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REVIEW-SYMPOSIUM

Carotid body hypersensitivity in intermittent hypoxia and obtructive sleep apnoea

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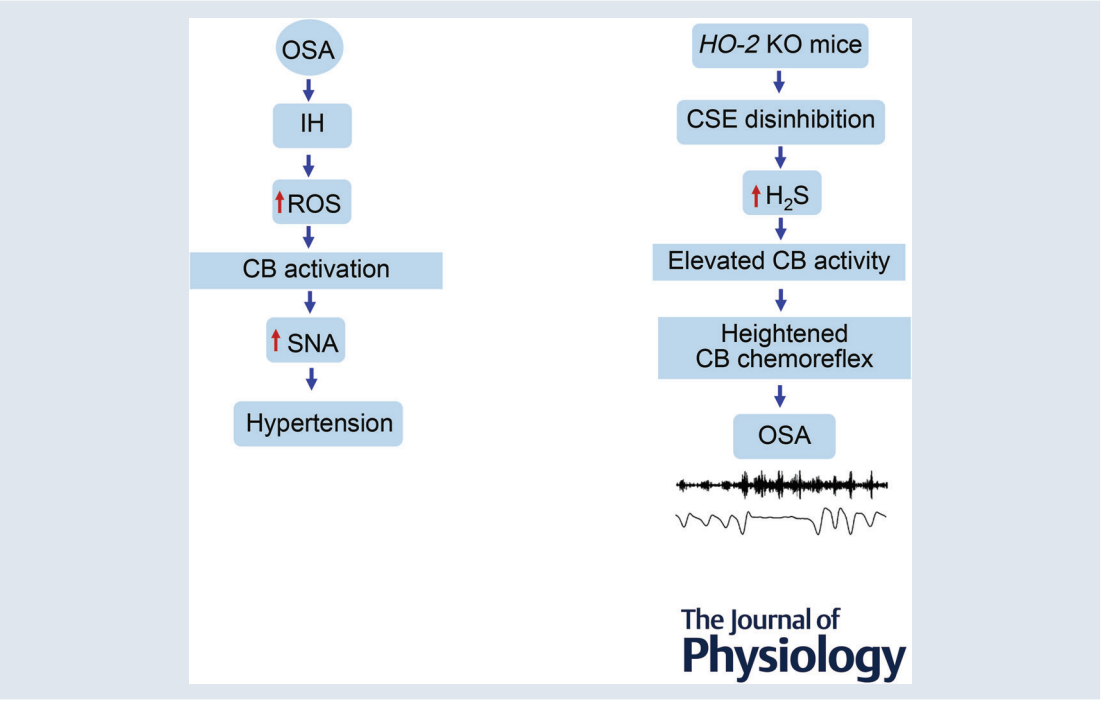

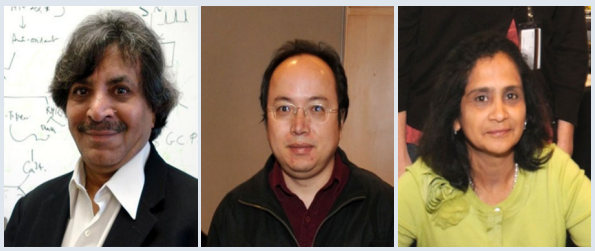

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Abstract Carotid bodies are the principal sensory organs for detecting changes in arterial blood oxygen concentration, and the carotid body chemoreflex is a major regulator of the sympathetic tone, blood pressure and breathing. Intermittent hypoxia is a hallmark manifestation of obstructive sleep apnoea (OSA), which is a widespread respiratory disorder. In the first part of this review, we discuss the role of carotid bodies in heightened sympathetic tone and hypertension in rodents treated with intermittent hypoxia, and the underlying cellular, molecular and epigenetic mechanisms. We also present evidence for hitherto-uncharacterized role of carotid body afferents in triggering cellular and molecular changes induced by intermittent hypoxia. In the second part of the review, we present evidence for a contribution of a hypersensitive carotid body to OSA and potential therapeutic intervention to mitigate OSA in a murine model.

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Abstract figure legend Hyperactive sympathetic nervous system (SNA) and hypertension are major comorbidities of obstructive sleep apnea (OSA). Intermittent hypoxia (IH) is a hallmark manifestation of OSA. First part of the review discuss the role of hyperactive CB in sympathetic activation and hypertension and underlying cellular and molecular mechanisms in rodents treated with IH (*left panel*). Second part of the review presents evidence for hyperactive CB as a cause of OSA in a murine model (*right panel*). Abbreviations: CSE, cystathionine-gama-lyase; HO2, hemeoxygease-2; KO, knockout; ROS, reactive oxygen species.

Introduction

Carotid bodies are sensory organs for monitoring arterial blood O_2 levels (Kumar & Prabhakar, 2012). Hypoxaemia increases carotid body sensory nerve activity, which is transmitted to brainstem neurons, triggering reflex stimulation of breathing and blood pressure to maintain homeostasis. A heightened carotid body chemoreflex has been implicated in several physiological (Kumar & Prabhakar, 2012; Prabhakar et al., 2022) and pathological conditions, such as heart failure and neurogenic hypertension (Iturriaga et al., 2021; Marcus et al., 2014) and metabolic dysfunction (Cunha-Guimaraes et al., 2020; Iturriaga et al., 2021; Marcus et al., 2014).

