JCI The Journal of Clinical Investigation

Hypoxia-inducible factors and obstructive sleep apnea

Nanduri R. Prabhakar, … , Ying-Jie Peng, Jayasri Nanduri

J Clin Invest. 2020;130(10):5042-5051. https://doi.org/10.1172/JCI137560.

Review Series

Intermittent hypoxia (IH) is a hallmark manifestation of obstructive sleep apnea (OSA), a widespread disorder of breathing. This Review focuses on the role of hypoxia-inducible factors (HIFs) in hypertension, type 2 diabetes (T2D), and cognitive decline in experimental models of IH patterned after O_2 profiles seen in OSA. IH increases HIF-1α and decreases HIF-2α protein levels. Dysregulated HIFs increase reactive oxygen species (ROS) through HIF-1–dependent activation of pro-oxidant enzyme genes in addition to reduced transcription of antioxidant genes by HIF-2. ROS in turn activate chemoreflex and suppress baroreflex, thereby stimulating the sympathetic nervous system and causing hypertension. We also discuss how increased ROS generation by HIF-1 contributes to IH-induced insulin resistance and T2D as well as disrupted NMDA receptor signaling in the hippocampus, resulting in cognitive decline.

The Journal of Clinical Investigation

Hypoxia-inducible factors and obstructive sleep apnea

Nanduri R. Prabhakar, Ying-Jie Peng, and Jayasri Nanduri

Institute for Integrative Physiology and Center for Systems Biology of Oxygen Sensing, Biological Science Division, University of Chicago, Chicago, Illinois, USA.

Intermittent hypoxia (IH) is a hallmark manifestation of obstructive sleep apnea (OSA), a widespread disorder of breathing. This Review focuses on the role of hypoxia-inducible factors (HIFs) in hypertension, type 2 diabetes (T2D), and cognitive decline in experimental models of IH patterned after O_2 profiles seen in OSA. IH increases HIF-1α and decreases HIF-2α protein levels. Dysregulated HIFs increase reactive oxygen species (ROS) through HIF-1–dependent activation of pro-oxidant enzyme genes in addition to reduced transcription of antioxidant genes by HIF-2. ROS in turn activate chemoreflex and suppress baroreflex, thereby stimulating the sympathetic nervous system and causing hypertension. We also discuss how increased ROS generation by HIF-1 contributes to IH-induced insulin resistance and T2D as well as disrupted NMDA receptor signaling in the hippocampus, resulting in cognitive decline.

Introduction

Obstructive sleep apnea (OSA) is a widespread respiratory disorder affecting 20%–30% of men and 10%–15% of women in the United States (1, 2). It is characterized by brief (tens of seconds) and repeated interruptions of breathing manifested as either complete (apnea) or partial (hypopnea) collapse of the upper airway during sleep. OSA prevalence varies with ethnicity and is higher in African Americans than in Whites of comparable age and body weight (3).

Interruption of breathing by OSA results in intermittent hypoxia (IH), mild hypercapnia, and arousals from sleep. OSA is associated with a number of comorbidities, including hypertension (2, 4–6), type 2 diabetes (T2D) (7–9), and cognitive decline (2, 10, 11). Recently developed rodent and cell culture models of IH patterned after blood O_2 saturation profiles during OSA have provided important insights into the molecular mechanisms underlying comorbidities associated with OSA. Hypoxia-inducible factor-1 (HIF-1) and HIF-2 belong to the HIF family of transcriptional activators. Activation of HIF-1 and HIF-2 mediates physiological adaptations to sustained hypoxia such as that experienced during extended sojourns to high altitudes (12). This Review focuses on emerging evidence implicating dysregulated transcription of HIF-1 and HIF-2 as a molecular mechanism underlying hypertension, T2D, and cognitive dysfunction stemming from OSA-induced IH.

OSA and hypertension

Using the apnea-hypopnea index (AHI; calculated as [{number of apnea events + hypopnea events}/total number of minutes of actual sleep time] × 60) as a measure of OSA severity, a population-based study found a strong correlation between severity of OSA and hypertension (4). According to this report, patients with an AHI of 5–15 events per hour and >15 events per hour are 2 and 3 times more at risk of developing hypertension, respectively. The correlation between the severity of OSA and hypertension was independent of confounding factors including BMI, age, and sex (4), and OSA was identified as a risk factor for resistant hypertension (13). Although arousals from sleep result in transient increases in systemic blood pressure, OSA-associated hypertension was independent of arousals as assessed by the sleep fragmentation index (a calculation that reflects the number of awakenings to stage 1 sleep from deeper stages of sleep relative to total sleep time) (14).

A recent study reported that the prevalence of cardiovascular pathologies, including coronary heart disease, heart failure, and stroke, depends on OSA patient subtypes (15). Based on daytime symptoms, four OSA subtypes were identified in a cohort of 1207 patients with an AHI index of ≥15 events per hour: (a) disturbed sleep, (b) minimally symptomatic, (c) excessively sleepy, and (d) moderately sleepy. Of these subtypes, the excessively sleepy subtype exhibited a greater risk of developing cardiovascular disease (hazard ratios, 1.7–2.4) than other subtypes. Whether the prevalence of hypertension depends on OSA subtype is not known.

Activation of the sympathetic nervous system constricts blood vessels and elevates blood pressure by increasing vascular resistance. Substantial evidence indicates that persistent activation of the sympathetic nervous system is a major contributing factor for OSA-associated hypertension. Several investigators recorded muscle sympathetic nerve activity (SNA), a reflection of systemic vascular resistance, in OSA patients (16–18). Normal subjects without OSA exhibited low levels of muscle SNA during sleep (19–21), while this phenotype was absent in OSA patients (22). OSA patients exhibit elevated SNA during daytime, wherein apneas are absent and arterial blood gases are normal (22). The elevated daytime SNA was independent of obesity, a common comorbidity in these patients (22). Circulating and urinary catecholamines (both norepinephrine and epinephrine), biomarkers of increased SNA, are also elevated in OSA patients (17, 18, 23–25).

Conflict of interest: NRP is a consultant to ANP Therapeutics Inc. and a coinventor on two pending patent applications, WO2018/119126 A1 and US 2013/0131028 A1.

Reference information: *J Clin Invest*. 2020;130(10):5042–5051.
https://doi.org/10.1172/JCI137560.

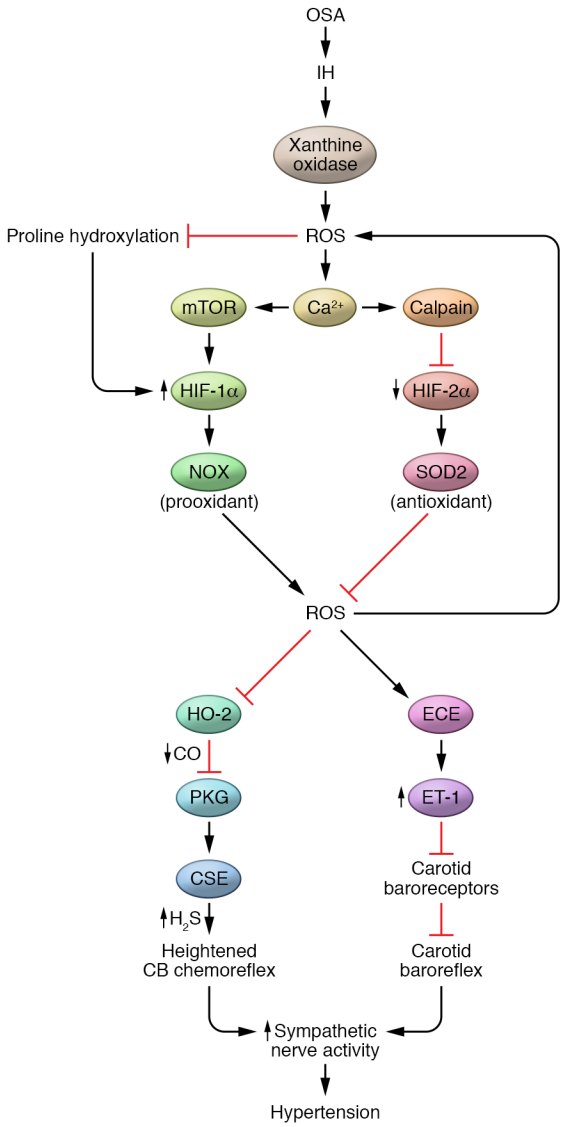

Figure 1. Schematic presentation of HIF-dependent signaling pathways in OSA-induced hypertension. Hypoxia-induced changes in HIF-1α and HIF-2α levels exacerbate increases in ROS levels. Within the carotid body, ROS elevations modify the balance between CO and H_2S (lower left) as well as attenuate the carotid baroreflex (lower right), resulting in increased sympathetic nerve activity that can drive hypertension. Ca^{2+}, calcium; CO, carbon monoxide; CSE, cystathionine-γ-lyase; ECE, endothelin-converting enzyme; ET-1, endothelin-1; H_2S, hydrogen sulfide; HO-2, heme oxygenase-2; NOX, NADPH oxidase.

