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Regulation of Carotid Body Oxygen Sensing by Hypoxia-Inducible Factors

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Abstract

Oxygen (O₂) sensing by the carotid body and its chemosensory reflex is critical for homeostatic regulation of breathing and blood pressure. Carotid body responses to hypoxia are not uniform but instead exhibit remarkable inter-individual variations. The molecular mechanisms underlying variations in carotid body O₂ sensing are not known. Hypoxia-inducible factor-1 (HIF-1) and HIF-2 mediate transcriptional responses to hypoxia. This article reviews the emerging evidence that proper expression of the HIF- α isoforms is a key molecular determinant for carotid body O₂ sensing. HIF-1 α deficiency leads to a blunted carotid body hypoxic response, which is due to increased abundance of HIF-2 α , elevated anti-oxidant enzyme activity, and a reduced intracellular redox state. Conversely, HIF-2 α deficiency results in augmented carotid body sensitivity to hypoxia, which is due to increased abundance of HIF-1 α , elevated pro-oxidant enzyme activity, and an oxidized intracellular redox state. Double heterozygous mice with equally reduced HIF-1 α and HIF-2 α showed no abnormality in redox state or carotid body O₂ sensing. Thus, mutual antagonism between HIF- α isoforms determines the redox state and thereby establishes the set point for hypoxic sensing by the carotid body.

Keywords

sleep apnea; NADPH oxidase 2; superoxide dismutase 2; hypertension; sympathetic nerve activity

Carotid bodies are sensory organs for detecting reduced arterial blood O₂ levels and the ensuing chemosensory reflex maintains cardio-respiratory homeostasis during hypoxemia [10]. However, the carotid body response to hypoxia is not uniform, but instead exhibits remarkable variation among different strains of rodents [20]. Consequently, the chemosensory reflex exhibits inter-individual variation, which has important physiological consequences [35]. Diminished hypoxic sensing results in poor adaptation to low O₂ environments and high-altitude pulmonary edema, whereas heightened carotid body sensitivity to hypoxia leads to increased sympathetic nerve activation and hypertension [20].

Despite the physiological significance, molecular mechanisms underlying the inherent variation in carotid body O₂ sensing are not known.

The hypoxia-inducible factor (HIF) family of transcriptional activators is critical for maintaining homeostasis under hypoxic conditions [31]. HIF-1 and HIF-2, which are the two best studied members of the HIF family, are heterodimers comprised of an O₂-regulated HIF-1 α or HIF-2 α subunit and a constitutively expressed HIF-1 β subunit. While HIF-1 α is expressed in all cells of all metazoan species, HIF-2 α is only expressed in certain cell types of vertebrate species [23]. In this article, we will summarize the emerging evidence demonstrating that proper expression of the HIF- α isoforms is a key molecular determinant for carotid body O₂ sensing, discuss the underlying cellular mechanisms, and consider the physiological relevance of these findings.

HIF- α isoform expression in the carotid body

The carotid body is comprised of two major cell types, including the Type I or glomus cells, which are of neuronal phenotype, and the glia-like Type II or sustentacular cells. Type I cells are the primary site of O₂ sensing and they work in concert with the nearby afferent nerve ending as a sensory unit [10]. Both Type I and Type II cells express HIF-1 α [27], whereas HIF-2 α expression is restricted to Type I cells [27, 34].

HIF-1 α deficiency impairs carotid body O₂ sensing

Complete deficiency of HIF-1 α leads to embryonic lethality, whereas mice with partial deficiency of HIF-1 α develop normally [6, 9]. The importance of HIF-1 α was assessed in mice with partial deficiency of HIF-1 α (*Hif1a*^{+/-}). The carotid body response to hypoxia was impaired in *Hif1a*^{+/-} mice, whereas chemoreceptor activation evoked either by cyanide or by CO₂ was unaltered [8,16,37]. Analysis of carotid body histology revealed no significant difference between *Hif1a*^{+/-} mice and wild-type littermates with respect to the number of Type I cells, mean Type I cell volume, or the ratio of Type I cell volume to total volume [8]. Digoxin is a potent inhibitor of HIF-1 α protein expression [39]. Wild-type mice treated with digoxin showed selective impairment of the carotid body response to hypoxia, similar to that observed in *Hif1a*^{+/-} mice [37].