In this review, we focus on the role of carotid bodies in rodents treated with intermittent hypoxia (IH) patterned after blood O_2 profiles occurring in obstructive sleep apnoea (OSA), which is a widespread breathing disorder during sleep (Dempsey et al., 2010; Peppard et al., 2013). Several co-morbidities are associated with OSA, most notably an overactive sympathetic nervous system and hypertension (Dempsey et al., 2010; Lavie et al., 2000; Nieto et al., 2000; Peppard et al., 2000). In the first part of this review, we discuss the evidence for a hyperactive carotid body chemoreflex as major contributor to activation of the sympathetic nervous system and hypertension in rodents treated with IH, a hallmark manifestation of OSA, and discuss the underlying cellular, molecular and epigenetic mechanisms. We also present evidence for a hitherto-uncharacterized role of the carotid body in triggering cellular and molecular changes induced by IH in the carotid body chemoreflex pathway. Emerging evidence in OSA patients suggests an increased loop gain of the chemoreflex as one of the factors contributing to OSA (Eckert et al., 2013). In the second part of the review, we present evidence that increased carotid body chemoreflex loop gain leads to OSA and that reducing the chemoreflex loop gain by pharmacological intervention prevents OSA in a murine model.

Hypertension and sympathetic nerve activity in OSA patients

Patients with OSA exhibit marked increases in arterial blood pressure during apnoeic episodes and exhibit daytime hypertension even in the absence of apnoeas (Peppard et al., 2000; Tamisier et al., 2011). Daytime hypertension is strongly correlated with the apnoea–hypopnoea index (Peppard et al., 2000). Hypertension in OSA patients is independent of obesity and periodic arousals from sleep (Morrell et al., 2000). Biomarkers of sympathetic nerve activation (SNA) are elevated in OSA patients, as indicated by elevated circulating and urinary catecholamines (Carlson et al., 1993; Marrone et al., 1993; Somers et al., 1995). Obstructive sleep apnoea patients exhibit elevated muscle sympathetic nerve activity (MSNA), which is an index of vascular tone (Narkiewicz & Somers, 1997; Okada et al., 1991). These findings suggest that heightened SNA and hypertension are two major cardiovascular co-morbidities associated with OSA.

Stimulus for OSA-associated SNA and hypertension

Obstructive sleep apnoea leads to IH, hypercapnia, periodic arousals and changes in intrathoracic pressure. Of these, IH is a hallmark manifestation of OSA, and severely affected patients exhibit nearly 50% reduction in arterial blood O_2 saturation. Two lines of evidence indicate IH as a major stimulus for causing hypertension and elevated SNA in OSA patients. First, continuous positive airway pressure, a treatment of choice for OSA, improves oxygenation in OSA patients and lowers blood pressure (Becker et al., 2003; Imadojemu et al., 2007; Somers et al., 1995). Second, in rodents subjected to IH patterned after blood O_2 desaturations in OSA subjects, alone is sufficient to cause hypertension and elevated SNA (Fletcher et al., 1992; Hui et al., 2003; Joyeux-Faure et al., 2005; Kanagy et al., 2001; Knight et al., 2011; Kumar et al., 2006; Silva & Schreihofer, 2011; Yuan et al., 2016; Zoccal et al., 2007).

How IH activates SNA and blood pressure

Hypoxaemia (reduction in blood O_2 level) activates the carotid body. It was proposed that OSA activates the carotid body chemoreflex, which mediates SNA and hypertension (Cistulli & Sullivan, 1994; Fig. 1). The following observations support such a possibility: (1) the carotid body chemoreflex is augmented in OSA patients (Hedner et al., 1992) and in rodents treated with IH (Peng et al., 2006; Rey et al., 2004); (2) hyperoxia, which inhibits the carotid body, reduces blood pressure and SNA in OSA patients (Narkiewicz et al., 1999); (3) surgical removal of the carotid bodies of OSA patients prevents hypertension (Somers & Abboud, 1993); and (4) either surgical ablation of the carotid sinus nerves (Fletcher et al., 1992) or selective ablation of the carotid body prevents elevated SNA and hypertension in IH-treated rats (Peng, Yuan et al., 2014).

Intermittent hypoxia activates the carotid bodies

The enhanced carotid body chemoreflex in OSA patients is attributable either to enhanced carotid body sensitivity to hypoxia and/or to processing of chemosensory information at the brainstem neurons. The carotid body sensory nerve activity was recorded in rodents subjected to 15 s of hypoxia and 5 min room air per episode; nine episodes per hour each day for 10 days. This protocol of IH decreased arterial blood O_2 saturation from 97 to 80% in rats (Peng, Yuan et al., 2014). IH-treated rats and mice showed sensitization of the carotid body neural response to hypoxia and sensory long-term facilitation (sLTF) manifested as a long-lasting increase in baseline sensory nerve activity following acute repetitive hypoxia (Peng et al., 2003; Fig. 2). Sensitization of the carotid body by IH was also reported not only in rats (Peng & Prabhakar, 2004), but also in cats (Rey et al., 2004). In anaesthetized rats, IH-induced sLTF was seen despite maintained arterial blood gases and blood pressure. In contrast, sLTF was not seen in rats subjected to either to a single episode of hypoxia for 4 h or to 4 h of hypoxia per day for 10 days (Peng & Prabhakar, 2004; Peng et al., 2003), suggesting that sLTF is unique to IH simulating blood O_2 levels during OSA. OSA patients (Somers et al., 1995) and IH-treated rodents (Peng, Yuan et al., 2014) exhibit elevated SNA in basal conditions. It is likely that sLTF might account for elevated basal SNA in IH-treated rodents and, possibly, in OSA patients (Prabhakar, 2013).