Intermittent hypoxia: stimulus for hypertension

Intermittent hypoxia (IH) associated with OSA is characterized by short and high-frequency bouts of blood O_2 desaturations as opposed to long, low-frequency hypoxic bouts seen with short ascents and descents from high altitude (26). Healthy humans subjected to 10 days of IH patterned after blood O_2 saturations during OSA exhibit increases in SNA (27). Exposing healthy subjects to hypobaric hypoxia for 4 weeks, simulating an altitude of 5260 m, also increases SNA, which persists for 3 days after return to sea level (28), whereas challenging subjects with a single episode of IH leads to a long-lasting increase in SNA (29–31), indicating that IH is a more potent stimulus for eliciting long-lasting SNA than hypobaric hypoxia. The persistent SNA evoked by IH may explain daytime elevation of SNA in OSA patients. Although OSA also results in mild hypercapnia, repetitive arousals, and changes in intrathoracic pressures, these findings suggest that IH is a major stimulus for evoking SNA and the ensuing hypertension in OSA patients. While the above-outlined studies indicate that IH is maladaptive as it causes hypertension, mild IH induces respiratory plasticity manifested as long-term facilitation of breathing, which may be beneficial for mitigating OSA by stabilizing upper airway function (32). However, long-term IH exposure might increase the number of apneas (32–34).

OSA-induced hypertension and sympathetic nerve excitation have also been observed in animal models. A canine model of OSA exhibits daytime hypertension (35). Rodent models of IH patterned after blood O_2 saturation profiles during OSA also develop hypertension (36–44), and the magnitude and the onset of hypertension depend on the paradigm of IH (Table 1 in ref. 45). IH increases the activity of cervical, thoracic, splanchnic, renal, and lumbar sympathetic nerves (44, 46–49). As in human subjects (29–31), acute IH results in long-lasting increases in SNA in anesthetized rats (50).

Norepinephrine released from sympathetic nerves constricts blood vessels and maintains vascular tone. IH-exposed rats exhibit elevated resting vascular tone (51). In addition, chronic exposure to IH leads to vascular remodeling of resistance vessels, as evidenced by attenuated vasoconstriction by norepinephrine (51) and impaired vasodilatation by acetylcholine (52).

Besides blood vessels, the adrenal medulla is another major target organ of the sympathetic nervous system. Adrenal medullary chromaffin cells (AMCs) are a major source of epinephrine and norepinephrine (40). AMCs of adult rats are normally insensitive to hypoxia, and catecholamine secretion evoked by low O_2 is neurogenic (40). Exposure to IH induces hypoxic sensitivity in adult rat AMCs and decreases neurogenic catecholamine release (40). By inducing hypoxic sensitivity, IH may facilitate catecholamine secretion from AMCs during each episode of apnea, which may contribute in part to the elevated circulating levels. These studies suggest that chronic exposure to IH results in remodeling of end organs innervated by the sympathetic nerves.

How relevant are the rodent models of IH in understanding OSA-associated hypertension and SNA? It appears that reoxygenation is more important than the hypoxic phase of IH (53, 54). It is likely that OSA patients exhibit substantial interindividual variations in the duration of apnea and the magnitude of O_2 desaturations. Moreover, there are no data showing what percentage of OSA subjects exhibit O_2 desaturations equivalent to those used in rodent studies and whether these subjects exhibit hypertension. Despite these limitations, the available evidence suggests that rodent models of IH mirror blood pressure and SNA phenotypes reported in OSA patients, and thus these models appear appropriate for elucidating the underlying mechanisms.

Physiological basis of OSA-dependent hypertension

How might IH increase SNA and blood pressure? Arterial blood O_2 levels are continuously monitored by peripheral chemoreceptors, in particular the carotid bodies (CBs) (55). Hypoxemia increases

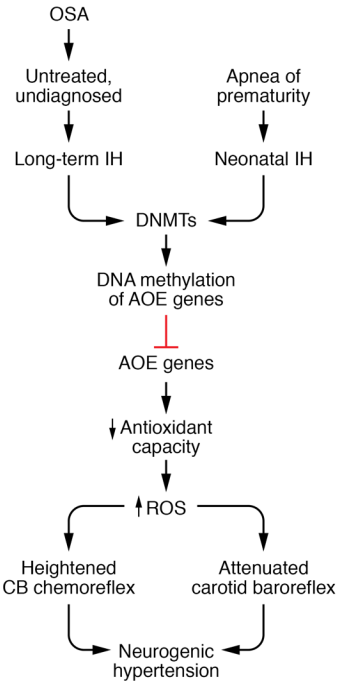

Figure 2. Activation of epigenetic mechanisms involving DNA methylation of antioxidant enzyme genes either in response to long-term IH associated with untreated and undiagnosed OSA or in young adults who had apnea of prematurity in neonatal life. AOE, antioxidant enzyme; DNMTs, DNA methyltransferases.

CB sensory nerve activity, which is transmitted to neurons in the nucleus tractus solitarius (nTS) and rostral ventrolateral medulla (RVLM) in the brainstem, from which the efferent signal is transmitted to the sympathetic nervous system. It was proposed that IH, by activating the CB chemoreflex, contributes to elevated SNA and hypertension in OSA patients (56). Supporting this possibility are the findings that (a) OSA patients exhibit augmented CB chemoreflex as indicated by exaggerated sympathetic nerve responses to acute hypoxia compared with normal subjects (57–59); (b) brief hyperoxia, which reduces CB sensory nerve activity, produces a more pronounced ventilatory depression (58, 60) and reduces blood pressure (59) in OSA patients but not in control subjects; and (c) OSA subjects with surgically ablated CBs do not develop hypertension (61).

In addition to the chemoreflex, arterial baroreflex is another major regulator of sympathetic tone and blood pressure (62). OSA patients exhibit an impaired baroreflex, especially during non–rapid eye movement (NREM) sleep (63, 64). These studies suggest that a combination of augmented CB chemoreflex and reduced baroreflex contribute to elevated SNA and hypertension in OSA subjects.

The carotid sinus nerve carries sensory information from the chemoreceptors in the CB as well as arterial baroreceptors located in the carotid sinus region. Studies on IH-exposed rodents have shown absence of sympathetic nerve activation and hypertension after sectioning of sinus nerves or selective ablation of the CB (65).

Rodent models provided further insights into the contribution of chemo- and baroreflexes to IH-evoked sympathetic nerve excitation and hypertension. Like OSA patients (57–59), IH-exposed rodents exhibit augmented hypoxic ventilatory response, a hallmark of the CB chemoreflex (66, 67). Neurophysiological studies revealed two major effects of IH on the CB: (a) enhanced sensitivity to hypoxia (68); and (b) progressive increases in baseline CB sensory nerve activity in response to IH, a phenomenon called sensory long-term facilitation (sLTF) (69). It was proposed that CB sLTF, by activating the chemoreflex, contributes to the daytime elevation of SNA seen in OSA patients (70).

Baroreflex activation inhibits SNA and causes bradycardia (decreased heart rate), and these responses are markedly attenuated in IH-treated rats (71). IH-treated rats exhibit attenuated activation of carotid baroreceptor in response to graded elevation of carotid sinus pressure (71). Thus, studies on rodents show that disrupted balance between chemo- and baroreflex is an important physiological basis for IH-evoked SNA and hypertension such as seen in OSA patients.

Molecular basis for OSA hypertension: hypoxia-inducible factors

Emerging evidence implicates transcriptional changes by hypoxia-inducible factors (HIFs) as an important molecular mechanism underlying alteration of chemo- and baroreflex functions by IH leading to SNA and hypertension. HIF-1 was the first identified member of the HIF family, followed by HIF-2 (72). While HIF-1 is expressed in all mammalian cells, HIF-2 expression is restricted to certain tissues, including developing blood vessels, lung, adrenal medulla, and CB (73–75). Both HIF-1 and HIF-2 are composed of an O_2-regulated α subunit and a constitutive β subunit (12).

Differential regulation of HIF-α isoforms by IH

Continuous hypoxia activates both HIF-1 and HIF-2 (76, 77). In striking contrast, IH results in differential regulation of HIF-1α and HIF-2α. IH alters HIF-α isoform expression in all three major components of the arterial chemoreflex pathway, including (a) the CB (sensor); (b) the nTS and RVLM (central component); and (c) the adrenal medulla (end organ of the sympathetic nervous system).

