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Forum Review

HIF-1–Dependent Respiratory, Cardiovascular, and Redox Responses to Chronic Intermittent Hypoxia

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ABSTRACT

Sleep-disordered breathing with recurrent apnea is a major cause of morbidity and mortality. Affected individuals have increased risk of systemic hypertension. Sleep apnea results in chronic intermittent hypoxia (CIH). Exposure of rodents to CIH is sufficient to induce hypertension by activation of the carotid body and sympathetic nervous system, leading to increased levels of circulating catecholamines. CIH induces increased levels of reactive oxygen species (ROS), and antioxidant treatment blocks CIH-induced hypertension. The transcriptional activator hypoxia-inducible factor 1 (HIF-1) plays an essential role in O_2 homeostasis. HIF-1 activity is induced when mice or cultured cells are subjected to CIH, an effect that is blocked by antioxidants. The carotid bodies from mice that are heterozygous for a null (knockout) allele at the locus encoding HIF-1α appear histologically normal but do not respond to continuous hypoxia or CIH. In contrast to wild-type littermates, when heterozygous-null mice are subjected to CIH, they do not develop hypertension or increased levels of HIF-1, catecholamines, or ROS. The data suggest the existence of a feed-forward mechanism in which CIH-induced ROS activate HIF-1, which then promotes persistent oxidative stress, which may further amplify HIF-1 activation, with its consequent effects on gene expression. *Antioxid. Redox Signal.* 9, 1391–1396.

SLEEP APNEA RESULTS IN CHRONIC INTERMITTENT HYPOXIA AND CARDIOVASCULAR DISEASE

Sleep-disordered breathing with recurrent apnea is a major cause of morbidity and mortality in the United States population, affecting an estimated 18 million people (36). In this condition, transient repetitive episodes of apnea (cessation of breathing) result in periodic hypoxemia (decreased Po_2 in arterial blood). In severely affected patients, the frequency of apnea may exceed 60 episodes per hour, and O_2 saturation of blood hemoglobin can be reduced to as low as 50%. Patients with sleep apnea have a greatly increased risk for the development of systemic hypertension and its sequelae (28, 49).

Sleep apnea results in both chronic intermittent hypoxia (CIH) and chronic intermittent hypercapnia. An important advance in the field of sleep apnea research was the demonstration that exposure of rats to CIH was sufficient to induce systemic hypertension (9). Studies in humans and rodents suggested that the carotid body, which is located at the bifurcation of the common carotid artery and is the primary chemoreceptor for detecting changes in arterial Po_2, mediates reflex increases in the activity of the sympathetic nervous system that result in elevated blood pressure (31). Rats in which the carotid bodies were surgically denervated or in which the sympathetic nervous system was inhibited by administration of 6-hydroxydopamine showed no increase in blood pressure in response to CIH (10, 11).

Plasma catecholamine (epinephrine and norepinephrine) levels and mean blood pressure are significantly elevated in rats and mice after 10 days of CIH consisting of alternating cycles of hypoxia (5% O_2 for 15 s) and normoxia (21% O_2 for 5 min),

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with nine episodes per hour for 8 h per day (23, 32). These results are consistent with the finding that patients with sleep apnea and hypertension have elevated urinary catecholamine levels before but not after tracheostomy (12). CIH exerts two major effects on the chemoreceptor reflex pathway: (a) augmentation of the carotid body and sympathetic effector responses to acute hypoxia, and (b) long-lasting activation of both the carotid body and sympathetic effector responses that persists for several hours after the termination of CIH (30). These results from rodent models of CIH are consistent with the persistent increase in sympathetic nervous system activity that is exhibited by patients with sleep apnea (4, 16, 42). In addition to its cardiovascular effects, CIH also induces changes in ventilatory adaptation (2, 30, 31, 33, 35).

