measure O₂ concentration. In the simplest model, the sensor binds O₂ directly, such that as O₂ concentration declines, the fraction of sensor molecules containing bound ligand declines (Figure 1A). In the bacterium *Rhizobium meliloti*, a two-component signaling system consists of FixL, a hemoprotein kinase that is active in the deoxy state, and FixJ, a transcription factor that is active when phosphorylated by FixL (Gong et al., 1998 and references therein). A hemoprotein O₂ sensor was also proposed for mammalian cells (Goldberg et al., 1988). There has been no experimental support for a ligand model, but it remains possible that mammalian O₂ sensing involves molecular interactions with one or more hemoproteins (see below). Besides heme, iron-sulfur clusters represent another intracellular target for O₂ (Figure 1B). In the presence of O₂, iron regulatory protein 1 is degraded via iron-sulfur cluster formation (Iwai et al., 1998). Although preliminary studies suggested that HIF-1 contained non-heme iron, the results could not be reproduced (Salceda and Caro, 1998). HIF-1 α is a basic-helix-loop-helix-PAS protein and the PAS domain is extensively utilized for O₂ and redox sensing in *Archaea*, *Bacteria* (e.g., FixL), and *Eucarya* (reviewed by Taylor and Zhulin, 1999), suggesting that HIF-1 activity may be directly affected by changes in O₂ concentration.

Alternatively, a metabolite of O₂ may be sensed. An NAD(P)H oxidase could convert O₂ to superoxide anion (O₂^{•−}), which is converted to hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD) (Figure 1C). As O₂ concentration declines, this model predicts that the production of ROS (O₂^{•−} and H₂O₂) declines, thus providing a redox signal for hypoxia (Fandrey et al., 1994; Ehleben et al., 1998; and references therein). Diphenylene iodonium (DPI), an inhibitor of NAD(P)H oxidases (and other flavoproteins) stimulates depolarization of carotid and neuroepithelial bodies, as predicted by the model. However,

Table 1. Examples of Acute and Chronic Responses to Hypoxia

Acute	Chronic
Increased cardiac output	Myocardial hypertrophy
Systemic arterial vasodilation	Neovascularization
Pulmonary arterial vasoconstriction	Pulmonary vascular remodeling
Decreased K ⁺ channel activity	Decreased K ⁺ channel mRNA/protein
Increased glycolytic enzyme activity	Increased glycolytic enzyme mRNA/protein

Figure 1. Models of O_2 Sensing

(A) The activity of a hemoprotein sensor is determined by the presence or absence of bound O_2 .

(B) The activity of a sensor protein containing an iron/sulfur-cluster is determined by its oxidation state.

(C) ROS are generated by a membrane-bound NAD(P)H oxidase in direct proportion to the O_2 concentration, and decreased cytosolic ROS generation signals hypoxia.

(D) Hypoxia inhibits the activity of cytochrome oxidase (cyt aa₃) leading to increased mitochondrial ROS generation. DPI is an inhibitor of flavoproteins, including NAD(P)H oxidases and ETC complex I, whereas rotenone is a specific inhibitor of the latter. In each of these models, O_2 or ROS could either directly interact with effector proteins (such as a K^+ channel or HIF-1) or instead activate a signal transduction pathway (such as a kinase cascade) leading to effector activation.

DPI inhibits induction of HIF-1 and downstream genes in response to hypoxia (Gleadle et al., 1995), an effect opposite to that predicted. Thus, the data are most consistent with involvement of an NAD(P)H oxidase in hypoxia-induced depolarization of chemoreceptor cells, but confirmatory genetic data are needed.

An alternative model proposes that mitochondrial electron transport chain (ETC) complex IV (cytochrome c oxidase) plays an important role in O_2 sensing. Data supporting this model have been obtained for both yeast and human cells (Wilson et al., 1994; Chandel et al., 1998; Kwast et al., 1999). Under hypoxic conditions the reduction of O_2 to H_2O by cytochrome oxidase is inhibited, resulting in release of electrons upstream at complex III and generation of O_2^- (Figure 1D). Inhibition of complex I (by DPI or rotenone) decreases O_2^- formation and inhibits induction of HIF-1 and downstream genes in response to hypoxia, as predicted by the model. In ρ^0 cells, which lack mitochondrial DNA and ETC activity, hypoxia does not induce ROS production or the expression of HIF-1 or downstream genes.

One might assume that measurement of ROS levels as a function of O_2 concentration would be sufficient to distinguish between these latter two models. Unfortunately, direct measurements of ROS are technically demanding and experiments utilizing different probes that become fluorescent upon oxidation by ROS (dihydrorhodamine 123, dichlorofluorescein diacetate) have yielded

data supporting one or the other model (Chandel et al., 1998; Ehleben et al., 1998). One interpretation is that different probes are measuring different oxidants which are generated in response to hypoxia and that these probes (and the catchword "ROS") lack sufficient precision to be useful in resolving this debate. Since conflicting results have been obtained using the same cell line, the differences are unlikely to reflect cell type specificity, although this is not meant to exclude the possibility of different O_2 -sensing mechanisms in different cell types or even within a single cell.