Both HIF-1α and HIF-2α are expressed in glomus cells, the primary O_2-sensing cells of the CB (76, 77). IH increases HIF-1α (78) and decreases HIF-2α (79) protein expression in the CB. Exposing rat pheochromocytoma (PC12) cell cultures, which share many similarities to glomus cells, to an IH paradigm similar to that employed in rodents increases HIF-1α protein (80) and decreases HIF-2α protein expression (79). Given that the CB receives the highest blood flow relative to tissue weight as compared with other organs (81–83), changes in HIF-α expression are likely due to direct effects of IH on glomus cells.

Studies in PC12 cells further showed that the increased HIF-1α is due to increased generation of reactive oxygen species (ROS) by xanthine oxidase, leading to subsequent activation of HIF-1α protein synthesis by mammalian target of rapamycin (mTOR) as well as decreased proline hydroxylation (84–86). The decrease of HIF-2α expression by IH is due to increased protein degradation by Ca^{2+}-dependent calpain proteases (79, 85). HIF-2α degradation by calpains involves the C-terminus part of the HIF-2α protein (85). Cell culture studies further revealed that IH-induced changes in

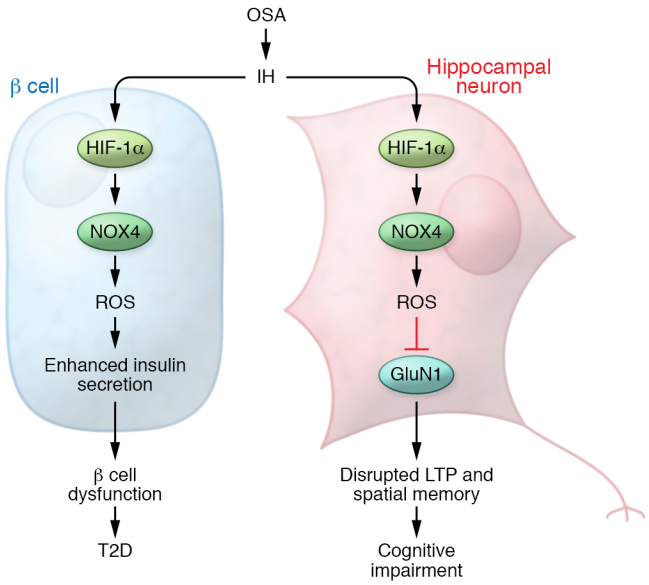

Figure 3. Schematic presentation of proposed mechanism(s) for HIF-1–dependent pancreatic β cell dysfunction manifesting as hypersecretion of insulin and insulin resistance and cognitive dysfunction evoked by OSA/IH. GluN1, glutamate ionotropic receptor NMDA type subunit 1; LTP, long-term potentiation.

HIF-α isoforms are associated with increased HIF-1–dependent and reduced HIF-2–dependent transcriptional activities (79, 80).

Differential regulation of HIF-α isoforms by IH was also seen in neurons of the nTS and RVLM as well as the adrenal medulla (65, 66, 79, 87). The effects of IH on the nTS, RVLM, and adrenal medulla are indirect and require sensory input from the CB, as evidenced by absence of HIF-α isoform changes by IH after selective ablation of the CB (65).

Physiological consequence of HIF-α dysregulation

Complete deficiency of HIF-1α is embryonically lethal at mid-gestation, whereas mice with heterozygous deficiency of *Hif1a* develop normally and are indistinguishable from WT littermate controls in normal oxygen conditions (88, 89). Unlike WT mice, IH-treated HIF-1α–heterozygous mice exhibit striking absences of augmented CB sensory nerve response to acute hypoxia, sLTF, sympathetic nerve excitation (evidenced by absence of elevated plasma catecholamine levels), and hypertension (66).

In contrast, HIF-2α–heterozygous (*Hif2a*$^{+/-}$) mice under basal room air conditions exhibit cardiorespiratory responses similar to those in WT mice treated with IH, including augmented CB responses to acute hypoxia, sympathetic nerve activation as indicated by elevated plasma catecholamines, hypertension, and increased incidence of apnea (90). Blocking IH-induced HIF-2α degradation with systemic administration of a calpain inhibitor prevents development of hypertension (79). These findings demonstrate that dysregulated HIF-α isoforms act as an important molecular mechanism underlying augmented CB chemoreflex, sympathetic nerve excitation, and hypertension caused by IH. Whether IH-induced attenuation of arterial baroreflex is altered in HIF-1α– and HIF-2α–heterozygous mice is not known.

Dysregulated HIFs increase ROS

OSA is characterized by periods of hypoxia and reoxygenation that resemble ischemia/reperfusion. It was proposed that increased ROS generated during IH contributes to hypertension associated with OSA (91). Supporting such a possibility, OSA patients exhibit elevated ROS levels in monocytes expressing integrin α_X chain protein (CD11C) (92). OSA patients exhibit elevated levels of biomarkers of ROS in plasma, urine, and the exhaled breath (93). Grebe et al. (94) reported that OSA patients exhibit decreased vasodilation of the brachial artery, and this response was normalized by antioxidant treatment, indicating contribution of ROS to increased vascular tone in OSA patients. A recent meta-analysis by Chen et al. (95) suggested that continuous positive airway pressure (CPAP) therapy lowers circulating ROS levels (as indicated by malondialdehyde measurements, an index of oxidized lipids) in elderly individuals with obesity, and patients with severe OSA.

IH-treated rodents exhibit elevated ROS levels in all three major components of the chemoreflex pathway, including the CB (69), the nTS and RVLM (65), and the adrenal medulla (40, 65), as evidenced by decreased aconitase enzyme activity (40, 65, 69), an established biochemical marker of ROS (96), and increased malondialdehyde levels (97). Likewise, IH increases ROS levels in the carotid sinus region, the primary site of carotid baroreceptors (71).

The following findings suggest that dysregulated HIF-α isoforms mediate ROS elevation by IH: (a) HIF-1α–heterozygous mice exposed to IH do not exhibit elevated ROS levels (66); (b) blocking HIF-1α expression in the nTS, RVLM, and adrenal medulla by CB ablation prevents ROS elevation by IH (65); and (c) HIF-2α–heterozygous mice, like IH-treated WT mice, exhibit elevated ROS levels in the CB and adrenal medulla under basal conditions, and antioxidant treatment prevents this response (90).

Normalizing ROS levels by antioxidant treatment prevents the following IH-induced responses: (a) augmented CB response to hypoxia and sLTF (69, 97–99); (b) attenuated carotid baroreceptor activity and baroreflex function (71); and (c) elevation of plasma catecholamine levels and hypertension (40). These findings suggest that increased ROS generation resulting from dysregulated HIF-α isoforms is an important cellular mechanism underlying enhanced chemoreflex and attenuated baroreflex leading to sympathetic nerve excitation and hypertension due to IH.

How do dysregulated HIFs lead to an increase in ROS levels by IH? Cellular ROS levels are balanced through generation by pro-oxidant enzymes and degradation by antioxidant enzymes. The following section summarizes studies showing that IH-induced dysregulation of HIF-α isoforms increases ROS by altering the transcription of genes encoding pro- and antioxidant enzymes.

HIF-1 mediates *Nox2* gene activation by IH

The family of NADPH oxidases (NOXs) are pro-oxidant enzymes and include NOX1, NOX2, NOX3, and NOX4 (100). Of the four isoforms, NOX2 is expressed in major components of the chemoreflex pathway (65, 98). IH increases *Nox2* mRNA in the CB and brainstem, areas associated with the chemoreflex, but not in the cerebellum, a brain area not associated with the chemoreflex (87). The IH-induced effect on *Nox2* mRNA is absent in HIF-1α–heterozygous mice after exposure to IH (66). Disrupting HIF-1α protein, either by RNA interference or by pharmacological approaches

(digoxin or YC-1), prevents upregulation of *Nox2* mRNA, protein, and enzyme activity in IH-treated PC12 cells and mouse embryonic fibroblasts (MEFs) (87). Conversely, increasing HIF-1α expression, either by treatment of PC12 cells with an iron chelator (desferoxamine) or by overexpression of HIF-1α, increases *Nox2* mRNA, protein expression, and enzyme activity (87) in a manner similar to that of IH. These findings suggest that HIF-1 mediates IH-induced upregulation of the major pro-oxidant enzyme NOX2.

Inhibition of complexes I and III of the mitochondrial electron transport chain also increases ROS generation (101). Complex I activity was inhibited in CBs of IH-treated rats (69), resulting in elevated ROS abundance (99). IH-evoked complex I inhibition was prevented by blocking NOX2 function (102), and was absent in mice deficient in gp91phox (the catalytic subunit of NOX2) (102), suggesting a crosstalk between NOX2 and the mitochondrial complex I. After termination of IH, ROS generation by NOX2 returns to baseline within 3 hours, whereas ROS generation by complex I inhibition persists as long as 16 hours (102), suggesting that a feed-forward ROS-induced ROS mechanism is responsible for long-lasting generation of ROS by IH.

