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Neural activation of molecular circuitry in intermittent hypoxia

Jayasri Nanduri, Ying-Jie Peng, Ning Wang and Nanduri R Prabhakar

People living at sea level experience intermittent hypoxia (IH) as a consequence of sleep apnea, which is a highly prevalent respiratory disorder. Sleep apnea patients and rodents exposed to IH exhibit autonomic dysfunction manifested as increased sympathetic nerve activity and hypertension. This article highlights physiologic basis of autonomic disturbances by IH, which involves abnormal activation of the carotid body (CB) chemo reflex by reactive oxygen species (ROS).We further evaluate major molecular mechanisms underlying IH-induced ROS generation including transcriptional activation of genes encoding pro-oxidant enzymes by hypoxia-inducible factor (HIF)-1 and transcriptional repression of anti-oxidant enzyme genes by DNA methylation. Lastly, evidence is presented for CB neural activity as a major regulator of HIF-1 activation and DNA methylation by IH in the chemo reflex pathway.

Address
Institute for Integrative Physiology and Center for Systems Biology for O 2 Sensing, Department of Medicine, University of Chicago, Chicago, IL, USA

Corresponding author: Prabhakar, Nanduri R (nanduri@uchicago.edu)

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Introduction

The circulatory and respiratory systems adapt to sustained hypoxia occurring at high altitude sojourns. Molecular basis of cardio-respiratory adaptations to altitude hypoxia has been well studied [1•,2–4]. People living at sea level often experience intermittent hypoxia (IH) as a consequence of sleep apnea (SA), which is a highly prevalent respiratory disorder. IH leads to mal-adaptations of autonomic functions manifested as heightened sympathetic nerve activity and hypertension [5–7]. Recent development of experimental models provided important insights into the molecular mechanisms underlying mal-adaptations to IH. This article presents the emerging role of hypoxia-inducible factors (HIFs) and epigenetic regulation of gene expression by DNA methylation in mal-adaptations of cardio-respiratory functions by IH.

Physiological basis of sympathetic activation and hypertension by IH

Arterial blood O_2 levels are continuously monitored by the carotid bodies (CB) [8]. Hypoxia increases the CB sensory nerve activity and the sensory information is transmitted to brainstem neurons in the nucleus tractus solitarii (nTS), and rostral ventrolateral medulla (RVLM) and then translated to sympathetic neurons in the spinal cord. Thus, the CB chemo reflex is a major regulator of sympathetic tone under hypoxia. Sleep apnea patients and rodents exposed to IH exhibit augmented CB chemo reflex [9–11]. IH produces two major effects on the CB: first, enhanced response to acute hypoxia; and second, progressive increase in baseline sensory activity following repetitive hypoxia, a phenomenon termed as sensory long-term facilitation (sLTF) [11–13]. IH also facilitates processing of the sensory information from the CB in the nTS, and RVLM by altering glutamatergic neurotransmission [14,15]. Adrenal medullary chromaffin cells (AMC), which are innervated by the sympathetic nervous system, are the major source of catecholamines. Hypoxia by activating sympathetic nerves release catecholamines from AMC, and this effect is markedly potentiated in IH exposed rodents [16]. Chronic ablation of CB prevents sympathetic activation by IH [17,18,19••]. Treating rats with a sympatholytic agent [17] or by ablating sympathetic innervation to the adrenal medulla [19••] prevent IH-evoked hypertension. These findings demonstrate that the CB chemo reflex is a major driver of IH-evoked sympathetic excitation and the ensuing hypertension.

Cellular mechanism(s) underlying chemo reflex activation by IH

Reactive oxygen species (ROS)-signaling is a major cellular mechanism mediating the augmented CB chemo reflex by IH. ROS generation is increased in all three major components of the chemo reflex pathway including the CB, nTS, RVLM and the adrenal medulla in IH treated rodents [19••]. IH evoked CB hypersensitivity is due to ROS-dependent inactivation of heme oxygenase-2 and reduced CO production resulting in increased hydrogen sulfide (H_2S) generation in the chemoreceptor tissue [20••]. The augmented catecholamine secretion from AMC of IH treated rodents involve ROS-dependent activation of Ca^{2+} channels and the ensuing Ca^{2+} influx [21] as well as protein kinase-C-dependent increase in readily releasable pool of secretory vesicles [22]. ROS scavengers prevent IH-induced hyperactive CB chemo reflex and the increased sympathetic nerve activity [11,16,19••,23].