The defective carotid body O₂ sensing caused by HIF-1 α deficiency has important consequences for cardio-respiratory homeostasis. Chronic hypoxia induces carotid body-dependent ventilatory adaptation that is manifested by an augmented response to subsequent acute hypoxia [10]. *Hif1a*^{+/-} mice, as well as wild-type littermates treated with digoxin, showed severely impaired ventilatory adaptation to chronic hypoxia and reduced sympathetic activation, which was manifested by low plasma norepinephrine levels [8, 37]. These findings demonstrate that HIF-1 α deficiency resulting either from genetic or pharmacological intervention leads to selective impairment of carotid body O₂ sensing and disrupted cardio-respiratory homeostasis.

HIF-2 α deficiency augments carotid body response to hypoxia

HIF-2 α , also known as endothelial PAS domain protein-1 (EPAS-1), shares 48% amino acid sequence identity with HIF-1 α [33]. Similar to HIF-1 α , continuous hypoxia leads to HIF-2 α accumulation and subsequent dimerization with HIF-1 β . Transcriptional activation by HIF-2 regulates some target genes in common with HIF-1 as well as other genes that are uniquely regulated by HIF-2 [3, 5, 29]. Despite the similarities between HIF-1 α and HIF-2 α , *Hif2a*^{+/-} mice exhibit a remarkable increase in carotid body sensitivity to hypoxia [19,37]. The heightened carotid body response to hypoxia was associated with increased sympathetic nerve activation as evidenced by elevated plasma norepinephrine levels, hypertension and irregular breathing with increased number of spontaneous apneas (transient cessation of breathing) [19, 37]. Similar to *Hif2a*^{+/-} mice, wild type mice treated with 2-methoxyestradiol (2ME2), an inhibitor of HIF-2 α protein expression, increased carotid body sensitivity to hypoxia and cardio-respiratory abnormalities, including increased plasma catecholamines, hypertension and irregular breathing [37]. These observations suggest that despite being an orthologue of HIF-1 α , HIF-2 α deficiency results in the opposite physiological effect of HIF-1 α deficiency, with augmented rather than impaired carotid body responses to hypoxia.

Reciprocal regulation of HIF- α isoforms in *Hif1a*^{+/-} and *Hif2a*^{+/-} mice

Hif1a^{+/-} mice and digoxin-treated wild-type mice showed increased abundance of HIF-2 α protein in Type I cells. The elevated HIF-2 α protein accumulation was due to decreased degradation of HIF-2 α protein by calpain proteases, with no change in HIF-2 α mRNA levels [37]. 2ME2 treatment normalized HIF-2 α protein levels in the carotid bodies of *Hif1a*^{+/-} mice and restored the carotid body hypoxic sensitivity as well as the ventilatory adaptation to chronic hypoxia.

In contrast, *Hif2a*^{+/-} mice and 2ME2-treated wild-type mice showed increased abundance of HIF-1 α protein in Type I cells, which was due to increased protein synthesis involving mammalian target of rapamycin (mTOR) and increased protein stability resulting from inhibition of prolyl hydroxylases [37]. Digoxin treatment normalized HIF-1 α protein levels, carotid body hypoxic sensing, plasma catecholamine levels, blood pressure, and breathing patterns. These findings establish that partial deficiency of HIF-1 α leads to increased HIF-2 α levels and reduced carotid body responses to hypoxia; whereas partial deficiency of HIF-2 α results in increased HIF-1 α levels and augmented carotid body sensitivity to hypoxia.

On the other hand, *Hif1a*^{+/-};*Hif2a*^{+/-} double heterozygous mice, which exhibited equally reduced levels of HIF-1 α and HIF-2 α , showed carotid body responses to hypoxia, blood pressure, plasma catecholamine levels, and ventilatory adaptation to chronic hypoxia that were indistinguishable from wild-type mice [37], suggesting that imbalanced levels of HIF-1 α and HIF-2 α , rather than absolute reductions in HIF- α isoform levels, lead to alterations in carotid body O₂ sensing with profound impact on cardio-respiratory homeostasis.

HIF- α isoforms affect carotid body O₂ sensing through redox regulation

Recent studies demonstrated that HIF-1 and HIF-2 regulate gene products with opposing effects on the cellular redox state. While HIF-1 regulates expression of genes encoding pro-oxidant enzymes, such as NADPH oxidase 2 (Nox2) [2, 36], HIF-2 is a potent activator of genes encoding anti-oxidant enzymes including superoxide dismutase 1 (Sod1), Sod2, and catalase [12, 29].