HIF-2 contributes to antioxidant enzyme decreases by IH

IH decreases the mRNA, protein, and enzyme activity of antioxidant enzymes (79). Scortegagna et al. reported that HIF-2 is a potent activator of genes encoding antioxidant enzymes (103). The following findings suggest that IH-induced degradation of HIF-2α protein contributes to downregulation of antioxidant enzymes, such as superoxide dismutase 2 (SOD2): (a) overexpression of transcriptionally active HIF-2α prevents IH-evoked decrease in *Sod2* mRNA and blocks increased ROS abundance in PC12 cells; and (b) treating IH-exposed rats with ALLM (*N*-acetyl-L-leucyl-L-leucyl-L-methionine), a calpain inhibitor, blocks HIF-2α degradation, restores SOD2 enzyme activity, normalizes ROS levels, and blocks the development of hypertension (79).

The following section summarizes signaling mechanisms by which ROS mediate activation of CBs and reduction of baroreceptor activity by IH.

Chemoreflex activation by IH requires ROS/H_2S signaling

Recent studies suggest that hypoxic sensing by the CB requires O_2-dependent interplay between carbon monoxide (CO) and hydrogen sulfide (H_2S). The enzyme heme oxygenase-2 (HO-2) generates CO in the CB (104). Hypoxia inactivates HO-2, leading to stimulus-dependent reduction in CO production (105). Given that CO is a physiological inhibitor of hypoxic sensing in the CB (105–107), and hypoxia reduces CO production (107), it was proposed that sensory nerve activation by hypoxia is due to release of the inhibitory effect of CO on the CB (104). Glomus cells of the CB also express cystathionine-γ-lyase (CSE), an enzyme that catalyzes H_2S synthesis (105, 106). Hypoxia increases H_2S generation in the CB in a stimulus-dependent manner (106). CO suppresses H_2S synthesis by inhibiting CSE activity in the CB through protein kinase G–dependent phosphorylation at the serine^{377} residue (107). Thus, CB hypoxic sensing uses a biochemical signaling mechanism involving O_2-dependent interplay between CO and H_2S.

IH increases H_2S production in the CB (44). The increased H_2S production by IH is due to ROS-dependent inactivation of HO-2 (44), thereby increasing CSE-dependent H_2S generation in the CB. Pharmacological or genetic blockade of H_2S synthesis prevents IH-evoked CB activation, sympathetic nerve excitation, and hypertension (44). These findings suggest that increased ROS generation resulting from dysregulated HIF-α isoforms mediates IH-induced CB hyperactivity and hypertension through oxidative inactivation of HO-2 and a consequent increase in H_2S production (Figure 1). HO-2–knockout mice exhibit greater abundance of CSE-derived H_2S in the CB, augmented chemoreflex, and OSA, and CSE inhibitor prevents OSA in HO-2–null mice (108).

Baroreflex attenuation by IH requires ROS/endothelin signaling

Peng et al. (71) examined the mechanism(s) underlying reduced carotid baroreflex function in IH-treated rats. They found elevated levels of the vasoconstrictor endothelin-1 (ET-1) in the carotid sinus region of IH-treated rats. The increased ET-1 levels were due to ROS-dependent activation of endothelin-converting enzyme (ECE), which generates biologically active ET-1. The reduction in carotid baroreceptor responses to increased carotid sinus pressure in IH-treated rats was due to the vasoconstrictor effect of ET-1 on the carotid sinus, as indicated by normalization of baroreceptor function by an ET_A receptor antagonist. Furthermore, antioxidant treatment blocked the effects of IH on ET-1 levels and ECE activity, reversed the attenuated carotid baroreceptor activity, and restored the baroreflex function in rats. These findings demonstrate that HIF-dependent ROS production contributes to IH-induced attenuation of carotid baroreflex function by activating ET-1 signaling (Figure 1).

Is OSA hypertension reversible?

CPAP is the current treatment of choice for OSA. However, meta-analysis studies indicate that CPAP is either ineffective (109) or minimally effective (110, 111) in reversing OSA hypertension. Lack of CPAP efficacy may in part be due to poor adherence rates (39%–50%) (112, 113). Also, studies on rodents suggest that IH leads to vascular remodeling (51, 52, 114), which may not be ameliorated by CPAP. It is possible that hypertension could be secondary to other OSA comorbidities that may not be addressed by CPAP therapy. Moreover, emerging evidence suggests that various factors contribute to the genesis of OSA, including (a) compromised pharyngeal anatomy; (b) inadequate upper airway muscle function; (c) hypersensitive chemoreflex feedback loop (i.e., high loop gain); and (d) low arousal threshold (115, 116). These findings suggest that OSA is a multifactorial disorder, and future development of therapies targeted to each of the contributing factors may be necessary to effectively control blood pressure in OSA patients.

In addition to the above possibilities, the effectiveness of CPAP may depend on the duration of OSA. For instance, CPAP may be less effective in normalizing blood pressure in undiagnosed and untreated patients experiencing OSA for several years. Such a possibility is partly supported by a recent study showing complete reversal of hypertension, sympathetic nerve activation, and augmented CB chemoreflex evoked by short-term IH (10 days of exposure) upon recovery in room air. In striking contrast, simi-

lar effects evoked by long-term IH (30 days of exposure) were not reversed even after 30 days of recovery in room air (34).

Though OSA is a condition affecting adults, infants born preterm also exhibit high incidence of apneas (apnea of prematurity), a major clinical problem in neonatology. CB chemoreflex is augmented in apnea of prematurity as indicated by enhanced hypoxic ventilatory response (117). Simulating apnea of prematurity by exposing neonatal rat pups to IH from ages P0 to P10 markedly enhances hypoxic response of the CB (118, 119) and augments chemoreflex (118, 119). Remarkably, the effects of neonatal IH were not reversed and persist into adulthood after return to normal air. Adult rats exposed to IH during neonatal life (from P0 to P10) exhibit hypertension, elevated plasma catecholamines, irregular breathing with high incidence of apneas, and augmented hypoxic response of the CB and chemoreflex (119, 120), findings reminiscent of high incidence of hypertension and sleep-disordered breathing in young adults born preterm (121, 122).

Long-term IH activates epigenetic mechanisms

Persistent hypertension, sympathetic excitation, and augmented chemoreflex seen in adult rats treated with long-term IH and rats treated with IH during the neonatal period are associated with persistent elevation of ROS levels and reduced expression of genes encoding antioxidant enzymes in the chemoreflex pathway (34, 120, 123). Long-lasting physiological responses to a given perturbation are attributed to gene regulation by epigenetic mechanisms. DNA hypermethylation is one such epigenetic mechanism that results in long-lasting suppression of gene expression (124). Rats treated with long-term IH as well as rats treated with IH in the neonatal period show DNA hypermethylation of genes encoding antioxidant enzymes in the CB chemoreflex pathway. This effect is accompanied by increased activity of the DNA methyltransferase enzyme, which catalyzes DNA hypermethylation (34, 120). Further analysis revealed hypermethylation of a single CpG dinucleotide in the region close to the transcription start site of the *Sod2* gene in rats exposed to long-term (34) or neonatal IH (34, 120). Treating rats with decitabine, a DNA-hypomethylating agent, during exposures to long-term and neonatal IH blocked DNA hypermethylation, restored antioxidant enzyme gene expression, normalized ROS levels in the chemoreflex pathway, prevented the development of hypertension, and normalized breathing irregularities (34, 120). These studies suggest that long-lasting suppression of antioxidant enzyme genes by DNA methylation results in persistent increase in ROS levels in the chemoreflex pathway leading to persistent hypertension in rats treated with long-term or neonatal IH (Figure 2). The mechanism(s) by which long-term and neonatal IH activates DNA methylation remains to be investigated.

The following section summarizes how, outside of hypertension, HIF-1–dependent ROS generation also contributes to development of T2D and cognitive dysfunction in rodent models of IH.

Type 2 diabetes and OSA

Type 2 diabetes (T2D) is another major comorbidity in OSA patients (7–9). T2D is characterized by initial insulin resistance followed by progressive loss of pancreatic β cell function (125). IH-treated mice manifest elevated basal plasma insulin levels and insulin resistance as evidenced by increased homeostatic model assessment (HOMA) index, an established method for assessing insulin resistance (126). ROS levels are elevated in pancreatic β cells of IH-treated mice, and antioxidant treatment blocks the elevated insulin secretion and normalizes the HOMA index (126). Pancreatic β cells express HIF-1α but not HIF-2α. Recent studies suggest that HIF-1 contributes to insulin secretion from β cells under basal conditions (127, 128). HIF-1α–heterozygous mice treated with 30 days of IH showed a remarkable absence of elevated fasting plasma insulin levels and absence of insulin resistance as assessed by HOMA (Figure 3). Further studies are needed to investigate the mechanism(s) by which HIF-1 contributes to pancreatic β cell function in the setting of chronic IH.

