HIGHLIGHTED TOPIC | Oxygen Sensing in Health and Disease

O₂-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1

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Semenza, Gregg L. O₂-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1. J Appl Physiol 96: 1173–1177, 2004; 10.1152/japplphysiol.00770.2003.—The cardiovascular and respiratory systems play key roles in O₂ homeostasis. Physiological responses to hypoxia involve changes in gene expression that are mediated by the transcriptional activator hypoxia-inducible factor (HIF)-1. Analysis of mice heterozygous for a knockout allele at the locus encoding the O₂-regulated HIF-1α or HIF-2α subunit has revealed that these proteins are required for multiple physiological responses to chronic hypoxia, including erythropoiesis and pulmonary vascular remodeling. In mice with partial HIF-2α deficiency, hypoxia-induced expression of endothelin-1 and norepinephrine is dramatically impaired, and the mice fail to develop pulmonary hypertension after 4 wk of exposure to 10% O₂. In mice with partial HIF-1α deficiency, the ability of the carotid body to sense and/or respond to acute or chronic hypoxia is lost. In wild-type mice, brief episodes of intermittent hypoxia are sufficient to induce production of erythropoietin (EPO), which protects the heart against apoptosis after ischemia-reperfusion, whereas in mice with partial HIF-1α deficiency, intermittent hypoxia does not induce EPO production or cardiac protection. Parenteral administration of EPO to rodents is sufficient to induce dramatic protection against ischemia-reperfusion injury in the heart. Thus HIF-1 mediates critical physiological responses to hypoxia, and the elucidation of these homeostatic mechanisms may lead to novel therapies for the most common causes of mortality in the US population.

hypoxia; pulmonary hypertension; erythropoietin; hypoxia-inducible factor

The function of the cardiorespiratory system is to deliver O₂ sufficient for each cell of the body to maintain normal metabolism. When O₂ delivery is impaired, the resulting hypoxia activates homeostatic mechanisms in the cardiovascular and respiratory systems. When hypoxia persists for prolonged periods of time (i.e., more than a few minutes), the response involves changes in gene expression. Several transcription factors have been shown to mediate physiological responses to hypoxia. Early growth response-1 and nuclear factor interleukin-6 appear to play important roles in mediating inflammatory and thrombotic responses to hypoxia in the lung (47–49). The activation of these transcription factors in response to hypoxia occurs only in restricted cell types, and the molecular mechanisms are not well established. In contrast, hypoxia-inducible factor (HIF)-1 functions as a global regulator of O₂ homeostasis. HIF-1 activity is induced by hypoxia in all nucleated cell types via a novel posttranslational mechanism and plays critical roles in the responses of the cardiovascular and respiratory systems to hypoxia.

The purification of HIF-1 revealed that it was a heterodimeric protein composed of HIF-1α and HIF-1β subunits (44). Nucleotide sequence analysis (43) revealed that both proteins contained basic helix-loop-helix-PAS domains [which were first identified in the proteins PER, aryl hydrocarbon nuclear translocator (ARNT), and SIM] and that HIF-1β was identical to ARNT, which was originally identified as a dimerization partner of the aryl hydrocarbon receptor. Subsequently, database searches led to the identification of a protein with sequence similarity to HIF-1α that was designated HIF-2α (14, 16, 17, 41). HIF-2α was shown to dimerize with HIF-1β and bind to the same DNA sequence as heterodimers containing HIF-1α and HIF-1β. Several other basic helix-loop-helix-PAS proteins have been identified that can dimerize with members of the ARNT family [HIF-1β (ARNT), ARNT2, and BMAL1 (ARNT3)], such as the neuronal PAS domain proteins NPAS1 and NPAS2 (52). However, these proteins do not mediate hypoxia-inducible gene expression.

The molecular basis for hypoxia-induced expression of HIF-1 is the O₂-dependent hydroxylation of proline residues in HIF-1α or HIF-2α, which promotes binding of the von Hippel-Lindau tumor suppressor protein, the recognition component of an E3 ubiquitin-protein ligase that targets the proteins for proteasomal degradation (15, 19, 21, 51). In addition, the O₂-dependent hydroxylation of an asparagine residue in HIF-1α and HIF-2α blocks the binding of the coactivator proteins CREB binding protein and p300 (24). Thus the expression and activity of HIF-1α and HIF-2α are O₂ regulated (Fig. 1).
HIF-1α and HIF-1β are expressed in most cell types, whereas HIF-2α shows a more restricted pattern of expression that includes the developing lung, vasculature, and catecholamine-producing cells. Homozygous targeted inactivation of the mouse Hif1α gene encoding HIF-1α resulted in embryonic lethality at midgestation (9, 20, 23, 32). In contrast, the effect of homozygous targeted inactivation of the gene encoding HIF-2α was less severe and more variable in its effects on the developing cardiovascular and respiratory systems (11, 29, 40). Whereas the analysis of homozygous knockout mice demonstrated the critical roles of HIF-1α and HIF-2α in development, analysis of heterozygous knockout mice has demonstrated critical roles for these factors in postnatal physiology.