Genetic Perspective

Further progress in this field would be greatly facilitated by genetic approaches utilizing somatic cells and/or model organisms. This is the first major contribution of the recent study by Wingrove and O'Farrell (1999) that utilized *Drosophila melanogaster* as a model organism to investigate molecular mechanisms of O_2 homeostasis. The second major contribution is their demonstration of a role for cGMP-dependent protein kinase (PKG) and/or nitric oxide synthase (NOS), using genetic and pharmacologic approaches in a remarkable spectrum of behavioral, cellular, developmental, and survival responses to hypoxia in this organism. Their data indicate that both acute and chronic responses to hypoxia are impaired in flies carrying a mutation in the gene encoding PKG or in flies exposed to the NOS inhibitor L-NAME, and these responses are amplified in transgenic flies

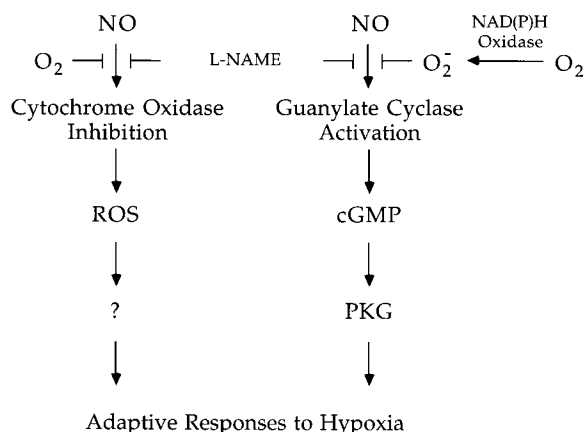


Figure 2. A Model for Hypoxia Signal Transduction Pathways in *Drosophila*

Although both pathways are NOS dependent, one is PKG independent (left) whereas the other is PKG dependent (right). Both pathways can be inhibited by either L-NAME or O_2^- . The two models are similar to those proposed for mammalian cells (Figure 1): the PKG-independent and -dependent pathways involve cytochrome oxidase and NAD(P)H oxidase, respectively.

that overexpress NOS. These results will stimulate interest in the use of model organisms to genetically dissect hypoxia signal transduction pathways and will also focus attention on the role of NO in mammalian O_2 sensing.

Can any connections be made between available data from *Drosophila* and mammalian cells? Analysis of isolated rat liver mitochondria (Giulivi, 1998) and cultured endothelial cells (Clementi et al., 1999) revealed that as O_2 concentration was reduced, the rate of O_2 consumption declined, whereas such an effect was not observed in the presence of a NOS inhibitor, suggesting that constitutive low-level mitochondrial production of NO may result in competitive inhibition of cytochrome oxidase at low O_2 concentrations. This is intriguing given the discovery of a novel mitochondrial NOS (mtNOS) isoform in rat liver (Giulivi, 1998 and references therein). These results have obvious implications for the mitochondrial model of O_2 sensing (Figure 1D). NO also binds to and activates the hemoprotein guanylate cyclase, which produces cGMP, the allosteric activator of PKG. In mammalian vascular smooth muscle cells, O_2^- produced by NADH oxidase inhibits activation of guanylate cyclase by NO (Gupte et al., 1999). The PKG-dependent and -independent effects of NO on O_2 homeostasis in *Drosophila* (Wingrove and O'Farrell, 1999) may therefore result from NO binding to guanylate cyclase and cytochrome oxidase, respectively (Figure 2). This model provides an interesting parallel to the two mammalian models (Figures 1C and 1D).

Inhibitors of NOS, guanylate cyclase, and PKG have been shown to decrease EPO expression in hypoxic human cells (Ohigashi et al., 1993), but the effects were modest. However, the NO/CO/cGMP pathway intersects with the hypoxia signal transduction pathway at multiple levels in mammalian cells, which may confound such analyses. Physiologic levels of NO and CO inhibit HIF-1 DNA binding activity (Liu et al., 1998; Sogawa et al., 1998) and pharmacologic levels also inhibit induction of

HIF-1 α protein expression and transactivation function (Huang et al., 1999) possibly via inhibition of ETC complex I. Finally, HIF-1 activates expression of genes encoding inducible NOS and heme oxygenase 1 resulting in the production of NO and CO, respectively (reviewed by Semenza, 1998). The further characterization and cloning of mammalian mtNOS and analysis of knockout mouse ES cells lacking expression of this or other NOS isoforms (or other components of the guanylate cyclase/PKG axis) may provide additional insights into mechanisms of O_2 sensing.

Whereas flies express only a single NOS isoform, mammals express three, and possibly four, different isoforms, which illustrates a potential limitation of utilizing invertebrates to understand mammalian biology. This difference in relative complexity is also observed with respect to development and physiology. Whereas *Drosophila* develop tracheal tubes that allow direct diffusion of O_2 to all cells, the greatly increased size of mammals necessitated development of complex circulatory and respiratory systems for O_2 delivery. It is likely that hypoxia signal transduction in mammals will also be more complex than in *Drosophila*. Just as we now appreciate that O_2 sensing is not restricted to specialized chemoreceptor cell types, the paradigm of a monolithic O_2 sensor responsible for initiating biological responses to hypoxia may represent an oversimplification. Given the fundamental importance of O_2 homeostasis for survival, multiple hypoxia signal-transduction and modulatory cross-talk pathways may eventually be identified. For now, understanding at least one of these pathways remains a formidable challenge.

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