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Hypoxia-inducible factors and obstructive sleep apnea

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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).

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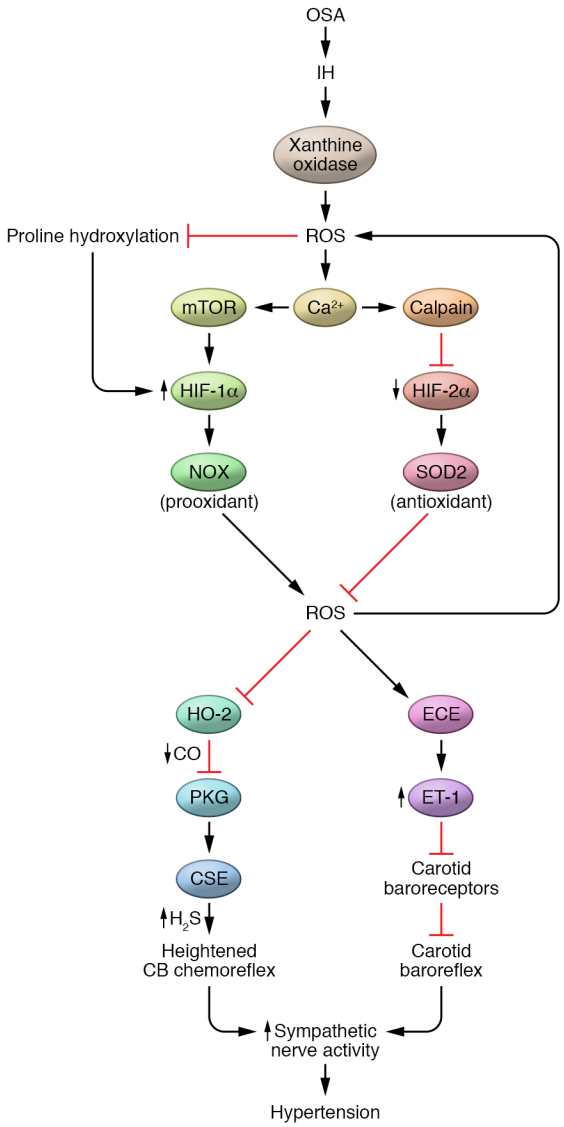

Figure 1. Schematic presentation of HIF-dependent signaling pathways in OSA-induced hypertension. Hypoxia-induced changes in HIF-1α and HIF-2α levels exacerbate increases in ROS levels. Within the carotid body, ROS elevations modify the balance between CO and H_2S (lower left) as well as attenuate the 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

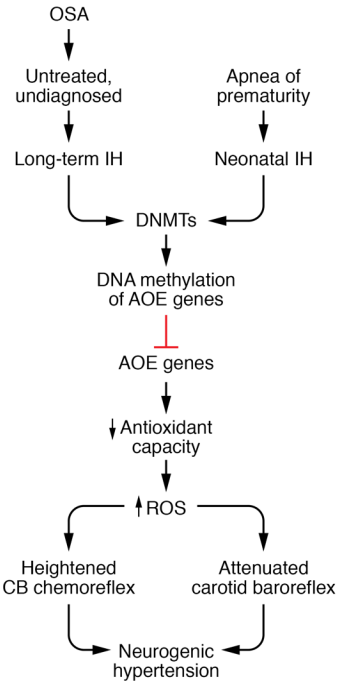

Figure 2. Activation of epigenetic mechanisms involving DNA methylation of antioxidant enzyme genes either in response to long-term IH associated with untreated and undiagnosed OSA or in young adults who had apnea of prematurity in neonatal life. AOE, antioxidant enzyme; DNMTs, DNA methyltransferases.

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

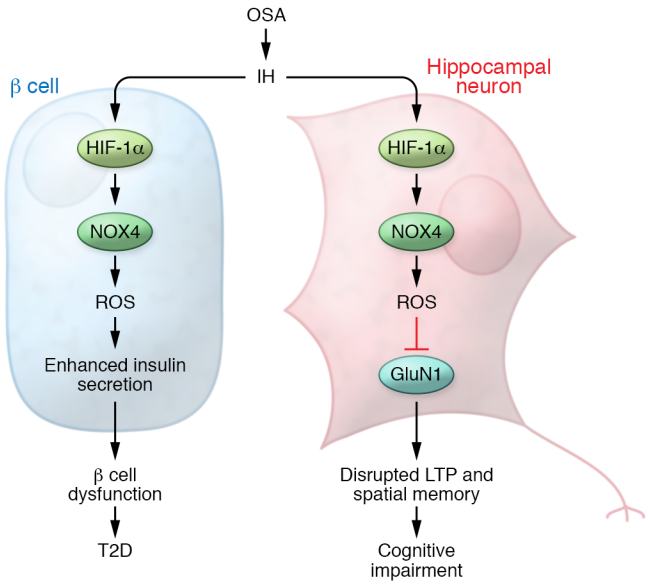

Figure 3. Schematic presentation of proposed mechanism(s) for HIF-1–dependent pancreatic β cell dysfunction manifesting as hypersecretion of insulin and insulin resistance and cognitive dysfunction evoked by OSA/IH. GluN1, glutamate ionotropic receptor NMDA type subunit 1; LTP, long-term potentiation.

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).

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