**PULMONARY AND SYSTEMIC RESPONSES TO CHRONIC HYPOXIA**

The first study in which this approach was utilized was the analysis of Hif1α+/− and wild-type littersmates exposed to 10% O2 for 1–6 wk. In response to chronic hypoxia, the development of polycythemia was significantly impaired in Hif1α+/− mice compared with wild-type littersmates (50). This effect was consistent with the original identification of HIF-1 as an activator of the EPO gene, which encodes erythropoietin (EPO), the peptide hormone that regulates red blood cell production (36). The development of hypoxia-induced pulmonary hypertension, as manifested by the presence of right ventricular hypertrophy and increased right ventricular pressure, was also impaired in Hif1α+/− mice (50). In Hif1α+/− mice exposed to 10% O2 for 3 wk, morphometric analysis of the small pulmonary arterioles that are subject to hypoxia-induced remodeling revealed a reduced number of completely muscularized vessels and reduced medial wall thickness in these vessels (50).

The luminal diameter of pulmonary arterioles is reduced by depolarization of the vascular smooth muscle cells, resulting in vessel constriction, and by hypertrophy of these same cells. To analyze these physiological responses, pulmonary arteriolar smooth muscle cells (PASMCs) were isolated from mice exposed to room air (21% O2) or 10% O2 for 3 wk. The hypoxia-induced depolarization of membrane potential that was demonstrated in PASMCs from wild-type mice was significantly blunted in cells from hypoxic Hif1α+/− mice (37). The hypoxia-induced depolarization of PASMCs from wild-type mice exposed to chronic hypoxia is due to a reduction in the activity of voltage-gated K+ channels. In contrast, PASMCs from hypoxic Hif1α+/− mice showed virtually no reduction in voltage-gated K+ channel activity (37). Analysis of capacitance, an electrophysiological measure of cell volume, demonstrated that the hypertrophy of PASMCs from hypoxic wild-type mice was also lost in PASMCs from Hif1α+/− mice (37). Thus partial HIF-1α deficiency had significant effects on critical physiological responses of the pulmonary vasculature to chronic hypoxia.

The same experimental paradigm was subsequently applied to the analysis of mice heterozygous for a knockout allele at the locus encoding HIF-2α. In this line, homozygous null mice died soon after birth of respiratory distress syndrome due to deficient surfactant production (11). The heterozygous null mice developed normally, and when they were exposed to 10% O2 for 4 wk, these mice did not develop right ventricular hypertension or hypertrophy and showed no evidence of pulmonary vascular remodeling (5). Expression of endothelin-1 (ET-1) is induced in the lungs of mice exposed to hypoxia (25). ET-1 promotes constriction and remodeling of pulmonary arterioles, and inhibitors of the endothelin type A (ETΑ) receptor block the development of hypoxic pulmonary hypertension (10, 12, 42). Et1 gene expression was induced in the lungs of wild-type mice exposed to hypoxia for 6 days but not in the lungs of heterozygous null mice. These results are consistent with the presence of an HIF-1 binding site in the Et1 gene promoter (18) and suggest that this gene is specifically activated by heterodimers containing HIF-2α.

In humans, high-altitude hypoxia induces increased plasma levels of norepinephrine (7), a potent vasoconstrictor. Complete HIF-2α deficiency resulted in deficient fetal production of catecholamines such as norepinephrine (40). Heterozygotes had normal plasma norepinephrine levels under normoxic conditions, but 4 wk of exposure to 10% O2 increased plasma...
norepinephrine levels only 3.5-fold in heterozygotes compared with 12-fold in wild-type mice (5). These results are consistent with HIF-1 regulation of the gene encoding tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis (33).