Cognitive decline and OSA

Cognitive decline is a recognized comorbidity of OSA (10, 129–134). Bucks et al. proposed two possible mechanisms by which OSA may cause cognitive decline: (a) cognitive impairment from OSA may be secondary to daytime sleepiness affecting attention; and (b) OSA may lead to cerebral vasculature remodeling, neural damage, and cell death, resulting in cognitive dysfunction (135). OSA has been shown to affect the hippocampus, which is a major brain structure associated with learning and memory (136–139).

A recent histopathological study using autopsy of brain tissues from OSA subjects showed a correlation between OSA severity and histopathological changes in the hippocampus including cortical thinning in the molecular layer of the dentate gyrus and the CA1 area as well as decreased myelin of the deep layers of entorhinal cortex (140). The regions of decreased cortical thickness and demyelination were seen in spatial memory pathways (140). Studies on rodents showed that IH impairs spatial learning and memory (141, 142) and weakens synaptic plasticity of the CA1 area of the hippocampus (143–147). The effects of IH were mediated by increased generation of ROS (148, 149). IH increased HIF-1α protein expression in hippocampal neurons (147, 149), upregulated *Nox4* mRNA, and elevated ROS levels (150). Increased ROS production, in turn, downregulated GluN1, an obligatory subunit of the *N*-methyl D-aspartate receptor (NMDAR), leading to disrupted long-term potentiation of hippocampal neuronal activity and impaired spatial memory function (150). IH-induced deficits in spatial memory were absent in HIF-1α–heterozygous mice and in WT mice treated with MnTMPyP, a membrane-permeable antioxidant (150). These findings suggest that IH results in HIF-1α–dependent destabilization of NMDAR-dependent synaptic physiology and spatial memory (Figure 3).

Perspective

Thus far, experimental models of IH have shown that imbalance of HIF-α isoform expression by activation of ROS signaling leads to maladaptation. However, it remains to be established whether HIF-1 and HIF-2 imbalances occur in OSA patients. Besides OSA, ROS-dependent activation of the chemoreflex has also been implicated in autonomic pathologies associated with congestive cardiac failure (CCF) (151, 152). Whether CCF leads to HIF-α isoform imbalance in the chemoreflex remains an interesting question. While studies on HIF-2α–heterozygous mice show heightened chemoreflex, studies on adult mice with inducible knockout of HIF-2α report absences of hypoxic ventilatory response, a hallmark of the CB chemoreflex (153), and loss of ventilatory adap-

tation to sustained hypoxia (154). Adult mice with inducible loss of HIF-2α showed selective loss of response to severe hypoxia (partial pressure of oxygen [pO_2] ~10–15 mmHg) by glomus cells (the primary O_2-sensing cells of the CB) (153). Thus, results from studies with inducible knockout of HIF-2α appear to be opposite to those obtained in mice with global partial knockdown of HIF-2α. Whether the differing phenotypic changes are due to absence of HIF-2 since birth, as is the case with the global partial knockout animals, as opposed to inducible complete loss of HIF-2α in adult life, remains to be investigated.

Although rodent models showed the involvement of HIF-1α in cognitive decline due to IH (150), the role of HIF-2α has not yet been investigated. Further studies are needed to establish whether HIF-1α activation in hippocampal neurons is due to a direct effect of IH or indirectly secondary to neural activation.

Given the modest efficacy of CPAP in mitigating OSA comorbidities, there is an unmet need for alternative strategies for preventing OSA-associated pathologies. Currently, inhibitors of HIF-2 signaling for kidney cancer are in clinical trials (155). Further development of pharmacological inhibitor(s) of HIF-1 may be one possibility for preventing some of the pathologies associated with OSA; pharmacological inhibitors of CSE-derived H_2S, a downstream target of HIF signaling, are another possibility. Indeed, a recent study provides proof of concept for the latter possibility, wherein systemic administration of CSE inhibitor blocks IH-induced sympathetic nerve activation and hypertension in rodents (108).

Acknowledgments

Studies in the authors' laboratory are supported by the NIH (National Heart, Lung, and Blood Institute grant P01-HL-44454). The authors are grateful to Benjamin Wang and Ganesh K. Kumar for their invaluable suggestions and help with editing.

Address correspondence to: Nanduri R. Prabhakar, Institute for Integrative Physiology, Center for Systems Biology of Oxygen Sensing, University of Chicago, MC 5068, 5841 South Maryland Avenue, Chicago, Illinois 60637, USA. Phone: 773.834.5480; Email: nanduri@uchicago.edu.

1. Peppard PE, Young T, Barnet JH, Palta M, Hagen EW, Hla KM. Increased prevalence of sleep-disordered breathing in adults. *Am J Epidemiol*. 2013;177(9):1006–1014.
2. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. *Physiol Rev*. 2010;90(1):47–112.
3. Redline S, Tishler PV, Hans MG, Tosteson TD, Strohl KP, Spry K. Racial differences in sleep-disordered breathing in African-Americans and Caucasians. *Am J Respir Crit Care Med*. 1997;155(1):186–192.
4. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med*. 2000;342(19):1378–1384.
5. Lavie P, Herer P, Hoffstein V. Obstructive sleep apnoea syndrome as a risk factor for hypertension: population study. *BMJ*. 2000;320(7233):479–482.
6. Nieto FJ, et al. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. *JAMA*. 2000;283(14):1829–1836.
7. Idris I, et al. Obstructive sleep apnoea in patients with type 2 diabetes: aetiology and implications for clinical care. *Diabetes Obes Metab*. 2009;11(8):733–741.
8. Laaban JP, et al. Prevalence and predictive factors of sleep apnoea syndrome in type 2 diabetic patients. *Diabetes Metab*. 2009;35(5):372–377.
9. Tasali E, Mokhlesi B, Van Cauter E. Obstructive sleep apnea and type 2 diabetes: interacting epidemics. *Chest*. 2008;133(2):496–506.
10. Wallace A, Bucks RS. Memory and obstructive sleep apnea: a meta-analysis. *Sleep*. 2013;36(2):203–220.
11. Jackson ML, Howard ME, Barnes M. Cognition and daytime functioning in sleep-related breathing disorders. *Prog Brain Res*. 2011;190:53–68.
12. 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(3):967–1003.
13. Logan AG, et al. High prevalence of unrecognized sleep apnoea in drug-resistant hypertension. *J Hypertens*. 2001;19(12):2271–2277.
14. Morrell MJ, Finn L, Kim H, Peppard PE, Badr MS, Young T. Sleep fragmentation, awake blood pressure, and sleep-disordered breathing in a population-based study. *Am J Respir Crit Care Med*. 2000;162(6):2091–2096.
15. Mazzotti DR, Keenan BT, Lim DC, Gottlieb DJ, Kim J, Pack AI. Symptom subtypes of obstructive sleep apnea predict incidence of cardiovascular outcomes. *Am J Respir Crit Care Med*. 2019;200(4):493–506.
16. Leuenberger U, Jacob E, Sweer L, Waravdekar N, Zwillich C, Sinoway L. Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxemia. *J Appl Physiol*. 1995;79(2):581–588.
17. Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest*. 1993;103(6):1763–1768.
18. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest*. 1995;96(4):1897–1904.
19. Hornyak M, Cejnar M, Elam M, Matousek M, Wallin BG. Sympathetic muscle nerve activity during sleep in man. *Brain*. 1991;114(pt 3):1281–1295.
20. Okada H, Iwase S, Mano T, Sugiyama Y, Watanabe T. Changes in muscle sympathetic nerve activity during sleep in humans. *Neurology*. 1991;41(12):1961–1966.
21. Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic-nerve activity during sleep in normal subjects. *N Engl J Med*. 1993;328(5):303–307.
22. Narkiewicz K, Somers VK. The sympathetic nervous system and obstructive sleep apnea: implications for hypertension. *J Hypertens*. 1997;15(12 pt 2):1613–1619.
23. Fletcher EC, Miller J, Schaaf JW, Fletcher JG. Urinary catecholamines before and after tracheostomy in patients with obstructive sleep apnea and hypertension. *Sleep*. 1987;10(1):35–44.
24. García-Río F, et al. Sleep apnea and hypertension. *Chest*. 2000;117(5):1417–1425.
25. Marrone O, Riccobono L, Salvaggio A, Mirabella A, Bonanno A, Bonsignore MR. Catecholamines and blood pressure in obstructive sleep apnea syndrome. *Chest*. 1993;103(3):722–727.
26. Dewan NA, Nieto FJ, Somers VK. Intermittent hypoxemia and OSA: implications for comorbidities. *Chest*. 2015;147(1):266–274.
27. Lusina SJ, Kennedy PM, Inglis JT, McKenzie DC, Ayas NT, Sheel AW. Long-term intermittent hypoxia increases sympathetic activity and chemosensitivity during acute hypoxia in humans. *J Physiol (Lond)*. 2006;575(pt 3):961–970.
28. Hansen J, Sander M. Sympathetic neural overactivity in healthy humans after prolonged exposure to hypobaric hypoxia. *J Physiol (Lond)*. 2003;546(pt 3):921–929.
29. Cutler MJ, Swift NM, Keller DM, Wasmund WL, Smith ML. Hypoxia-mediated prolonged elevation of sympathetic nerve activity after periods of intermittent hypoxic apnea. *J Appl Physiol*. 2004;96(2):754–761.
30. Morgan BJ, Crabtree DC, Palta M, Skatrud JB. Combined hypoxia and hypercapnia evokes long-lasting sympathetic activation in humans. *J Appl Physiol*. 1995;79(1):205–213.
31. Xie A, Skatrud JB, Puleo DS, Morgan BJ. Exposure to hypoxia produces long-lasting sympathetic activation in humans. *J Appl Physiol*. 2001;91(4):1555–1562.
32. Mateika JH, Syed Z. Intermittent hypoxia, respiratory plasticity and sleep apnea in humans: present knowledge and future investigations. *Respir Physiol Neurobiol*. 2013;188(3):289–300.
33. Mateika JH, Narwani G. Intermittent hypoxia and respiratory plasticity in humans and other animals: does exposure to intermittent hypoxia promote or mitigate sleep apnoea? *Exp Physiol*. 2009;94(3):279–296.
34. Nanduri J, et al. Epigenetic regulation of redox