Peng et al. (2003) were unable to elicit sLTF in control rats reared in room air. However, Cummings & Wilson (2005) reported sLTF in control rats challenged with repetitive hypoxia combined with severe hypercapnia (P_{CO_2} of ~70 mmHg). The significance of sLTF in control rats is not known.

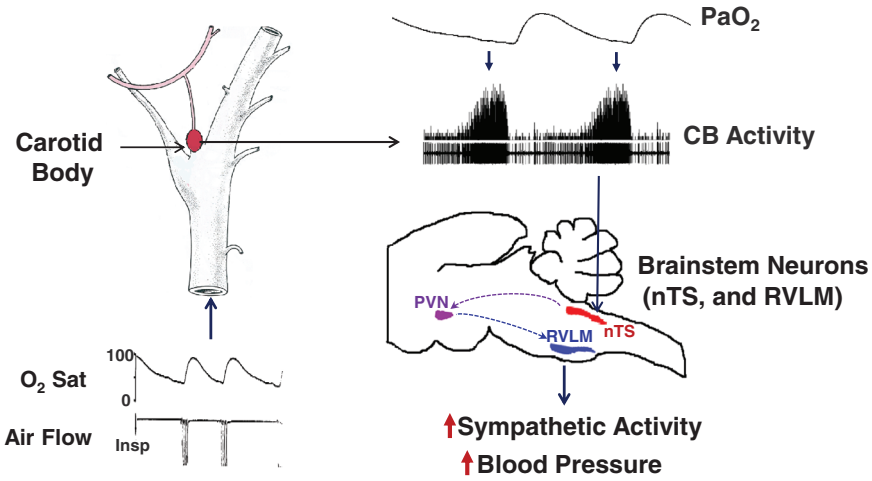


Figure 1. Schematic presentation of contribution of the carotid body chemoreflex to elevated sympathetic nerve activity and blood pressure in obstructive sleep apnoea
Abbreviations: CB activity, carotid body neural activity; NTS, nucleus tractus solitarii; PaO_2, arterial partial pressure of oxygen; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla.

Mechanism(s) of carotid body activation by IH

How might IH activate carotid body? A number of signalling molecules in the carotid body are affected by IH, including Two-pore domain (TASK) K^+ channels (Ortiz et al., 2013) and inflammatory cytokines (Del Rio et al., 2012). However, their contribution to the carotid body activation by IH is not known. Intermittent hypoxia has no effect on the number of glomus cells or the total volume of the carotid body (Del Rio et al., 2011; Peng et al., 2003), indicating that altered morphology is not likely to account for the carotid body activation by IH.

Reactive oxygen species. The following observations suggest that reactive oxygen species (ROS) mediate carotid body activation by IH. First, ROS scavengers prevent carotid body sensitization to hypoxia and sLTF in IH-treated rodents (Del Rio et al., 2010; Peng & Prabhakar, 2004; Peng et al., 2003, 2012). Second, IH increases ROS abundance in the carotid body as measured by aconitase enzyme activity (Peng et al., 2003) or malondialdehyde levels (Peng et al., 2013), which are indices of oxidized lipids. Likewise, IH-treated carotid bodies exhibit elevated biomarker levels of oxidative stress (i.e. serum 8-isoprostane and nitrotyrosine) (Lam et al., 2014).

The mechanisms associated with ROS generation were examined in rodents and cell culture subjected to IH. Generation of ROS by IH is complex, involving distinct inter-related mechanisms, and depends on the duration of IH, as described below.

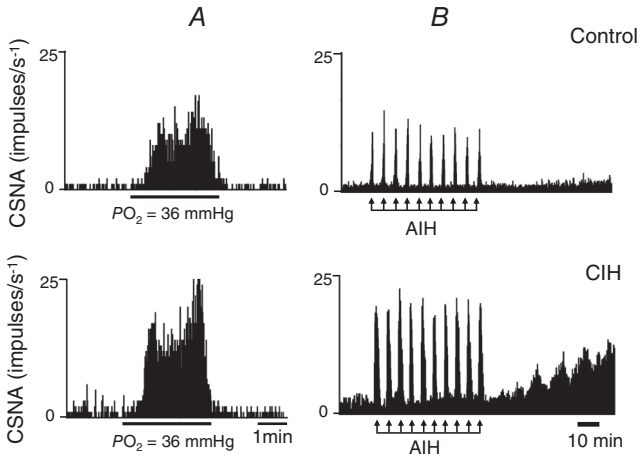


Figure 2. Examples of sensitization of carotid body response to hypoxia (*A*) and sensory long-term facilitation (*B*) in rats subjected to 10 days of intermittent hypoxia
Each episode of intermittent hypoxia consists of a cycle of 15 s of hypoxia followed by 5 min normoxia, with nine cycles per hour for 8 h each day. Abbreviations: AIH, acute intermittent hypoxia; CSNA, carotid body sensory nerve activity. Adapted from *Respir. Physiol. Neurobiol.*, *157*, 148–153, 2007.