The blunted hypoxic sensitivity of the carotid bodies in *Hif1a*^{+/-} mice was associated with decreased pro-oxidant capacity as evidenced by low abundance of Nox2 mRNA and enzyme activity, and high abundance of Sod2 mRNA and enzyme activity. As a result, the carotid bodies in *Hif1a*^{+/-} mice exhibited a reduced cellular redox state. Normalizing HIF-2 α levels by 2ME2 administration decreased Sod2 mRNA and enzyme activity and corrected the redox state [37]. In contrast, the augmented carotid body hypoxic sensitivity in *Hif2a*^{+/-} mice was associated with an oxidized redox state, decreased Sod2 mRNA and enzyme activity, and increased Nox2 mRNA and pro-oxidant enzyme activity. Digoxin treatment, which normalized HIF-1 α levels, alleviated oxidative stress by restoring Nox2 enzyme activity, and abrogated the carotid body hypersensitivity to hypoxia [37]. These findings demonstrate that dysregulation of HIF- α isoform expression in the carotid body leads to imbalanced expression of pro-oxidant and anti-oxidant enzymes that alter the cellular redox state and thereby affect response of the carotid body to hypoxia.

Studies from an experimental model of sleep apnea establish the clinical significance of dysregulated HIF- α isoform expression

Sleep-disordered breathing with obstructive sleep apnea (OSA) is a major clinical disorder that is reaching epidemic proportions [13, 32]. In severely affected patients, the frequency of apnea may exceed 60 episodes/h and arterial blood O₂ saturation can be reduced to as low as 50%. Patients with sleep apnea exhibit elevated sympathetic nerve activity, as well as increased plasma and urinary catecholamines, and are prone to develop hypertension. Emerging evidence suggests that a heightened carotid body chemosensory reflex mediates autonomic dysfunction in sleep apnea patients [7, 22].

Chronic intermittent hypoxia (IH) is a hallmark manifestation of sleep apnea. Rodents exposed to chronic IH exhibit selective augmentation of the carotid body response to hypoxia [14-16, 25, 26], and sensory long-term facilitation (LTF) manifested by a long-lasting increase in baseline sensory activity [14, 16]. Carotid bodies from chronic IH-exposed rodents show increased HIF-1 α [11, 16, 36] and decreased HIF-2 α levels [12]. The increased HIF-1 α levels were due to increased HIF-1 α protein synthesis, which was mediated by Ca²⁺- and protein kinase C-dependent activation of mTOR, and decreased HIF-1 α degradation as a result of impaired prolyl hydroxylation [36]. The decreased HIF-2 α levels in the carotid bodies of mice exposed to chronic IH were due to increased degradation of HIF-2 α protein by Ca²⁺-activated calpain proteases [12].

HIF-1 activation by chronic IH was associated with increased abundance of Nox2 mRNA, protein and enzyme activity in the carotid body [17]. The decreased HIF-2 activation was

associated with reduced abundance of Sod2 mRNA, protein, and enzyme activity [12]. ROS levels were elevated in chronic IH-exposed carotid bodies [12, 17]. The following findings suggest that HIF-mediated ROS contribute to altered carotid body function by chronic IH: 1) anti-oxidant treatment prevented chronic-IH-induced carotid body sensitization to hypoxia and sensory LTF [14]; 2) carotid body responses to chronic IH were absent in *Hif1a*^{+/-} mice [16]; and 3) *Hif2a*^{+/-} mice exhibited augmented hypoxic carotid body sensitivity, which was similar to chronic IH-exposed mice and could also be prevented by anti-oxidant treatment [19].

HIF-mediated changes in the carotid body contribute to cardio-respiratory pathology associated with chronic IH. Following each episode of apnea, the increased carotid body sensitivity to hypoxia results in a greater magnitude of hyperventilation, thus driving the respiratory controller below the apneic threshold for CO₂, leading to a greater number of apneas [21]. Thus, the heightened hypoxic sensitivity of the carotid body might act as a “positive feedback,” leading to greater incidence of apneas. Indeed, chronic IH-exposed rats with intact carotid bodies exhibit greater incidence of spontaneous apneas and this effect was absent in carotid body-ablated rats exposed to chronic IH [24]. Patients with OSA exhibit elevated sympathetic nerve activity even during the day-time when apneas are absent [7]. Since chronic IH-induces sensory LTF of the carotid body, it is likely that increased sensory nerve activity by activating the chemo-sensory reflex mediates the elevated sympathetic nerve activity. Supporting this possibility is the finding that following bilateral sectioning of the carotid sinus nerves, chronic IH-exposed rats no longer showed elevated sympathetic nerve activity [24]. *Hif1a*^{+/-} mice, whose carotid bodies are insensitive to hypoxia, exhibit a remarkable absence of hypertension and elevated plasma catecholamines in response to chronic IH. *Hif2a*^{+/-} mice, on the other hand, like chronic IH-exposed mice, exhibit an increased number of apneas, hypertension and elevated plasma catecholamine levels. Taken together, these findings suggest that dysregulation of HIF- α isoform expression contributes to cardio-respiratory pathology in the setting of chronic IH through a heightened chemosensory reflex arising from augmented carotid body sensitivity to hypoxia.