state mediates persistent cardiorespiratory abnormalities after long-term intermittent hypoxia. *J Physiol (Lond)*. 2017;595(1):63–77.
35. Brooks D, Horner RL, Kozar LF, Render-Teixeira CL, Phillipson EA. Obstructive sleep apnea as a cause of systemic hypertension. Evidence from a canine model. *J Clin Invest*. 1997;99(1):106–109.
36. Fletcher EC, Lesske J, Behm R, Miller CC, Stauss H, Unger T. Carotid chemoreceptors, systemic blood pressure, and chronic episodic hypoxia mimicking sleep apnea. *J Appl Physiol*. 1992;72(5):1978–1984.
37. Kanagy NL, Walker BR, Nelin LD. Role of endothelin in intermittent hypoxia-induced hypertension. *Hypertension*. 2001;37(2 pt 2):511–515.
38. Hui AS, et al. Regulation of catecholamines by sustained and intermittent hypoxia in neuroendocrine cells and sympathetic neurons. *Hypertension*. 2003;42(6):1130–1136.
39. Joyeux-Faure M, et al. Chronic intermittent hypoxia increases infarction in the isolated rat heart. *J Appl Physiol*. 2005;98(5):1691–1696.
40. Kumar GK, et al. Chronic intermittent hypoxia induces hypoxia-evoked catecholamine efflux in adult rat adrenal medulla via oxidative stress. *J Physiol (Lond)*. 2006;575(pt 1):229–239.
41. Zoccal DB, Bonagamba LG, Oliveira FR, Antunes-Rodrigues J, Machado BH. Increased sympathetic activity in rats submitted to chronic intermittent hypoxia. *Exp Physiol*. 2007;92(1):79–85.
42. Knight WD, Little JT, Carreno FR, Toney GM, Mifflin SW, Cunningham JT. Chronic intermittent hypoxia increases blood pressure and expression of FosB/DeltaFosB in central autonomic regions. *Am J Physiol Regul Integr Comp Physiol*. 2011;301(1):R131–R139.
43. Silva AQ, Schreihofer AM. Altered sympathetic reflexes and vascular reactivity in rats after exposure to chronic intermittent hypoxia. *J Physiol (Lond)*. 2011;589(pt 6):1463–1476.
44. Yuan G, et al. H2S production by reactive oxygen species in the carotid body triggers hypertension in a rodent model of sleep apnea. *Sci Signal*. 2016;9(441):ra80.
45. Prabhakar NR, Peng YJ, Kumar GK, Nanduri J. Peripheral chemoreception and arterial pressure responses to intermittent hypoxia. *Compr Physiol*. 2015;5(2):561–577.
46. Greenberg HE, Sica A, Batson D, Scharf SM. Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia. *J Appl Physiol*. 1999;86(1):298–305.
47. Huang J, et al. Sympathetic response to chemostimulation in conscious rats exposed to chronic intermittent hypoxia. *Respir Physiol Neurobiol*. 2009;166(2):102–106.
48. Zoccal DB, et al. Increased sympathetic outflow in juvenile rats submitted to chronic intermittent hypoxia correlates with enhanced expiratory activity. *J Physiol (Lond)*. 2008;586(13):3253–3265.
49. Marcus NJ, Li YL, Bird CE, Schultz HD, Morgan BJ. Chronic intermittent hypoxia augments chemoreflex control of sympathetic activity: role of the angiotensin II type 1 receptor. *Respir Physiol Neurobiol*. 2010;171(1):36–45.
50. Dick TE, Hsieh YH, Wang N, Prabhakar N. Acute intermittent hypoxia increases both phrenic and sympathetic nerve activities in the rat. *Exp Physiol*. 2007;92(1):87–97.
51. Phillips SA, Olson EB, Lombard JH, Morgan BJ. Chronic intermittent hypoxia alters NE reactivity and mechanics of skeletal muscle resistance arteries. *J Appl Physiol*. 2006;100(4):1117–1123.
52. Phillips SA, Olson EB, Morgan BJ, Lombard JH. Chronic intermittent hypoxia impairs endothelium-dependent dilation in rat cerebral and skeletal muscle resistance arteries. *Am J Physiol Heart Circ Physiol*. 2004;286(1):H388–H393.
53. Yuan G, Adhikary G, McCormick AA, Holcroft JJ, Kumar GK, Prabhakar NR. Role of oxidative stress in intermittent hypoxia-induced immediate early gene activation in rat PC12 cells. *J Physiol (Lond)*. 2004;557(pt 3):773–783.
54. Lim DC, et al. Simulating obstructive sleep apnea patients' oxygenation characteristics into a mouse model of cyclical intermittent hypoxia. *J Appl Physiol*. 2015;118(5):544–557.
55. Kumar P, Prabhakar NR. Peripheral chemoreceptors: function and plasticity of the carotid body. *Compr Physiol*. 2012;2(1):141–219.
56. Cistulli PA, Sullivan CE. Pathophysiology of sleep apnea. In: Saunders NA, Sullivan CE, eds. *Sleep and Breathing*. 2nd ed. Marcel Dekker; 1994:405–448.
57. Hedner JA, Wilcox I, Laks L, Grunstein RR, Sullivan CE. A specific and potent pressor effect of hypoxia in patients with sleep apnea. *Am Rev Respir Dis*. 1992;146(5 pt 1):1240–1245.
58. Kara T, Narkiewicz K, Somers VK. Chemoreflexes—physiology and clinical implications. *Acta Physiol Scand*. 2003;177(3):377–384.
59. Narkiewicz K, van de Borne PJ, Pesek CA, Dyken ME, Montano N, Somers VK. Selective potentiation of peripheral chemoreflex sensitivity in obstructive sleep apnea. *Circulation*. 1999;99(9):1183–1189.
60. Tafil-Klawe M, Thiele AE, Raschke F, Mayer J, Peter JH, von Wichert W. Peripheral chemoreceptor reflex in obstructive sleep apnea patients; a relationship between ventilatory response to hypoxia and nocturnal bradycardia during apnea events. *Pneumologie*. 1991;45(suppl 1):309–311.
61. Somers VK, Abboud FM. Chemoreflexes—responses, interactions and implications for sleep apnea. *Sleep*. 1993;16(8 suppl):S30–S33; discussion S33.
62. Guyenet PG. The sympathetic control of blood pressure. *Nat Rev Neurosci*. 2006;7(5):335–346.
63. Bonsignore MR, et al. Baroreflex control of heart rate during sleep in severe obstructive sleep apnoea: effects of acute CPAP. *Eur Respir J*. 2006;27(1):128–135.
64. Parati G, et al. Autonomic cardiac regulation in obstructive sleep apnea syndrome: evidence from spontaneous baroreflex analysis during sleep. *J Hypertens*. 1997;15(12 pt 2):1621–1626.
65. Peng YJ, et al. Regulation of hypoxia-inducible factor-α isoforms and redox state by carotid body neural activity in rats. *J Physiol (Lond)*. 2014;592(17):3841–3858.
66. Peng YJ, et al. Heterozygous HIF-1alpha deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol (Lond)*. 2006;577(pt 2):705–716.
67. Rey S, Del Rio R, Alcayaga J, Iturriaga R. Chronic intermittent hypoxia enhances cat chemosensory and ventilatory responses to hypoxia. *J Physiol (Lond)*. 2004;560(pt 2):577–586.
68. Peng YJ, Prabhakar NR. Effect of two paradigms of chronic intermittent hypoxia on carotid body sensory activity. *J Appl Physiol*. 2004;96(3):1236–1242; discussion 1196.
69. 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(17):10073–10078.
70. Prabhakar NR. Sensory plasticity of the carotid body: role of reactive oxygen species and physiological significance. *Respir Physiol Neurobiol*. 2011;178(3):375–380.
71. Peng YJ, et al. Endothelin-1 mediates attenuated carotid baroreceptor activity by intermittent hypoxia. *J Appl Physiol*. 2012;112(1):187–196.
72. Semenza GL. Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology. *Annu Rev Pathol*. 2014;9:47–71.
73. Flamme I, Fröhlich T, von Reutern M, Kappel A, Damert A, Risau W. HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor-1 alpha and developmentally expressed in blood vessels. *Mech Dev*. 1997;63(1):51–60.
74. Wiesener MS, et al. Widespread hypoxia-inducible expression of HIF-2alpha in distinct cell populations of different organs. *FASEB J*. 2003;17(2):271–273.
75. Hampton-Smith RJ, Peet DJ. From polyps to people: a highly familiar response to hypoxia. *Ann N Y Acad Sci*. 2009;1177:19–29.
76. Lam SY, Tipoe GL, Liong EC, Fung ML. Differential expressions and roles of hypoxia-inducible factor-1alpha, -2alpha and -3alpha in the rat carotid body during chronic and intermittent hypoxia. *Histol Histopathol*. 2008;23(3):271–280.
77. 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(1):53–57.
78. Lam SY, Tipoe GL, Liong EC, Fung ML. Hypoxia-inducible factor (HIF)-1alpha and endothelin-1 expression in the rat carotid body during intermittent hypoxia. *Adv Exp Med Biol*. 2006;580:21–27; discussion 351.
79. Nanduri J, et al. Intermittent hypoxia degrades HIF-2alpha via calpains resulting in oxidative stress: implications for recurrent apnea-induced morbidities. *Proc Natl Acad Sci U S A*. 2009;106(4):1199–1204.
80. Yuan G, Nanduri J, Bhasker CR, Semenza GL, Prabhakar NR. Ca2+/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. *J Biol Chem*. 2005;280(6):4321–4328.
81. Barnett S, Mulligan E, Wagerle LC, Lahiri S. Measurement of carotid body blood flow in cats by use of radioactive microspheres. *J Appl Physiol*. 1988;65(6):2484–2489.
82. Clarke JA, de Burgh Daly M, Ead HW. Dimensions and volume of the carotid body in the adult cat, and their relation to the specific blood flow through the organ. A histological and morphometric study. *Acta Anat (Basel)*. 1986;126(2):84–86.
83. De Burgh Daly M, Lambertsen CJ, Schweitzer A. Observations on the volume of blood flow and