Increased ROS generation by IH involves activation of pro-oxidant enzymes and reduced anti-oxidant enzymes activities. As short as few cycles of IH activate xanthine oxidase (XO), a pro-oxidant enzyme in rat pheochromocytoma (PC)12 cells, and this effect is mediated by increased proteolytic processing of XO [24,25••]. ROS generated by XO in turn, activates NADPH oxidase (Nox) 2 by facilitating the translocation of p47phox and p67phox subunits to the plasma membrane and their interaction with membrane bound gp91phox subunit [24]. ROS generated by Nox leads to further increase in ROS generation by inhibiting the Complex I of the mitochondrial electron transport chain through increased Ca^{2+} influx in to the mitochondria and S-glutathionylation of 75-kDa and 50-kDa subunits of the Complex I [26]. ROS generated by XO and Nox return to baseline within couple of hours after terminating IH, whereas ROS generated by the Complex I inhibition recovers 24 hours after terminating IH [24]. Thus, IH leads to progressive recruitment of pro-oxidant mechanisms resulting in ROS-induced ROS generation (positive feed-forward mechanism) (Figure 1). In addition, anti-oxidant enzyme activities of superoxide dismutase-2 (Sod2) and catalase are reduced by IH [27].

Molecular mechanisms of redox regulation by IH

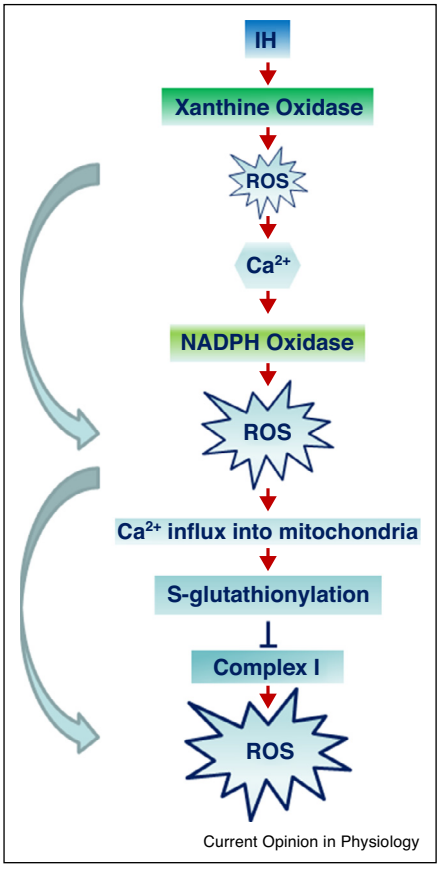
Transcriptional regulation of genes encoding pro-oxidant and anti-oxidant enzymes by hypoxia-inducible factors (HIFs)

Genes encoding pro-oxidant enzymes are upregulated and anti-oxidant enzyme genes are down regulated in the CB, nTS, RVLM and adrenal medulla of IH exposed rodents [19••]. Emerging evidence suggests that HIF-1 and HIF-2, the major members of the HIF family of transcriptional activators play a major role in regulation of genes encoding pro-oxidant and anti-oxidant enzymes by IH [28]. IH *increases* HIF-1α and *decreases* HIF-2α, the O_2-regulated alpha subunits of the HIF complex [13,19••,27]. The IH evoked increase in HIF-1α protein is due to increased protein synthesis by Ca^{2+}-dependent activation of mammalian target of rapamycin (mTOR) and decreased proline hydroxylation [25••,29]. HIF-2α degradation by IH is mediated by Ca^{2+}-dependent protease, calpain [27].

Complete HIF-1α deficiency results in embryonic lethality at mid-gestation, whereas *Hif1a*$^{+/-}$ heterozygous (het) mice, which are partially deficient in HIF-1α, develop normally and are indistinguishable from wild-type (WT) littermates [30,31]. IH treated *Hif1a*$^{+/-}$ het mice exhibit remarkable absence of increased ROS generation, CB hyperactivity, sympathetic excitation and hypertension [13]. IH increases *Nox2* mRNA abundance and this response was abolished by blocking HIF-1 activity either with RNA interference or by pharmacologic inhibition of HIF-1α protein by digoxin or YC-1 in cell cultures [29]. IH-evoked increase in *Nox2* mRNA was absent in *Hif1a*$^{+/-}$ mice [29]. These findings demonstrate that HIF-1 mediates transcriptional upregulation of Nox2 by IH.

HIF-2α, which also known as endothelial PAS domain protein-1 (EPAS-1) shares ~50% amino acid sequence identity with HIF-1α and interacts with HIF-1β, the constitutively expressed subunit of the HIF complex [32,33]. In contrast to *Hif1a*$^{+/-}$ mice, *Hif-2α*$^{+/-}$ het mice exhibit elevated ROS levels, heightened CB response to hypoxia, elevated plasma catecholamines, higher incidence of apnea and hypertension under basal conditions, similar to responses seen in IH treated wild type mice [34]. Cell culture studies showed that HIF-2α protein degradation by IH contributes to decreased transcription of genes encoding anti-oxidant enzymes [27]. Systemic administration of ALLM, a calpain inhibitor, which prevents HIF-2α degradation, restores Sod2 enzyme activity and protein and prevents elevated ROS levels in the chemo reflex pathway of rats exposed to IH [27].