Perspective and future directions

HIFs regulate hundreds of genes, including those encoding ion channels and enzymes associated with neurotransmitter synthesis or degradation [23, 30], which have been implicated in the carotid body response to hypoxia [10]. Dysregulation of HIF- α isoform expression affects the transcription of genes other than those associated with redox regulation remains to be investigated. While ROS resulting from imbalanced expression of HIF- α isoforms mediate the carotid body response to hypoxia, the downstream targets of ROS signaling have not yet been identified. Recent studies implicate carbon monoxide (CO)-sensitive hydrogen sulfide (H₂S) generation as critical mediators of the carotid body response to hypoxia [18, 38]. Whether altered ROS levels result from dysregulated HIF- α isoform expression target CO and H₂S signaling in the carotid body remains to be studied. In addition to sleep apnea, a hyperactive carotid body chemosensory reflex is implicated in autonomic dysfunction associated with congestive heart failure [28] and neurogenic hypertension [1]. The blunted hypoxic sensitivity of the carotid body has adverse physiological consequences, including impaired ventilatory adaptation to high altitude

hypoxia that is manifested by pulmonary edema [4]. Whether imbalanced expression of HIF- α isoforms in the carotid body also contributes to these pathologies remains an interesting possibility. Studies described above utilized digoxin and 2ME-2 to block the HIF-1 α and HIF-2 α protein accumulation, respectively. However, the precise mechanism of action and the specificity of these compounds to HIF- α isoforms have not been determined. Exposure to continuous hypobaric hypoxia results in initial augmentation, followed by desensitization of the carotid body to hypoxia [10]. Whether differential regulation of HIF- α isoforms contribute to the diametrically opposed carotid body responses during the initial and late phase of continuous hypobaric hypoxia remains to be studied.

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References

1. Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, Sobotka PA, Gourine AV, Paton JF. Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. *J Physiol.* 2012; 590:4269–4277. [PubMed: 22687617]
2. Diebold I, Petry A, Djordjevic T, Belaiba RS, Fineman J, Black S, Schreiber C, Fratz S, Hess J, Kietzmann T, Gorch A. Reciprocal regulation of Rac1 and PAK-1 by HIF-1 α : a positive-feedback loop promoting pulmonary vascular remodeling. *Antioxid Redox Signal.* 2010; 13:399–412. [PubMed: 20001745]
3. Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii-Kuriyama Y. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1 α regulates the VEGF expression and is potentially involved in lung and vascular development. *Proc Natl Acad Sci U S A.* 1997; 94:4273–4278. [PubMed: 9113979]
4. Hohenhaus E, Paul A, McCullough RE, Kucherer H, Bartsch P. Ventilatory and pulmonary vascular response to hypoxia and susceptibility to high altitude pulmonary edema. *Eur Respir J.* 1995; 8:1825–1833. [PubMed: 8620946]
5. Holmquist-Mengelbier L, Fredlund E, Lofstedt T, Noguera R, Navarro S, Nilsson H, Pietras A, Vallon-Christersson J, Borg A, Gradin K, Poellinger L, Pahlman S. Recruitment of HIF-1 α and HIF-2 α to common target genes is differentially regulated in neuroblastoma: HIF-2 α promotes an aggressive phenotype. *Cancer Cell.* 2006; 10:413–423. [PubMed: 17097563]
6. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O₂ homeostasis by hypoxia-inducible factor 1 α . *Genes Dev.* 1998; 12:149–162. [PubMed: 9436976]
7. Kara T, Narkiewicz K, Somers VK. Chemoreflexes--physiology and clinical implications. *Acta Physiol Scand.* 2003; 177:377–384. [PubMed: 12609009]
8. Kline DD, Peng YJ, Manalo DJ, Semenza GL, Prabhakar NR. Defective carotid body function and impaired ventilatory responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 α . *Proc Natl Acad Sci U S A.* 2002; 99:821–826. [PubMed: 11792862]
9. Kotch LE, Iyer NV, Laughner E, Semenza GL. Defective vascularization of HIF-1 α -null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev Biol.* 1999; 209:254–267. [PubMed: 10328919]
10. Kumar P, Prabhakar NR. Peripheral chemoreceptors: function and plasticity of the carotid body. *Compr Physiol.* 2012; 2:141–219. [PubMed: 23728973]
11. Lam SY, Tipoe GL, Liong EC, Fung ML. Differential expressions and roles of hypoxia-inducible factor-1 α , -2 α and -3 α in the rat carotid body during chronic and intermittent hypoxia. *Histol Histopathol.* 2008; 23:271–280. [PubMed: 18072084]