OXIDANTS MEDIATE THE PATHOPHYSIOLOGIC EFFECTS OF CHRONIC INTERMITTENT HYPOXIA

In rats and mice, the effects of CIH on catecholamine production, blood pressure elevation, long-term facilitation of respiratory motor activity, and sensory long-term facilitation in the carotid body can all be blocked by concurrent treatment of the animals with the superoxide scavenger MnTMPyP, indicating that the generation of reactive oxygen species (ROS) plays a critical role in mediating the pathophysiologic effects of CIH on the cardiovascular and respiratory systems (23, 30, 32, 33).

Rat PC12 cells share many properties with glomus cells of the carotid body, including O_2-regulated neurotransmitter release and expression of tyrosine hydroxylase, the rate-limiting enzyme for catecholamine production (6, 7, 22). Exposure of PC12 cells to CIH consisting of alternating cycles of hypoxia (1.5% O_2 for 15 s) and reoxygenation (20% O_2 for 4 min) for 120 cycles induced increased expression of mRNAs encoding c-Fos and tyrosine hydroxylase, an effect that was blocked by treating the cells with MnTMPyP (51).

HYPOXIA-INDUCIBLE FACTOR 1 MEDIATES CELLULAR AND SYSTEMIC RESPONSES TO CONTINUOUS HYPOXIA

The transcriptional activator hypoxia-inducible factor 1 (HIF-1) plays an essential role in O_2 sensing by the carotid body (21, 32). HIF-1 is a global regulator of oxygen homeostasis that controls multiple key developmental and physiologic processes including angiogenesis and erythropoiesis (17). Hundreds of HIF-1–regulated genes have been identified by microarray assays of gene expression (8, 26). More than 50 of these genes have been identified as direct targets of HIF-1–mediated transactivation. HIF-1 has been shown to bind directly to *cis*-acting hypoxia-response elements in these genes, which include those encoding erythropoietin (EPO) and vascular endothelial growth factor (VEGF), and to activate their transcription (13, 38).

HIF-1 is a heterodimeric protein that is composed of a constitutively expressed HIF-1β subunit and an O_2-regulated HIF-1α subunit (44, 45). HIF-1 activity is induced under conditions of continuous hypoxia as a result of a decreased rate of O_2-dependent proline hydroxylation, ubiquitination, and proteasomal degradation of the HIF-1α subunit (3, 27, 47). HIF-1α transcriptional activity is also regulated *via* O_2-dependent asparagine hydroxylation that blocks coactivator recruitment (29). HIF-2α is the protein product of a distinct genetic locus, which also is regulated by O_2-dependent hydroxylation and heterodimerizes with HIF-1β to activate a set of target genes that overlaps with those activated by HIF-1α: HIF-1β heterodimers (8).

Mice that are homozygous for a knockout allele at the locus encoding HIF-1α (*Hif1a*$^{-/-}$) die at midgestation with cardiac and vascular malformations (5, 19, 37) and impaired erythropoiesis (48). *Hif1a*$^{+/-}$ mice that are heterozygous for the knockout allele develop normally but have impaired responses when subjected to long-term continuous hypoxia by exposure to 10% O_2 for 3 weeks, including reduced hypoxia-induced pulmonary vascular remodeling (39, 40, 46, 50). Carotid bodies from *Hif1a*$^{+/-}$ mice are markedly impaired in the ability to sense or respond to hypoxia or both (21). Carotid body histology is normal, including the presence of glomus cells, which perform the O_2-sensing function of the carotid body, and *Hif1a*$^{+/-}$ carotid bodies respond normally to cyanide, indicating a specific defect in O_2 sensing (21).