Figure 1



Progressive recruitment of pro-oxidant mechanisms by IH results in reactive oxygen species (ROS)-induced ROS generation (positive feed-forward) in the carotid body chemo reflex pathway.

Neural regulation of HIFs during CIH

Prolonged hypoxia is an established physiological activator of HIFs [35]. However, the duration of hypoxia occurring during each episode of IH (simulating sleep apnea) is brief (15 s) and the magnitude is modest. A recent study showed that arterial pO_2 decreases from 100 to 80 mmHg (range between 15–20 mmHg) during each episode of IH, in unsedated rats [19••]. Because CB receives highest blood flow and is exquisitly sensitive to changes in arterial blood O_2, it is likely that changes in HIF-α isoforms are due to direct effects of IH on glomus tissue. On the other hand, basal pO_2 levels of most tissues including the brain and peripheral organs range between 30 and 60 mmHg [36], which are much below the level of hypoxia seen during each episode of IH. Thus, it is unlikely that altered HIF-α isoform expressions in neurons of nTS and RVLM and adrenal medulla are due to direct effects of IH. Neural activity is a potent regulator of gene expression in the nervous system [37–39]. A recent study showed that CB ablated rats exhibit remarakable absence of IH-evoked increase in HIF-1α protein and Nox2 activity and decreased HIF-2α protein and Sod2 activity in the nTS, RVLM [19••]. Likewise, either CB ablation or chronic sectioning of sympathetic nerves eliminated IH-evoked changes in HIFα isoform proteins, pro-oxidant and anti-oxidant enzymes in the adrenal medulla [19••].

Sympathetic nerves innervating AMCs release acetyl choline (ACh), which binds to muscarinic and/or nicotinic ACh receptors. Treating rats with atropine, a muscarinic receptor blocker, during IH treatment blocked increased HIF-1α and decreased HIF-2α proteins, upregulation of Nox and reduced Sod-2 activities as well as increased ROS production. Further analysis revealed that stimulating muscarinic receptors elevate $[Ca^{2+}]_i$, which by activating mTOR increases HIF-1α protein and decreases HIF-2α protein through activation of Ca^{2+}-dependent protease, calpain [19••]. The mechanisms underlying CB neural activity-dependent changes in HIF-α proteins in the nTS and RVLM neurons by IH remain to be investigated. Nonetheless, these findings demonstrate that regulation of HIFα isoforms by CB neural activity mediate transcriptional changes of pro-oxidant and anti-oxidant enzyme genes in the central and efferent components of the CB chemo reflex (Figure 2).