12. Nanduri J, Wang N, Yuan G, Khan SA, Souvannakitti D, Peng YJ, Kumar GK, Garcia JA, Prabhakar NR. Intermittent hypoxia degrades HIF-2 α via calpains resulting in oxidative stress: implications for recurrent apnea-induced morbidities. *Proc Natl Acad Sci U S A*. 2009; 106:1199–1204. [PubMed: 19147445]
13. Nieto FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline S, D'Agostino RB, Newman AB, Lebowitz MD, Pickering TG. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. *Sleep Heart Health Study*. *JAMA*. 2000; 283:1829–1836. [PubMed: 10770144]
14. Peng YJ, Overholt JL, Kline D, Kumar GK, Prabhakar NR. Induction of sensory long-term facilitation in the carotid body by intermittent hypoxia: implications for recurrent apneas. *Proc Natl Acad Sci U S A*. 2003; 100:10073–10078. [PubMed: 12907705]
15. Peng YJ, Prabhakar NR. Effect of two paradigms of chronic intermittent hypoxia on carotid body sensory activity. *J Appl Physiol*. 2004; 96:1236–1242. [PubMed: 14660510]
16. Peng YJ, Yuan G, Ramakrishnan D, Sharma SD, Bosch-Marce M, Kumar GK, Semenza GL, Prabhakar NR. Heterozygous HIF-1 α deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol*. 2006; 577:705–716. [PubMed: 16973705]
17. Peng YJ, Nanduri J, Yuan G, Wang N, Deneris E, Pendyala S, Natarajan V, Kumar GK, Prabhakar NR. NADPH oxidase is required for the sensory plasticity of the carotid body by chronic intermittent hypoxia. *J Neurosci*. 2009; 29:4903–4910. [PubMed: 19369559]
18. Peng YJ, Nanduri J, Raghuraman G, Souvannakitti D, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. H₂S mediates O₂ sensing in the carotid body. *Proc Natl Acad Sci U S A*. 2010; 107:10719–10724. [PubMed: 20556885]
19. Peng YJ, Nanduri J, Khan SA, Yuan G, Wang N, Kinsman B, Vaddi DR, Kumar GK, Garcia JA, Semenza GL, Prabhakar NR. Hypoxia-inducible factor 2 α (HIF-2 α) heterozygous-null mice exhibit exaggerated carotid body sensitivity to hypoxia, breathing instability, and hypertension. *Proc Natl Acad Sci U S A*. 2011; 108:3065–3070. [PubMed: 21288809]
20. Peng YJ, Makarenko VV, Nanduri J, Vasavda C, Raghuraman G, Yuan G, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. Inherent variations in CO-H₂S-mediated carotid body O₂ sensing mediate hypertension and pulmonary edema. *Proc Natl Acad Sci U S A*. 2014; 111:1174–1179. [PubMed: 24395806]
21. Prabhakar NR. Oxygen sensing during intermittent hypoxia: cellular and molecular mechanisms. *J Appl Physiol*. 2001; 90:1986–1994. [PubMed: 11299293]
22. Prabhakar NR, Dick TE, Nanduri J, Kumar GK. Systemic, cellular and molecular analysis of chemoreflex-mediated sympathoexcitation by chronic intermittent hypoxia. *Exp Physiol*. 2007; 92:39–44. [PubMed: 17124274]
23. Prabhakar NR, Semenza GL. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. *Physiol Rev*. 2012; 92:967–1003. [PubMed: 22811423]
24. Prabhakar NR. Sensing hypoxia: physiology, genetics and epigenetics. *J Physiol*. 2013; 591:2245–2257. [PubMed: 23459758]
25. Rey S, Del Rio R, Alcayaga J, Iturriaga R. Chronic intermittent hypoxia enhances cat chemosensory and ventilatory responses to hypoxia. *J Physiol*. 2004; 560:577–586. [PubMed: 15319419]
26. Rey S, Del Rio R, Iturriaga R. Contribution of endothelin-1 to the enhanced carotid body chemosensory responses induced by chronic intermittent hypoxia. *Brain Res*. 2006; 1086:152–159. [PubMed: 16595126]
27. Roux JC, Brismar H, Aperia A, Lagercrantz H. Developmental changes in HIF transcription factor in carotid body: relevance for O₂ sensing by chemoreceptors. *Pediatr Res*. 2005; 58:53–57. [PubMed: 15879294]
28. Schultz HD, Li YL. Carotid body function in heart failure. *Respir Physiol Neurobiol*. 2007; 157:171–185. [PubMed: 17374517]
29. Scortegagna M, Ding K, Oktay Y, Gaur A, Thurmond F, Yan LJ, Marck BT, Matsumoto AM, Shelton JM, Richardson JA, Bennett MJ, Garcia JA. Multiple organ pathology, metabolic