Stage 1: non-transcriptional mechanisms. In the early stages, IH generates ROS by directly activating xanthine oxidase (XO), a pro-oxidant enzyme, through proteolytic conversion of xanthine dehydrogenase (XDH) to XO (Nanduri et al., 2013). The XO-generated ROS increase cytosolic $[Ca^{2+}]_i$, which facilitates translocation of p47phox and p67phox subunits from the cytosol to the plasma membrane, thereby activating another pro-oxidant enzyme, NADPH oxidase 2 (Nox2). The ROS generated by Nox2 facilitate Ca^{2+} influx into mitochondria, inhibiting complex I of the mitochondrial electron transport chain (ETC), leading to further ROS generation (Khan et al., 2011). After terminating IH, ROS generation by Nox2 is reversed within few hours, whereas ROS generated by complex I inhibition lasts as long as 18 h (Khan et al., 2011). Intermittent hypoxia decreased complex I activity in the carotid body, and this effect was associated with an augmented carotid body neural response to hypoxia and sLTF (Peng et al., 2003). The effects of IH on the carotid body involving mitochondrial complex I (MCI) appear opposite to the reported impairment of the glomus cell and breathing rate responses to acute hypoxia with inducible knockdown of MCI in mice (Arias-Mayenco et al., 2018). These authors suggested that glomus cell responses to acute hypoxia depend on the $CoQH_2/CoQ$ ratio, NADH accumulation and compartmentalization of ROS at MCI. Arias-Mayenco et al. (2018) studied the glomus cell response to severe hypoxia (P_{O_2} of ∼10–15 mmHg) with inducible deletion of *Ndufs2 gene* in mice, whereas Peng et al. (2003) examined the carotid body sensory nerve response to acute hypoxia in rats subjected 10 days of IH. It is possible that the methodological differences between both studies account for the discrepancy in their results. Nonetheless, these observations suggest that ROS generation in the early stages of IH involves a non-transcriptional and positive feed-forward mechanism involving ROS-induced ROS (Fig. 3).

Stage 2: transcriptional mechanisms. As IH continues, ROS generated by non-transcriptional mechanisms recruit transcriptional regulation of ROS involving hypoxia-inducible factor (HIF)-1 and HIF-2 transcription factors (Fig. 3).

Hypoxia-inducible factor-1. Prolonged hypoxia increases the accumulation of HIF-1α protein, an essential pre-requisite for HIF-1-dependent transcription (Prabhakar & Semenza, 2012). Despite a brief duration of hypoxia, lasting only tens of seconds, IH increases HIF-1α protein in all three major components of the carotid body chemoreflex, including the carotid body (Lam et al., 2006), brainstem neurons [nucleus tractus solitarii (NTS) and rostral venrolateral medulla (RVLM), representing the central

component] and adrenal medulla (the efferent limb of the chemoreflex) (Nanduri et al., 2018). Intermittent hypoxia-induced HIF-1α accumulation requires ROS generated by non-transcriptional mechanisms. Reactive oxygen species increase HIF-1α protein synthesis through Ca^{2+}-dependent activation of mammalian target of rapamycin (mTOR) and by inhibition of its degradation by prolyl hydroxylases (Nanduri et al., 2013, 2015; Yuan et al., 2008). Hypoxia-inducible factor-1-dependent transcription activates genes encoding NADPH oxidase (*Nox*)*2* and *Nox4*, resulting in further ROS generation in the carotid body chemoreflex pathway (Nanduri et al., 2018) and in IH-treated cell cultures (Yuan et al., 2011). Mice partly deficient in HIF-1α exhibit an absence of IH-induced ROS generation, carotid body activation, hypertension and biomarkers of SNA (Peng et al., 2006).

Hypoxia-inducible factor-2. Hypoxia-inducible factor-2α, also called E-PAS, is an orthologue of HIF-1α. Continuous hypoxia increases HIF-2α protein, whereas IH decreases HIF-2α protein and HIF-2-dependent transcription (Nanduri et al., 2009). HIF-2α degradation by IH is mediated by ROS-dependent activation of Ca^{2+}-dependent calpain proteases (Nanduri et al., 2013). Calpains degrade HIF-2α by targeting the C-terminal trans-activation domain (CTAD) of HIF-2α (Nanduri et al., 2013). Intermittent hypoxia-exposed rats treated with calpain inhibitor exhibit absence of HIF-2α degradation, unaltered antioxidant enzyme activity and ROS levels and absence of hypertension (Nanduri et al., 2009). In summary, insufficient transcription of anti-oxidant enzymes and the resulting decrease in antioxidant capacity combined with increased oxidative capacity via HIF-1-dependent pro-oxidant enzymes contribute to ROS generation by IH (Nanduri et al., 2009).

Stage 3: epigenetic mechanisms. Following recovery in room air, ROS generation, carotid body activation, elevated SNA and hypertension were completely reversed in rats subjected to 10 days of IH. In contrast, all these responses evoked by long-term IH (30 days) persisted even after 30 days of recovery in room air (Nanduri et al., 2012, 2018; Nanduri, Peng et al., 2017). Long-lasting