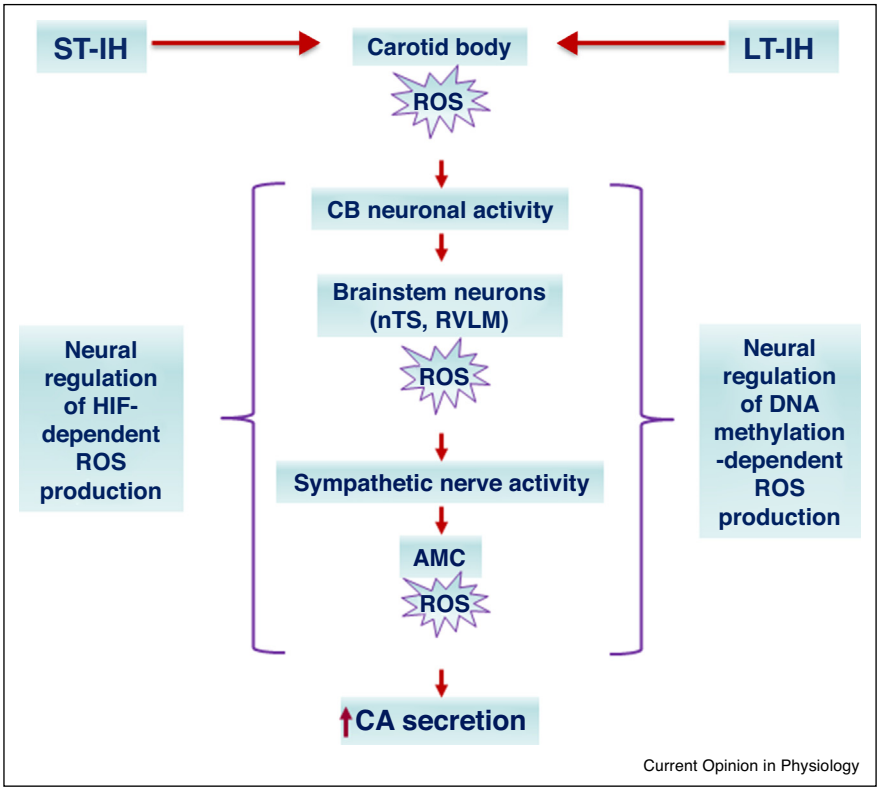
Prolonged CIH activates DNA methylation

The reversal of redox-dependent CB-reflex activation depends on the duration of IH exposure. The effects produced by ten day exposure to IH are completely reversed during recovery in room air, whereas the effects produced by long-term (30 days)-exposure to IH (LT-IH) persist despite recovery in room air for 30-days [40••]. What molecular mechanisms mediate the persistent effects of long-term IH? Emerging evidence suggests that epigenetic regulation of gene expression leads to long-lasting changes in physiological functions. Epigenetic changes are heritable modifications of DNA including DNA methylation and histone modifications [41,42]. DNA methylation occurs predominantly on cytosine bases that are part of a 5′-CpG-3′ dinucleotide [43]. DNA hypermethylation repress gene transcription, whereas hypomethylation activates transcription [44].

Rats exposed to LT-IH showed increased DNA methylation and repression of genes encoding several anti-oxidant enzymes including superoxide dismutase 1 and 2 (*Sod1*, *Sod2*), catalase (*Cat*), thioredoxin reductase 2 (*Txnrd2*), peroxiredoxin 4 (*Prdx4*), and glutathione peroxidase 2 (*Gpx2*) in the CB, nTS, RVLM and adrenal medulla [40••]. Bisulphite sequencing of the promoter region of the *Sod2* gene showed methylation of a single CpG dinucleotide at +157 bp (relative to the transcription site), out of the 25 CpG sites analyzed [40••]. LT-IH evoked DNA methylation was tissue-selective and cell-selective and was not seen in brainstem regions that do not participate in the CB reflex [40••]. Brainstem regions that showed absence of DNA methylation showed unaltered anti-oxidant enzyme gene expression and ROS levels in LT-IH exposed rats [40••]. On the other hand, ten day exposure to IH had no effect on DNA methylation of anti-oxidant enzyme genes [40••], suggesting that prolonged exposure to IH is necessary to trigger DNA methylation.

DNA methyl transferases (Dnmts) catalyze DNA cytosine methylation. Several Dnmts have been identified, including Dnmt1, Dnmt2, Dnmt3a, and Dnmt3b [45]. Dnmt1 is responsible for the maintenance of pre-existing DNA methylation, whereas Dnmt3a and Dnmt3b are *de novo* methyltransferases [46]. LT-IH increased the aundances of Dnmt1 and Dnmt3b proteins and elevated Dnmt enzyme activity. The increased Dnmt protein expression was due to post-translational rather than transcriptional activation [40••]. Systemic administration of decitabine, an inhibitor of DNA methylation, either during exposure to LT-IH or during recovery from LT-IH, prevented DNA methylation; normalized anti-oxidant enzyme gene expression, ROS levels, CB chemo reflex, and blood pressure [40••]. These findings suggest that DNA methylation-dependent repression anti-oxidant enzyme genes and the resulting increase in ROS levels contribute to persistent CB chemo reflex activation by LT-IH. CB ablation blocked LT-IH-induced DNA methylation in the nTS, RVLM, and adrenal medulla and normalized the expressions of genes encoding anti-oxidant enzymes as well as ROS levels [47••]. These observations demonstrate that CB neural activity mediates LT-IH-induced DNA methylation in the central (nTS and RVLM) and efferent (adrenal medulla) components of the chemo reflex (Figure 2).

Figure 2



Neural regulation of molecular mechanisms in the carotid body (CB) chemo reflex pathway under short-term (ST; 10 days) and log-term (LT; 30 days) exposure to intermittent hypoxia (IH). ST-IH increases HIF-1α and decreases HIF-2α proteins leading increased transcription of genes encoding pro-oxidant enzymes and decreased transcription of genes encoding anti-oxidant enzymes (AOE) in the CB chemo reflex pathway, respectively. The effects of ST-IH on the CB are likely to be direct, whereas in nucleus tractus solitarri (nTS) and rostral ventrolateral medulla (RVLM) and adrenal medullary chromaffin cells (AMC) are indirect and require CB neural activity and ensuing increase in sympathetic nerve activity, respectively. LT-IH triggers long-lasting suprresion of genes encoding AOE and persistent elevation of ROS. The effects of LT-IH on nTS, RVLM and AMC require CB neural activity and the ensuing increase in sympathetic nerve activity, respectively. CA, catecholamines.

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Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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