oxygen utilization of the carotid body in the cat. *J Physiol (Lond)*. 1954;125(1):67–89.
84. Yuan G, Nanduri J, Khan S, Semenza GL, Prabhakar NR. Induction of HIF-1alpha expression by intermittent hypoxia: involvement of NADPH oxidase, Ca2+ signaling, prolyl hydroxylases, and mTOR. *J Cell Physiol*. 2008;217(3):674–685.
85. Nanduri J, Vaddi DR, Khan SA, Wang N, Makerenko V, Prabhakar NR. Xanthine oxidase mediates hypoxia-inducible factor-2α degradation by intermittent hypoxia. *PLoS One*. 2013;8(10):e75838.
86. Nanduri J, et al. HIF-1α activation by intermittent hypoxia requires NADPH oxidase stimulation by xanthine oxidase. *PLoS One*. 2015;10(3):e0119762.
87. 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(11):2925–2933.
88. Iyer NV, et al. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev*. 1998;12(2):149–162.
89. Yu AY, et al. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. *J Clin Invest*. 1999;103(5):691–696.
90. Peng YJ, et al. Hypoxia-inducible factor 2α (HIF-2α) heterozygous-null mice exhibit exaggerated carotid body sensitivity to hypoxia, breathing instability, and hypertension. *Proc Natl Acad Sci U S A*. 2011;108(7):3065–3070.
91. Prabhakar NR. Oxygen sensing during intermittent hypoxia: cellular and molecular mechanisms. *J Appl Physiol*. 2001;90(5):1986–1994.
92. Dyugovskaya L, Lavie P, Lavie L. Increased adhesion molecules expression and production of reactive oxygen species in leukocytes of sleep apnea patients. *Am J Respir Crit Care Med*. 2002;165(7):934–939.
93. Prabhakar NR, Kumar GK, Nanduri J, Semenza GL. ROS signaling in systemic and cellular responses to chronic intermittent hypoxia. *Antioxid Redox Signal*. 2007;9(9):1397–1403.
94. Grebe M, et al. Antioxidant vitamin C improves endothelial function in obstructive sleep apnea. *Am J Respir Crit Care Med*. 2006;173(8):897–901.
95. Chen Q, et al. The effect of continuous positive airway pressure on circulating malondialdehyde among obstructive sleep apnea patients: a meta-analysis [published online December 4, 2019]. *Sleep Breath*. https://doi.org/10.1007/s11325-019-01998-x.
96. Gardner PR. Aconitase: sensitive target and measure of superoxide. *Meth Enzymol*. 2002;349:9–23.
97. Peng YJ, Nanduri J, Raghuraman G, Wang N, Kumar GK, Prabhakar NR. Role of oxidative stress-induced endothelin-converting enzyme activity in the alteration of carotid body function by chronic intermittent hypoxia. *Exp Physiol*. 2013;98(11):1620–1630.
98. Peng YJ, et al. NADPH oxidase is required for the sensory plasticity of the carotid body by chronic intermittent hypoxia. *J Neurosci*. 2009;29(15):4903–4910.
99. Pawar A, et al. Reactive oxygen species-dependent endothelin signaling is required for augmented hypoxic sensory response of the neonatal carotid body by intermittent hypoxia. *Am J Physiol Regul Integr Comp Physiol*. 2009;296(3):R735–R742.
100. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev*. 2007;87(1):245–313.
101. Ambrosio G, et al. Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. *J Biol Chem*. 1993;268(25):18532–18541.
102. Khan SA, et al. NADPH oxidase 2 mediates intermittent hypoxia-induced mitochondrial complex I inhibition: relevance to blood pressure changes in rats. *Antioxid Redox Signal*. 2011;14(4):533–542.
103. Scortegagna M, et al. Multiple organ pathology, metabolic abnormalities and impaired homeostasis of reactive oxygen species in Epas1-/- mice. *Nat Genet*. 2003;35(4):331–340.
104. Prabhakar NR, Dinerman JL, Agani FH, Snyder SH. Carbon monoxide: a role in carotid body chemoreception. *Proc Natl Acad Sci U S A*. 1995;92(6):1994–1997.
105. Peng YJ, et al. Inherent variations in CO-H2S-mediated carotid body O2 sensing mediate hypertension and pulmonary edema. *Proc Natl Acad Sci U S A*. 2014;111(3):1174–1179.
106. Prabhakar NR. Sensing hypoxia: physiology, genetics and epigenetics. *J Physiol (Lond)*. 2013;591(9):2245–2257.
107. Yuan G, et al. Protein kinase G-regulated production of H2S governs oxygen sensing. *Sci Signal*. 2015;8(373):ra37.
108. Peng YJ, et al. Complementary roles of gasotransmitters CO and H2S in sleep apnea. *Proc Natl Acad Sci U S A*. 2017;114(6):1413–1418.
109. Alajmi M, et al. Impact of continuous positive airway pressure therapy on blood pressure in patients with obstructive sleep apnea hypopnea: a meta-analysis of randomized controlled trials. *Lung*. 2007;185(2):67–72.
110. Montesi SB, Edwards BA, Malhotra A, Bakker JP. The effect of continuous positive airway pressure treatment on blood pressure: a systematic review and meta-analysis of randomized controlled trials. *J Clin Sleep Med*. 2012;8(5):587–596.
111. Bazzano LA, Khan Z, Reynolds K, He J. Effect of nocturnal nasal continuous positive airway pressure on blood pressure in obstructive sleep apnea. *Hypertension*. 2007;50(2):417–423.
112. Kushida CA, et al. Effects of continuous positive airway pressure on neurocognitive function in obstructive sleep apnea patients: The Apnea Positive Pressure Long-term Efficacy Study (APPLES). *Sleep*. 2012;35(12):1593–1602.
113. Rosen CL, et al. A multisite randomized trial of portable sleep studies and positive airway pressure autotitration versus laboratory-based polysomnography for the diagnosis and treatment of obstructive sleep apnea: the HomePAP study. *Sleep*. 2012;35(6):757–767.
114. Philippi NR, Bird CE, Marcus NJ, Olson EB, Chesler NC, Morgan BJ. Time course of intermittent hypoxia-induced impairments in resistance artery structure and function. *Respir Physiol Neurobiol*. 2010;170(2):157–163.
115. Eckert DJ, White DP, Jordan AS, Malhotra A, Wellman A. Defining phenotypic causes of obstructive sleep apnea. Identification of novel therapeutic targets. *Am J Respir Crit Care Med*. 2013;188(8):996–1004.
116. Wellman A, et al. A method for measuring and modeling the physiological traits causing obstructive sleep apnea. *J Appl Physiol*. 2011;110(6):1627–1637.
117. Nock ML, Difiore JM, Arko MK, Martin RJ. Relationship of the ventilatory response to hypoxia with neonatal apnea in preterm infants. *J Pediatr*. 2004;144(3):291–295.
118. Peng YJ, Rennison J, Prabhakar NR. Intermittent hypoxia augments carotid body and ventilatory response to hypoxia in neonatal rat pups. *J Appl Physiol*. 2004;97(5):2020–2025.
119. Pawar A, Peng YJ, Jacono FJ, Prabhakar NR. Comparative analysis of neonatal and adult rat carotid body responses to chronic intermittent hypoxia. *J Appl Physiol*. 2008;104(5):1287–1294.
120. Nanduri J, et al. Epigenetic regulation of hypoxic sensing disrupts cardiorespiratory homeostasis. *Proc Natl Acad Sci U S A*. 2012;109(7):2515–2520.
121. Paavonen EJ, et al. Very low birth weight increases risk for sleep-disordered breathing in young adulthood: the Helsinki Study of Very Low Birth Weight Adults. *Pediatrics*. 2007;120(4):778–784.
122. Hibbs AM, et al. Prenatal and neonatal risk factors for sleep disordered breathing in school-aged children born preterm. *J Pediatr*. 2008;153(2):176–182.
123. Nanduri J, Peng YJ, Wang N, Khan SA, Semenza GL, Prabhakar NR. DNA methylation in the central and efferent limbs of the chemoreflex requires carotid body neural activity. *J Physiol (Lond)*. 2018;596(15):3087–3100.
124. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature*. 2007;447(7143):433–440.
125. Asghar Z, Yau D, Chan F, Leroith D, Chan CB, Wheeler MB. Insulin resistance causes increased beta-cell mass but defective glucose-stimulated insulin secretion in a murine model of type 2 diabetes. *Diabetologia*. 2006;49(1):90–99.
126. Wang N, Khan SA, Prabhakar NR, Nanduri J. Impairment of pancreatic β-cell function by chronic intermittent hypoxia. *Exp Physiol*. 2013;98(9):1376–1385.
127. Cantley J, et al. Deletion of the von Hippel-Lindau gene in pancreatic beta cells impairs glucose homeostasis in mice. *J Clin Invest*. 2009;119(1):125–135.
128. Cheng K, et al. Hypoxia-inducible factor-1alpha regulates beta cell function in mouse and human islets. *J Clin Invest*. 2010;120(6):2171–2183.
129. Varga AW, et al. Apnea-induced rapid eye movement sleep disruption impairs human spatial navigational memory. *J Neurosci*. 2014;34(44):14571–14577.
130. Gildeh N, Drakatos P, Higgins S, Rosenzweig I, Kent BD. Emerging co-morbidities of obstructive sleep apnea: cognition, kidney disease, and cancer. *J Thorac Dis*. 2016;8(9):E901–E917.
131. Devita M, et al. Obstructive sleep apnea and its controversial effects on cognition. *J Clin Exp Neuropsychol*. 2017;39(7):659–669.
132. Devita M, et al. Cognitive and motor reaction times in obstructive sleep apnea syndrome: a study based on computerized measures. *Brain*