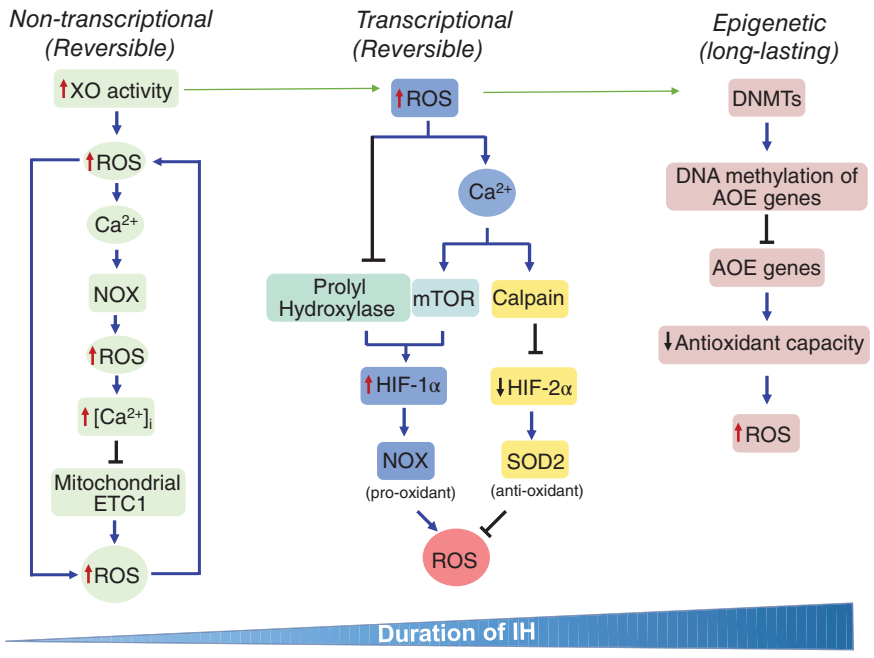


Figure 3. Schematic presentation of signalling pathways associated with time-dependent generation of reactive oxygen species by intermittent hypoxia
Non-transcriptional mechanisms contribute to ROS generation in early stages of IH. As IH continues, ROS generated by non-transcriptional mechanisms recruit transcriptional regulation of ROS by the hypoxia-inducible factors HIF-1 and HIF-2. Generation of ROS by non-transcriptional and transcriptional mechanisms is reversed by recovery in room air. Long-term IH activates epigenetic regulation of ROS by DNA hypermethylation, which persists even after several weeks of recovery in room air. Abbreviations: AOE, antioxidant enzyme; DNMT, DNA methyl transferase; HIF-1α, hypoxia-inducible factor 1 alpha subunit; HIF-2α, hypoxia-inducible factor 2 alpha subunit; IH, intermittent hypoxia; mitochondrial ETC1, mitochondrial electron transport chain at complex I; mTOR, mammalian target of rapamycin; NOX, NADPH oxidase; ROS, reactive oxygen species; XO, xanthine oxidase. Adapted from *Handbook of Clin. Neurol.*, *188*, 103–123, 2022.

physiological responses are often attributed to gene regulation by epigenetic mechanisms that involve altered chromatin affecting the accessibility of the DNA for transcription factors without changes in the coding sequence of DNA *per se* (Feinberg, 2007). DNA methylation is one such epigenetic mechanism regulating the gene expression. Increased DNA methylation suppresses gene transcription, and DNA hypomethylation activates gene transcription (for references, see Nanduri, Semenza et al., 2017). DNA methylation is catalysed by DNA methyl transferases (Dnmts), including Dnmt1, Dnmt3a and Dnmt3b (Bird, 2002). Rats treated with long-term IH (30 days) showed elevated Dnmt enzyme activity, Dnmt1, Dnmt3a and Dnmt3b proteins, and DNA hypermethylation of genes encoding antioxidant enzymes in the carotid body, NTS, RVLM and adrenal medulla (Nanduri et al., 2012; Nanduri, Peng et al., 2017). Further analysis of the *Sod2* gene showed hypermethylation of a single CpG dinucleotide in the region close to the transcription start site in rats subjected to long-term IH (Nanduri et al., 2012; Nanduri, Peng et al., 2017). Treatment of rats with decitabine, a DNA hypomethylating agent, during long-term IH blocked DNA hypermethylation, restored gene expressions of antioxidant enzymes, normalized ROS levels in the chemoreflex pathway and prevented hypertension (Nanduri et al., 2012; Nanduri, Peng et al., 2017) (Fig. 3).

How might long-term IH activate DNA methylation? Dnmts are central for DNA methylation. Long-term IH increases Dnmt protein through post-translational mechanisms involving ROS-dependent activation of Akt (protein kinase B) and subsequent inactivation of glycogen synthase 3β (GSK3β) (Nanduri et al., 2018; Nanduri, Peng et al., 2017). Blockade of GSK3β inactivation with GSK690693 (10 mg/kg I.P.) normalized Dnmt protein levels, preventing DNA hypermethylation of antioxidant enzyme genes, sympathetic activation and hypertension in rats subjected to long-term IH (Nanduri et al., 2018).

The above findings suggest that epigenetic regulation by DNA methylation represents a molecular basis of time-dependent progression of autonomic changes by IH from a reversible to a long-lasting or quasi-permanent phenotype.

Epigenetic regulation of pro-oxidant enzymes. Besides DNA methylation, other epigenetic mechanisms include acetylation and methylation of lysine residues in histones. Recent studies have shown that IH regulates histone lysine acetylation and methylation (Nanduri et al., 2021; Wang et al., 2021). Histone lysine acetylation involves reduced activity of histone deacetylases (HDACs), whereas histone lysine methylation increased activity of Jumanji C (JmjC)-containing histone lysine demethylases (JmjC-KDMs) (Nanduri et al., 2021). Intermittent hypoxia-evoked histone modifications facilitate HIF-1-dependent transcription of *Nox4* and thereby increase ROS generation (Nanduri et al., 2021; Wang et al., 2021).

Effect of IH on the central component of the carotid body chemoreflex

Afferent information from the carotid body is transmitted to the CNS for translation of the sensory information to activate SNA. Carotid body afferent input is transmitted to the NTS, especially the commissural part of the NTS (Chitravanshi & Sapru, 1995; Zhang & Mifflin, 1993), and the RVLM (Knight et al., 2011), a major site of preganglionic sympathetic premotor neurons.