In contrast to the effects of partial HIF-1α loss of function in mice, humans with Chuvash polycythemia have HIF-1α gain of function as a result of homozygosity for a missense mutation that decreases the binding of VHL to hydroxylated HIF-

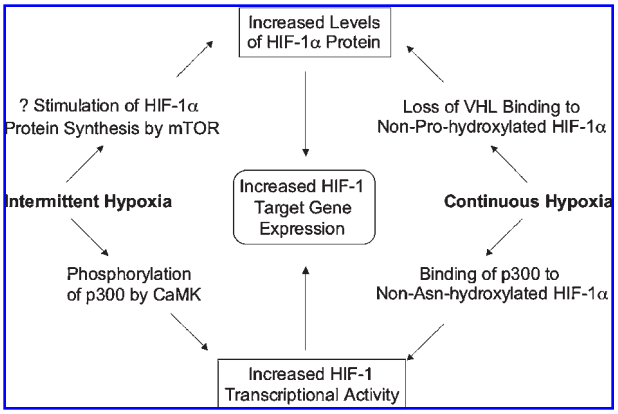

FIG. 1. Activation of HIF-1 in response to continuous and intermittent hypoxia. In continuous hypoxia, inhibition of hydroxylation of proline (Pro) residue 402 or 564 or both results in decreased ubiquitination and degradation of HIF-1α mediated by binding of the von Hippel–Lindau tumor-suppressor protein (VHL). Hydroxylation of asparagine (Asn) residue 803 is also inhibited under continuous hypoxia, resulting in increased binding of the coactivators p300 and CBP, leading to transcriptional activation. Under intermittent hypoxia, Ca^{2+}-dependent activation of calcium-calmodulin protein kinase (CaMK) stimulates HIF-1 transcriptional activity by phosphorylation of p300. HIF-1α protein levels also are induced by intermittent hypoxia. The molecular mechanisms underlying the increase in HIF-1α levels have not been determined but may involve increased translation of HIF-1α mRNA resulting from activation of a signal-transduction pathway involving the mammalian target of rapamycin (mTOR).

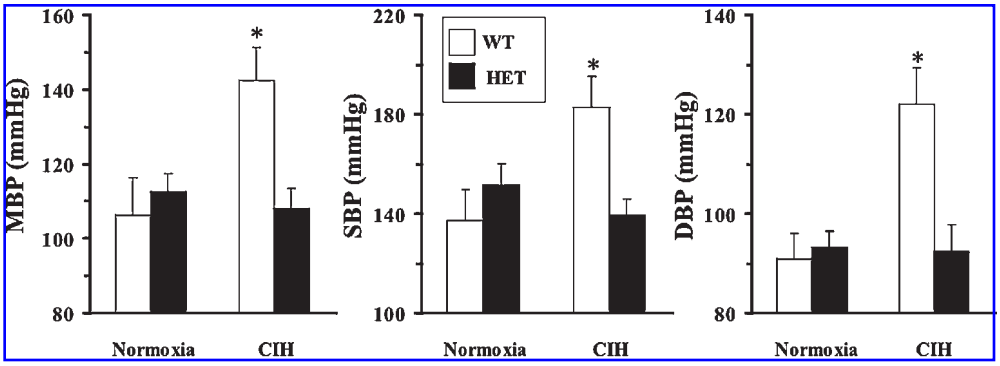

FIG. 2. Prolonged intermittent hypoxia induces systemic hypertension in wild-type adult mice but not in *Hif1a*$^{+/-}$ littermates. Mean (MBP), systolic (SBP), and diastolic (DBP) blood pressures were determined before and after exposure of eight wild-type and *Hif1a*$^{+/-}$ littermates to 10 days of CIH. (Data from ref. 32.)

1α (1). Analysis of three affected individuals revealed abnormalities in both ventilatory control and pulmonary vascular regulation (41). Basal ventilation and pulmonary vascular tone were elevated, and ventilatory, pulmonary vasoconstrictive, and heart-rate responses to acute hypoxia were greatly increased. Taken together, the mouse loss-of-function laboratory experiment and the human gain-of-function "experiment of nature" provide compelling evidence that HIF-1 plays a major role in coordinating ventilatory and cardiovascular responses to continuous hypoxia at the level of gene transcription.