Whereas partial HIF-2α deficiency resulted in a more dramatic impairment of hypoxia-induced pulmonary vascular remodeling than did partial HIF-1α deficiency, only the latter was associated with impaired development of hypoxia-induced polycythemia (5, 50). Finally, partial HIF-1α deficiency was associated with increased weight loss in response to chronic hypoxia (50), whereas weight loss was decreased in mice with partial HIF-2α deficiency relative to wild-type littermates (5). These data indicate that HIF-1α and HIF-2α play distinct, critical roles in mediating physiological responses to chronic hypoxia. One interesting possibility is that HIF-2α plays a critical role in pulmonary arterial endothelial cells (e.g., in stimulating ET-1 production), whereas HIF-1α plays an important role in PASMCs (Fig. 2).

**CAROTID BODY-MEDIATED HYPOXIC VENTILATORY RESPONSES**

An essential adaptation to acute and chronic hypoxia is an increase in ventilation that depends on the activity of peripheral chemoreceptors, particularly those within the carotid body, which detect changes in arterial O2 concentration and relay sensory information to the brain stem neurons that regulate minute ventilation in response to acute hypoxia (reduction in arterial O2 concentration and relays sensory information to the brain stem neurons that regulate breathing (30). Hif1α+/− mice and wild-type littermates showed similar increases in respiratory rate, tidal volume, and minute ventilation in response to acute hypoxia (reduction in fraction of inspired O2 from 1.00 to 0.12) or hypercarbia (5% CO2) (22). Chronic hypoxia induces ventilatory adaptation as manifested by an augmented response to subsequent acute hypoxia, an effect that is mediated by the carotid body (30). Wild-type mice showed a significantly increased ventilatory response to 12% O2 after, compared with before, exposure to chronic hypobaric hypoxia (0.4 atm for 3 days). In contrast, Hif1α−/− mice showed a significantly decreased acute hypoxic ventilatory response after, compared with before, exposure to chronic hypoxia (22). The Dejours test, which uses the magnitude of the transitory ventilatory decline in response to a brief hyperoxic exposure as an index of peripheral chemoreceptor, especially carotid body, sensitivity, was performed. This response was markedly blunted in Hif1α−/− mice.

These results provided evidence for impaired carotid body function. Because chemoreceptors innervated by the vagus nerve contribute to hypoxic ventilatory responses, especially under conditions of impaired carotid body function, ventilatory responses were monitored in wild-type and Hif1α−/− littermates exposed to hypoxia before and after bilateral vagotomy. Whereas hypoxia-induced increases in respiratory rate and integrated phrenic nerve activity were not impaired by vagotomy in wild-type mice, these responses were dramatically reduced in Hif1α−/− mice, indicating that these mice depend on chemoreceptors other than the carotid body for O2 sensing (22).

Remarkably, isolated carotid bodies from Hif1α−/− mice superfused with 12% O2 showed little increase in carotid sinus nerve activity. In contrast, carotid bodies of both genotypes showed a dramatic increase in neural activity when exposed to sodium cyanide, demonstrating that partial HIF-1α deficiency resulted in a dramatic and specific loss of O2 sensing in the carotid body (22). Immunohistochemical analysis of carotid body sections demonstrated normal expression of chromogranin A and tyrosine hydroxylase, two markers of the glomus cells that mediate O2 sensing. Morphometric analysis of carotid bodies from wild-type and Hif1α−/− littermates revealed no difference in the number of glomus cells per section, mean glomus cell volume, glomeric volume, total volume per body, or the ratio of glomeric volume to total volume (22).

These data indicate that partial HIF-1α deficiency specifically impairs the ability of the carotid body to sense and/or respond to hypoxia. Because HIF-2α is also expressed in the carotid body (40), these results suggest that HIF-1α plays unique roles in the carotid body. Presumably, target genes that are transactivated only by heterodimers containing HIF-1α encode proteins that play critical roles in carotid body O2 sensing. In contrast, the functioning of noncarotid peripheral chemoreceptors is less impaired by partial HIF-1α deficiency, indicating differences in the genetic mechanisms underlying the physiological functioning of these different chemoreceptors. Carotid sinus nerve transection initially abolishes acute hypoxic ventilatory responses but subsequently leads to a central reorganization of the chemoreflex pathway and recovery of the response (3, 26, 31). In Hif1α−/− mice, a functional denervation of the carotid sinus nerves may lead to similar physiological compensation.