Intermittent hypoxia activates NTS and RVLM neuronal activity, as indicated by increased expression of the Fos family of proteins (FosB/ΔfosB) (Knight et al., 2011) and c-Fos protein in the NTS (Sica et al., 2000). Activity of NTS neurons depends on a balance between the excitatory transmitter glutamate and the inhibitory transmitter dopamine. Intermittent hypoxia increases expression of *N*-methyl-D-aspartate receptor 1 (NMDA-R1), which mediates excitatory effects of glutamate (Reeves et al., 2003), whereas IH decreases expression of tyrosine hydroxylase, the rate-limiting enzyme of dopamine synthesis in the NTS (Gozal et al., 2005). Intermittent hypoxia increases postsynaptic NTS neuronal activity (Kline et al., 2007).

Neurons in the NTS relay information to the hypothalamic paraventricular nucleus and sympathoexcitatory neurons in the RVLM (Herman & Cullinan, 1997; Sawchenko et al., 1996). A recent study reported that IH increases RVLM neuronal activity linked to presympathetic neurons (Karlen-Amarante et al., 2023). On the one hand, Zoccal et al. (2011) believe that IH enhances purinergic but not glutamatergic transmission in the RVLM. On the other hand, Silva & Schreihofer (2011) found that glutamatergic transmission is crucial for RVLM activation by IH. These studies indicate that the exaggerated chemoreflex-dependent sympathetic activation by IH involves altered neurotransmitter profiles in brainstem neurons.

The carotid body mediates molecular changes by IH

The above-described studies suggest that the carotid body chemoreflex mediates systemic responses to IH manifested as SNA and hypertension. However, emerging evidence suggests that sensory information from the carotid body is also crucial for IH-evoked transcriptional and epigenetic changes in the central component and efferent limb of the chemoreflex. Given the exquisite sensitivity of glomus tissue to hypoxia,

molecular changes (transcriptional and epigenetic) are likely to be attributable to direct effects of IH on the carotid body. In contrast, IH-evoked molecular changes in the central component and efferent limb of the chemoreflex require afferent input from the carotid body. Evidence indicates that selective ablation of the carotid bodies prevents HIF-1α protein accumulation, HIF-1-dependent transcription and DNA methylation in the NTS and RVLM and in the adrenal medulla of IH-treated rats (Nanduri et al., 2018; Peng, Yuan et al., 2014). These findings indicate a hitherto-uncharacterized role for carotid body sensory input for initiating molecular mechanisms induced by IH in addition to the well-established role of the carotid bodies in evoking systemic autonomic responses. Further studies are needed to delineate the mechanisms by which carotid body sensory input activates transcriptional and epigenetic mechanisms.

How ROS mediate carotid body activation by IH

Studies described above suggest that ROS signalling is crucial for carotid body activation by IH. In this section, we summarize recent studies examining the signalling downstream from ROS associated with carotid body activation.

Recent evidence suggests that carotid body neural activation by acute hypoxia requires an O_2-dependent interaction between the gaseous messengers carbon monoxide (CO) and hydrogen sulphide (H_2S). CO inhibits Prabhakar et al. 1995, and H_2S stimulates the carotid body sensory nerve activity (Peng et al., 2010; Prabhakar, 2013). Carbon monoxide and H_2S are the products of the enzymes heme oxygenase 2 (HO-2) and cystathionine-γ-lyase (CSE), respectively. In normoxia, CO production is high and H_2S production is low in the carotid body (Peng, Makarenko et al., 2014). Lower H_2S abundance in normoxia is attributable to inhibition of CSE-dependent H_2S production by CO involving protein kinase G-dependent phosphorylation at the serine 377 residue in CSE (Yuan et al., 2015). In contrast, hypoxia increases H_2S production, and this effect is mediated by decreased CO production through inactivation of HO-2 by low O_2 (Peng, Makarenko et al., 2014). *CSE* null mice exhibit impaired carotid body sensory nerve, glomus cell and breathing response to hypoxia (Makarenko et al., 2012; Peng et al., 2010).

The role of CO–H_2S signalling in IH-induced carotid body activation, SNA and hypertension was examined by Yuan et al. (2016). Intermittent hypoxia increases H_2S production in the carotid body, and this effect involves ROS-dependent inactivation of HO-2. Either pharmacological (Fig. 4) or genetic blockade of H_2S synthesis prevents IH-evoked carotid body sensitization to hypoxia, sLTF, splanchnic SNA and hypertension (Yuan et al., 2016).

Murine carotid bodies express a high abundance of olfactory receptor 78 (Olfr78), a G-protein-coupled receptor (Chang et al., 2015; Zhou et al., 2016). Carotid body, glomus cell and breathing responses to hypoxia are impaired in *Olfr78* null mice (Chang et al., 2015). A later report claimed unaltered glomus cell responses to severe hypoxia (P_{O_2} of ∼10–15 mmHg) in *Olfr78* null mice (Torres-Torrelo et al., 2018). However, a subsequent study showed that *Olfr78* null mice do exhibit impaired sensory nerve and glomus cell responses to a wide range of hypoxia but not to severe hypoxia (P_{O_2} of ∼15 mmHg) (Peng et al., 2020). Moreover, IH-evoked carotid body sensitization to hypoxia and sLTF, and biomarkers of SNA and hypertension, were absent in *Olfr78* null mice