Cogn. 2017;117:26–32.
133. Leng Y, McEvoy CT, Allen IE, Yaffe K. Association of sleep-disordered breathing with cognitive function and risk of cognitive impairment: a systematic review and meta-analysis. *JAMA Neurol*. 2017;74(10):1237–1245.
134. Bucks RS, Olaithe M, Eastwood P. Neurocognitive function in obstructive sleep apnoea: a meta-review. *Respirology*. 2013;18(1):61–70.
135. Bucks RS, Olaithe M, Rosenzweig I, Morrell MJ. Reviewing the relationship between OSA and cognition: where do we go from here? *Respirology*. 2017;22(7):1253–1261.
136. Sforza E, Celle S, Saint-Martin M, Barthélémy JC, Roche F. Hippocampus volume and subjective sleepiness in older people with sleep-disordered breathing: a preliminary report. *J Sleep Res*. 2016;25(2):190–193.
137. Cha J, et al. The effects of obstructive sleep apnea syndrome on the dentate gyrus and learning and memory in children. *J Neurosci*. 2017;37(16):4280–4288.
138. Macey PM, et al. Sex-specific hippocampus volume changes in obstructive sleep apnea. *Neuroimage Clin*. 2018;20:305–317.
139. Song X, et al. Altered resting-state hippocampal and caudate functional networks in patients with obstructive sleep apnea. *Brain Behav*. 2018;8(6):e00994.
140. Owen JE, BenediktsdÓttir B, Gislason T, Robinson SR. Neuropathological investigation of cell layer thickness and myelination in the hippocampus of people with obstructive sleep apnea. *Sleep*. 2019;42(1):zsy199.
141. Gozal D, et al. Temporal aspects of spatial task performance during intermittent hypoxia in the rat: evidence for neurogenesis. *Eur J Neurosci*. 2003;18(8):2335–2342.
142. Row BW, Kheirandish L, Neville JJ, Gozal D. Impaired spatial learning and hyperactivity in developing rats exposed to intermittent hypoxia. *Pediatr Res*. 2002;52(3):449–453.
143. Goldbart A, Cheng ZJ, Brittian KR, Gozal D. Intermittent hypoxia induces time-dependent changes in the protein kinase B signaling pathway in the hippocampal CA1 region of the rat. *Neurobiol Dis*. 2003;14(3):440–446.
144. Payne RS, Goldbart A, Gozal D, Schurr A. Effect of intermittent hypoxia on long-term potentiation in rat hippocampal slices. *Brain Res*. 2004;1029(2):195–199.
145. Xie H, et al. Brain-derived neurotrophic factor rescues and prevents chronic intermittent hypoxia-induced impairment of hippocampal long-term synaptic plasticity. *Neurobiol Dis*. 2010;40(1):155–162.
146. Zhang SX, Wang Y, Gozal D. Pathological consequences of intermittent hypoxia in the central nervous system. *Compr Physiol*. 2012;2(3):1767–1777.
147. Wall AM, Corcoran AE, O'Halloran KD, O'Connor JJ. Effects of prolyl-hydroxylase inhibition and chronic intermittent hypoxia on synaptic transmission and plasticity in the rat CA1 and dentate gyrus. *Neurobiol Dis*. 2014;62:8–17.
148. Nair D, Dayyat EA, Zhang SX, Wang Y, Gozal D. Intermittent hypoxia-induced cognitive deficits are mediated by NADPH oxidase activity in a murine model of sleep apnea. *PLoS One*. 2011;6(5):e19847.
149. Chou YT, et al. C/EBP homologous binding protein (CHOP) underlies neural injury in sleep apnea model. *Sleep*. 2013;36(4):481–492.
150. Arias-Cavieres A, Khuu MA, Nwakudu CU, Barnard JE, Dalgin G, Garcia AJ. A HIF1a-dependent pro-oxidant state disrupts synaptic plasticity and impairs spatial memory in response to intermittent hypoxia. *eNeuro*. 2020;7(3):ENEURO.0024-20.2020.
151. Schultz HD, Li YL. Carotid body function in heart failure. *Respir Physiol Neurobiol*. 2007;157(1):171–185.
152. Sun SY, Wang W, Zucker IH, Schultz HD. Enhanced activity of carotid body chemoreceptors in rabbits with heart failure: role of nitric oxide. *J Appl Physiol*. 1999;86(4):1273–1282.
153. Moreno-Domínguez A, et al. Acute O_2 sensing through HIF2α-dependent expression of atypical cytochrome oxidase subunits in arterial chemoreceptors. *Sci Signal*. 2020;13(615):eaay9452.
154. Fielding JW, et al. PHD2 inactivation in Type I cells drives HIF-2α-dependent multilineage hyperplasia and the formation of paraganglioma-like carotid bodies. *J Physiol (Lond)*. 2018;596(18):4393–4412.
155. Courtney KD, et al. Phase I dose-escalation trial of PT2385, a first-in-class hypoxia-inducible factor-2α antagonist in patients with previously treated advanced clear cell renal cell carcinoma. *J Clin Oncol*. 2018;36(9):867–874.