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**Fig. 2. Involvement of HIF-1α and HIF-2α in physiological responses of pulmonary arterioles to chronic hypoxia.** Hypoxia-induced production of endothelin 1 (ET-1) within the pulmonary arteriolar endothelial cell (PAEC) is dependent on the presence of normal levels of HIF-2α. ET-1 is secreted by PAEC, binds to endothelin type A receptors located on the pulmonary arteriolar smooth muscle cell (PASMC), and stimulates membrane depolarization and hypertrophy. Hypoxia also directly stimulates membrane depolarization and hypertrophy of PASMCs, and these effects may require the presence of normal levels of HIF-1α. [Ca²⁺]i, intracellular Ca²⁺ concentration; Kc channel, voltage-gated K+ channel.
SURVIVAL OF ISCHEMIC CELLS MEDIATED BY EPO

In the heart, survival of tissue subjected to prolonged lethal ischemia can be increased by prior exposure to repeated brief periods of sublethal ischemia induced by transient coronary artery occlusion and reperfusion, a phenomenon known as preconditioning (28). After the preconditioning stimulus, there is an early window of acute protection that lasts 1–2 h followed by a late window of delayed protection that lasts ~12–72 h (45). Systemic exposure of animals to chronic hypoxia also induces protection against myocardial ischemia (1, 39). More recently, short episodes of intermittent or continuous hypoxia have been shown to induce delayed cardiac protection (6, 46).

Wild-type mice were exposed to five cycles of 6% O2 for 6 min and reoxygenation for 6 min and then allowed to recover for 24 h. The hearts were isolated for Langendorff perfusion and subjected to 30 min of global ischemia followed by 2 h of reperfusion. The hearts of mice subjected to intermittent hypoxia showed a dramatically increased recovery of left ventricular developed pressure and a dramatic reduction in infarct size after ischemia-reperfusion compared with controls (6). In contrast, prior exposure of Hif1a+/− mice to intermittent hypoxia had no protective effect on the heart. Immediately after the 1 h of intermittent hypoxia, EPO mRNA expression in the kidneys of wild-type mice was induced more than sixfold, resulting in a greater than ninefold increase in plasma EPO levels 1 h later. In contrast, no significant increase in EPO mRNA expression was detected in the kidneys of Hif1a+/− mice immediately after intermittent hypoxia (6). Intrapleural administration of a single dose of recombinant human EPO (rhEPO) 24 h after ischemia-reperfusion was sufficient to induce increased functional recovery, as measured by left ventricular developed pressure, and decreased apoptosis, as measured by TdT-mediated dUTP nick end labeling and activated caspase-3 assays (6). Daily rhEPO administration for 7 days was also shown to result in functional improvement and decreased myocyte loss after in situ coronary artery occlusion and reperfusion (8).

Exposure of rats to continuous hypoxia (3 h at 8% O2) or administration of a chemical inducer of HIF-1 activity (cobalt chloride or desferrioxamine) 24 h before cerebral hypoxia-ischemia also dramatically reduced infarct size (2). The protective effect may be mediated via HIF-1-induced EPO expression, because administration of rhEPO also protects against ischemic brain injury in rodents (4, 38). However, whereas cardiac protection appears to involve the endocrine production of EPO in the kidney, cerebral protection may involve a paracrine mechanism, in which hypoxic astrocytes produce EPO, which binds to EPO receptors on neurons (27).

FUTURE RESEARCH DIRECTIONS

Like a blind man touching an elephant, the studies summarized in this review represent only small pieces of the large and rapidly growing body of data regarding the role of HIF-1 in human physiology and pathophysiology. Other important areas of investigation, such as the role of HIF-1 in angiogenesis and cancer, have been reviewed recently elsewhere (34, 35). The many critical roles of HIF-1 generate excitement that the factor may represent a therapeutic target in several common disease states, but these data also indicate that it may be difficult to achieve desired therapeutic efficacy without incurring unwanted side effects. In this regard, the demonstration that partial deficiency of HIF-1α or HIF-2α does not result in disease but does protect against hypoxia-induced pulmonary hypertension suggests that inhibitors of HIF-1 activity may be of therapeutic utility in patients with chronic lung disease. However, such inhibitors are likely to target both HIF-1α and HIF-2α, and the consequences of combined inhibition are unknown.

The administration of EPO to patients presenting with acute heart attack or stroke may provide a means of reducing infarct size, mortality, and morbidity. Prophylactic use of EPO is less likely to be useful, because the development of polycythemia resulting from chronic administration would itself increase the risk of heart attack and stroke. If encouraging preliminary data (13) are confirmed in larger clinical trials, EPO therapy could have a tremendous impact on public health, given the fact that heart attack and stroke combined represent the most common cause of death in the US population. The studies described in this review illustrate how the combined application of molecular and physiological methods represents a powerful means to elucidate fundamental homeostatic mechanisms that can then be exploited therapeutically.

REFERENCES