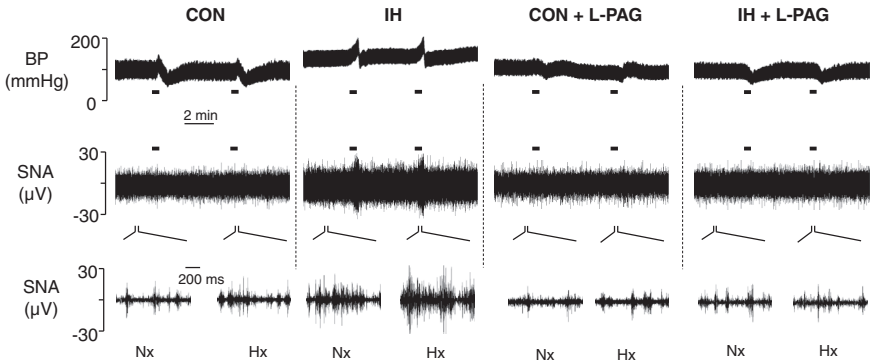


Figure 4. H_2S mediates chemosensory reflex-dependent sympathetic activation and hypertension in intermittent hypoxia-treated rats
Example of experiments from anaesthetized rats reared in room air (CON = control), subjected to IH (intermittent hypoxia for 10 days) treated with L-propargyl glycine, an H_2S synthesis inhibitor (L-PAG; 30 mg/kg/day for 10 days; I.P.). The bottom panels represent SNA on an expanded time scale. Black bars represent brief hypoxic change for 15 s. Abbreviations: BP, arterial blood pressure (in millimetres of mercury); Hx, 15 s of hypoxia represented by black bars; Nx, normoxia; SNA, splanchnic sympathetic nerve activity. Adapted from *Sci. Sig.*, *9*(441), ra80, 2016.

(Peng et al., 2021). *HO-2* null mice, which have a higher apnoea index (58 ± 1.2 apnoeas/h) than wild-type mice (8 ± 0.8 apnoeas/h), exhibit elevated blood pressure and plasma noradrenaline levels and a heightened carotid body response to hypoxia and sLTF, and all these responses are either absent or markedly attenuated in *HO-2*/*Olfr78* double null mice (Peng et al., 2021). These results suggest that Olfr78 participates in IH-induced SNA, hypertension and heightened carotid body activity. How might IH activate Olfr78? Olfr78 belongs to a family of sensory receptors detecting odorant stimuli. H_2S, which mediates carotid body activation, SNA and hypertension (Yuan et al., 2016), is an odorant gas. It is likely that Olfr78 activation by H_2S might contribute to carotid body activation by IH, an intriguing possibility that requires further studies. Signalling pathways associated with ROS-dependent CO–H_2S signalling in carotid body activation by IH are summarized in Fig. 5.

Heightened carotid body activity causes OSA

Obstructive sleep apnoea is a multifactorial disease. Factors contributing to OSA include compromised pharyngeal anatomy, inadequate upper airway muscle function, low arousal threshold and heightened chemoreflex feedback loop (i.e. enhanced loop gain) (Eckert & Younes, 2014; Eckert et al., 2009, 2013; Wellman et al., 2011). In this section, we present evidence that a heightened carotid body chemoreflex causes OSA in a murine model.

Murine model of OSA. Mice with genetic deletion of *HO-2* (*HO-2* null mice) exhibit irregular breathing, with apnoea and hypopnoea (i.e reduced tidal volume), compared with wild-type mice (Peng et al., 2017). Sixty per cent of apnoeas in *HO-2* null mice appear to be obstructive, as indicated by an absence of breathing movements with increased inspiratory intercostal muscle activity [inspiratory intercostal muscle EMG (I-EMG)], whereas 40% of apnoeas seem to be of central origin, as indicated by cessation of breathing with complete absence of I-EMG activity (Peng et al., 2017). Apnoea and hypopnoea indices are higher in non-rapid eye movement (NREM) and rapid eye movement (REM) sleep in *HO-2* null mice compared with wild-type controls and wakefulness (Table 1). These findings demonstrate that *HO-2* null mice manifest obstructive and central apnoea during NREM and REM sleep.

Evidence for carotid body mediation of OSA in *HO-2* null mice. *HO-2* null mice exhibit greater carotid body sensitivity to hypoxia and hypoxic ventilatory response (Peng et al., 2017). Hyperoxia (90% O_2), which inhibits carotid body activity, reduces obstructive and central apnoea indices. In contrast, mild hypoxia (15% O_2), which stimulates the carotid body, increases the incidence of obstructive and central apnoea by three- and twofold, respectively (Peng et al., 2017). Systemic administration of CORM-3, a CO donor, reduces the enhanced carotid body sensitivity to hypoxia and normalizes breathing in *HO-2* null mice (Peng et al., 2017). In contrast, mild hypercapnia (2% CO_2) reduces the only central apnoea index (Peng et al., 2017).

Increased H_2S production (from CSE) mediates the enhanced carotid body sensitivity in *HO-2* null mice (Yuan et al., 2015). *HO-2*$^{-/-}$ and *CSE*$^{-/-}$ double null mice exhibit stable breathing, without apnoeas or hypopnoeas (Peng et al., 2017). Either systemic or oral administration of L-propargyl glycine (L-PAG), a CSE inhibitor, reduces carotid body hypersensitivity and normalizes the breathing of *HO-2* null mice (Fig. 6), and the effects of the CSE inhibitor are dose dependent and reversible