HIF-1 ACTIVITY IS INDUCED BY INTERMITTENT HYPOXIA

When PC12 cells were exposed to CIH (1.5% O_2 for 30 s followed by 20% O_2 for 4 min), HIF-1α protein expression and HIF-1 transcriptional activity increased in a dose-dependent manner as the duration of IH was increased from 10 to 30 to 60 cycles (52). Ca^{2+}/calmodulin-dependent (CaM) kinase activity was increased fivefold in cells subjected to CIH. The induction of HIF-1 transcriptional activity in response to CIH was blocked by the intracellular Ca^{2+} chelator BAPTA-AM or by the CaM kinase inhibitor KN93. Whereas exposure of PC12 cells to either CIH or continuous hypoxia induced expression of tyrosine hydroxylase mRNA, only CIH-induced expression was blocked KN93, demonstrating that HIF-1 activity is induced by different mechanisms in cells exposed to continuous hypoxia, as opposed to CIH. Increased CaM kinase activity in PC12 cells leads to the phosphorylation of the coactivator p300, which appears to increase its interaction with HIF-1α under nonhypoxic conditions, when asparagine hydroxylation of HIF-1α would otherwise prevent their interaction (52).

Induction of HIF-1α protein expression by CIH was not blocked by KN93, indicating that the effects of CIH on HIF-1 were mediated by at least two different signal-transduction pathways (52). The increased HIF-1α protein levels in response to CIH may be mediated *via* the signal-transduction pathway that includes mammalian target of rapamycin (mTOR), as has been demonstrated for growth factor–induced HIF-1α protein expression (14, 24, 43). In support of this hypothesis, exposure of PC12 cells to 1% O_2 for 3 h was shown to increase intracellular Ca^{2+} and stimulate the activities of protein kinase C-α and mTOR, which led to increased HIF-1α protein synthesis (18). Further studies are required to determine whether this pathway is activated in response to CIH. The mechanisms regulating HIF-1α protein expression and transcriptional activity in response to chronic and intermittent hypoxia are summarized in Fig. 1. As described later, ROS are required for induction of HIF-1α in response to intermittent hypoxia *in vivo*, but the mechanisms by which this occurs have not been delineated.

PATHOPHYSIOLOGIC RESPONSES TO CHRONIC INTERMITTENT HYPOXIA ARE IMPAIRED IN MICE WITH PARTIAL HIF-1α DEFICIENCY

To investigate the physiologic significance of CIH-induced HIF-1 transcriptional activity in intact animals, wild-type and

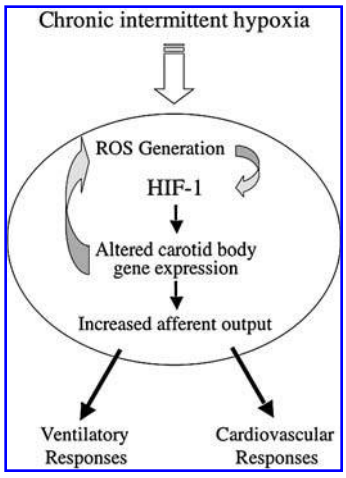

FIG. 3. ROS– and HIF-1–dependent effects of CIH on cardiovascular and ventilatory homeostasis. (Adapted from ref. 32.)

heterozygous *Hif1a*$^{+/-}$ littermate male mice were exposed to either CIH (15 s of hypoxia followed by 5 min of normoxia × nine episodes/h × 8 h/day × 10 day) or to normoxia (controls). Wild-type mice exposed to CIH exhibited augmented hypoxic ventilatory response; long-term facilitation (LTF) of breathing; enhanced carotid body response to graded hypoxia and sensory LTF; increased diastolic, systolic, and mean arterial blood pressures (Fig. 2); and elevated plasma norepinephrine levels. In striking contrast, in *Hif1a*$^{+/-}$ mice exposed to CIH, carotid body responses to hypoxia were absent, and all measured cardiorespiratory responses were either absent or markedly attenuated (32).