- abnormalities and impaired homeostasis of reactive oxygen species in *Epas1*^{-/-} mice. *Nat Genet.* 2003; 35:331–340. [PubMed: 14608355]
30. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell.* 2012; 148:399–408. [PubMed: 22304911]
 31. Semenza GL. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu Rev Pathol.* 2014; 9:47–71. [PubMed: 23937437]
 32. Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Nieto FJ, O'Connor GT, Boland LL, Schwartz JE, Samet JM. Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med.* 2001; 163:19–25. [PubMed: 11208620]
 33. Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev.* 1997; 11:72–82. [PubMed: 9000051]
 34. Tian H, Hammer RE, Matsumoto AM, Russell DW, McKnight SL. The hypoxia-responsive transcription factor EPAS1 is essential for catecholamine homeostasis and protection against heart failure during embryonic development. *Genes Dev.* 1998; 12:3320–3324. [PubMed: 9808618]
 35. Weil JV. Variation in human ventilatory control-genetic influence on the hypoxic ventilatory response. *Respir Physiol Neurobiol.* 2003; 135:239–246. [PubMed: 12809623]
 36. Yuan G, Khan SA, Luo W, Nanduri J, Semenza GL, Prabhakar NR. Hypoxia-inducible factor 1 mediates increased expression of NADPH oxidase-2 in response to intermittent hypoxia. *J Cell Physiol.* 2011; 226:2925–2933. [PubMed: 21302291]
 37. Yuan G, Peng YJ, Reddy VD, Makarenko VV, Nanduri J, Khan SA, Garcia JA, Kumar GK, Semenza GL, Prabhakar NR. Mutual antagonism between hypoxia-inducible factors 1 α and 2 α regulates oxygen sensing and cardio-respiratory homeostasis. *Proc Natl Acad Sci U S A.* 2013; 110:E1788–1796. [PubMed: 23610397]
 38. Yuan G, Vasavda C, Peng YJ, Makarenko VV, Raghuraman G, Nanduri J, Gadalla MM, Semenza GL, Kumar GK, Snyder SH, Prabhakar NR. Protein kinase G-regulated production of H₂S governs oxygen sensing. *Sci Signal.* 2015; 8:ra37. [PubMed: 25900831]
 39. Zhang H, Qian DZ, Tan YS, Lee K, Gao P, Ren YR, Rey S, Hammers H, Chang D, Pili R, Dang CV, Liu JO, Semenza GL. Digoxin and other cardiac glycosides inhibit HIF-1 α synthesis and block tumor growth. *Proc Natl Acad Sci U S A.* 2008; 105:19579–19586. [PubMed: 19020076]

Figure 1.

Chronic intermittent hypoxia associated with obstructive sleep apnea dysregulates HIF- α isoform expression, leading to increased ROS, carotid body activation, increased sympathetic activation, and systemic hypertension. NOX2, NADPH oxidase 2; ROS, reactive oxygen species; SOD2, superoxide dismutase 2 ; PKC, protein kinase C; mTOR, mammalian target of rapamycin; $[Ca^{2+}]_i$, intracellular calcium ion concentration. HIF-1 α and HIF-2 α are mutually antagonistic: HIF-1 α deficiency results in decreased NOX2, decreased ROS, decreased $[Ca^{2+}]_i$, and decreased calpain activity, which leads to increased HIF-2 α levels; HIF-2 α deficiency causes decreased SOD2 activity, increased ROS, increased $[Ca^{2+}]_i$, and increased mTOR activity, which leads to increased HIF-1 α levels.