Immunoblot analysis of cerebral cortical tissue lysates prepared from normoxic wild-type and heterozygous *Hif1a*$^{+/-}$ littermate mice revealed that the heterozygotes manifested a partial deficiency in the expression of HIF-1α protein, as expected. Analysis of samples that were prepared from mice exposed to CIH revealed a marked increase in HIF-1α protein expression in wild-type mice (52). In contrast, *Hif1a*$^{+/-}$ littermate mice showed no increase in HIF-1α protein levels in response to CIH. Thus, the virtually complete absence of ventilatory and cardiovascular responses to CIH in *Hif1a*$^{+/-}$ mice could be attributed to the failure to induce HIF-1α protein expression in these mice.

In addition to the development of systemic hypertension, in individuals with intermittent hypoxia due to sleep apnea, a metabolic syndrome develops, consisting of insulin resistance, hypercholesterolemia, and hypertriglyceridemia. These responses are also observed in wild-type mice exposed to CIH for 5 days, whereas in *Hif1a*$^{+/-}$ mice, the development of hypertriglyceridemia was impaired and was associated with impaired activation of sterol response element–binding protein 1, a key activator of triglyceride synthesis (25).

IMPAIRED OXIDATIVE STRESS RESPONSE TO CHRONIC INTERMITTENT HYPOXIA IN *Hif1a*$^{+/-}$ MICE

CIH increases ROS and administration of antioxidants prevents systemic and cellular responses to CIH (33). To assess whether HIF-1 contributes to CIH-induced oxidative stress, the levels of thiobarbituric acid reactive substances (TBARSs) were determined as a measure of oxidized proteins (34). TBARS assays were performed in the same cerebral cortical tissue samples used for the analysis of HIF-1α described earlier. Basal levels of TBARS in tissue from normoxic wild-type and *Hif1a*$^{+/-}$ mice were comparable. TBARS were significantly elevated in response to CIH in wild-type mice. In contrast, the levels of TBARS were not increased in *Hif1a*$^{+/-}$ mice after CIH.

To confirm that the increased levels of TBARS reflected increased protein oxidation, wild-type mice were treated with the antioxidant MnTMPyP, which is a potent scavenger of superoxide. Antioxidant treatment blocked the increase in TBARS induced by CIH in wild-type mice, whereas it had no effect on basal TBARS. Thus, CIH induces oxidative stress in wild-type mice but not in *Hif1a*$^{+/-}$ mice (32). MnTMPyP, which blocks responses to CIH in cell culture and whole-animal models (33), completely prevented CIH-induced HIF-1α upregulation, whereas it had no effect on basal HIF-1α or HIF-1β expression.

Taken together, these results indicate that HIF-1 plays a critical role in mediating cardiorespiratory responses to CIH and that CIH-induced oxidative stress involves complex positive interactions between HIF-1 and oxidants (Fig. 3). This feed-forward mechanism provides a potential explanation for the failure to induce HIF-1α expression in CIH-exposed *Hif1a*$^{+/-}$ mice. CIH may initially trigger oxidative stress *via* mitochondrial ROS generation (33). The increased ROS in turn upregulates HIF-1α, as has been described in other experimental contexts (15, 20). Once HIF-1 is activated, it may promote persistent oxidative stress by stimulating oxidant production or by inhibiting antioxidant production. This mechanism may amplify the induction of HIF-1α and ROS in wild-type mice while amplifying the deficiency of CIH-induced HIF-1α and ROS in *Hif1a*$^{+/-}$ mice. Further analysis of *Hif1a*$^{+/-}$ mice may provide additional novel insights into the molecular mechanisms underlying ROS generation and cardiorespiratory responses to CIH.

ABBREVIATIONS

Asn, asparagine; CaMK, calcium-calmodulin protein kinase; CIH, chronic intermittent hypoxia; DBP, diastolic blood pressure; EPO, erythropoietin; HIF-1, hypoxia-inducible factor 1; LTF, long-term facilitation; MBP, mean blood pressure; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; Po_2, partial pressure of oxygen; Pro, proline; ROS, reactive oxygen species; SBP; systolic blood pressure; TBARS, thiobarbituric acid–reactive substances; VEGF, vascular endothelial growth factor; VHL, von Hippel–Lindau tumor-suppressor protein.

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