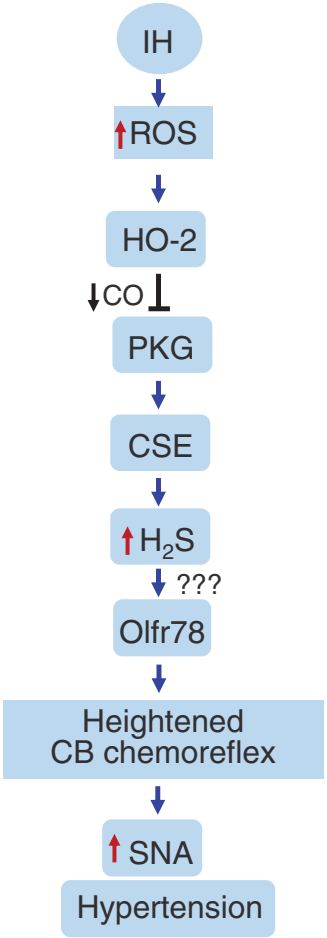
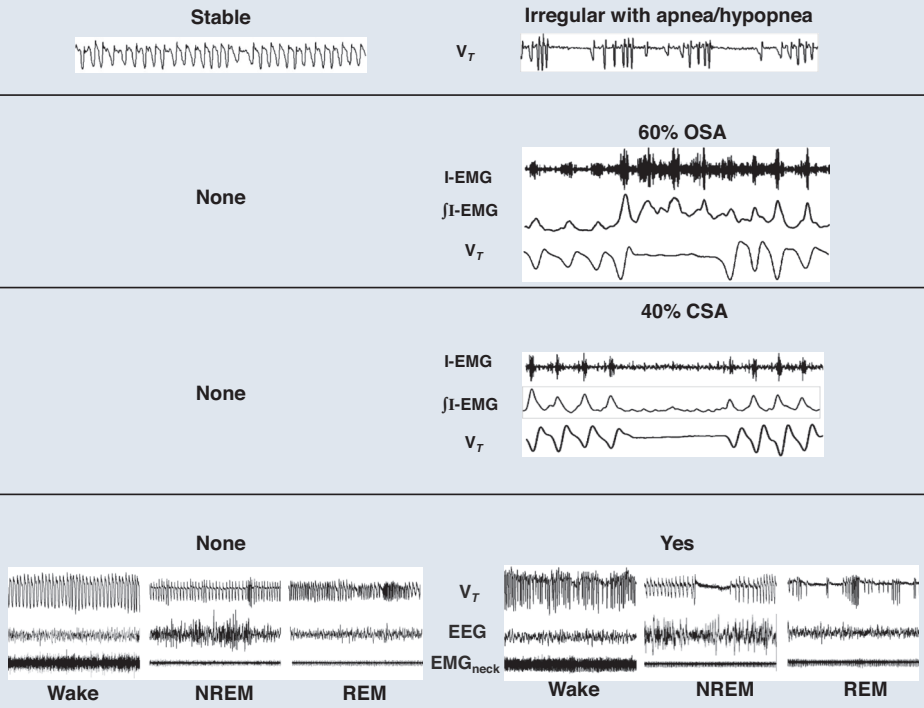


Figure 5. Schematic presentation of reactive oxygen species-dependent signalling mechanisms contributing to carotid body activation by intermittent hypoxia
Abbreviations: CO, carbon monoxide; CSE, cytathionine-γ-lyase; HO-2, heme oxygenase 2; Olfr78, olfactory receptor 78; PKG, protein kinase G; SNA, sympathetic nerve activity.

Table 1. *HO-2* null mice exhibit obstructive sleep apnoea and central apnoea during rapid eye movement and non-rapid eye movement sleep compared with wild-type control mice on the same genetic background

Characteristic	Wild-type mice	*HO-2* null mic
	Wild-Type	**HO-2 Null**
Breathing Stability	**Stable**	**Irregular with apnea/hypopnea**
Obstructive Apnea	**None**	**60% OSA**
Central Apnea	**None**	**40% CSA**
State-Dependent Apnea	**None**	**Yes**



Adapted from Peng et al. *Proc. Natl. Acad. Sci. USA* 114:1413–1418, 2017.
Abbreviations: CSA, central sleep apnea; HO2 null, HO-2 knockout mice; ∫I-EMG, integrated intercostal electromyography. I-EMG, intercostal electromyography; NREM, non-rapid eyme movement sleep; OSA, obstructive sleep apnea; REM, rapid eye movement sleep. Vt, tidal volume;

(Peng et al., 2017). These findings suggest that pharmacological blockade of carotid body hypersensitivity might be a new therapeutic approach for mitigating OSA in patients with high carotid body chemoreflex loop gain.

Gaps in knowledge and future directions

Obstructive sleep apnoea with IH is a major clinical problem affecting a substantial number of adult men and women globally. In the first part of this review,

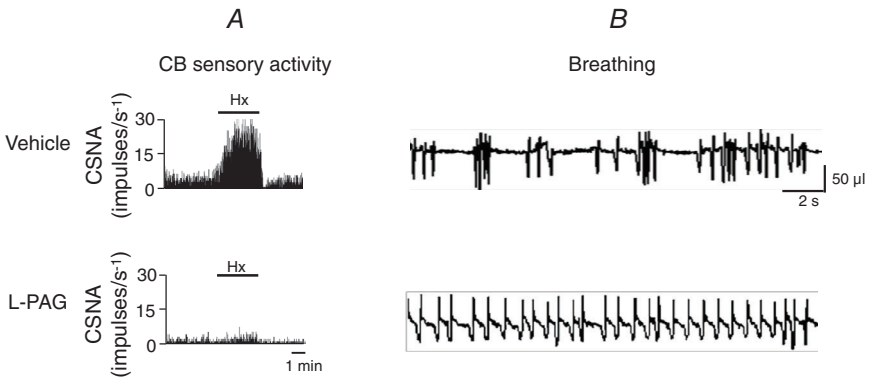


Figure 6. H_2S synthesis inhibitor blocks carotid body sensitivity to hypoxia (*A*) and normalizes breathing (*B*) in *HO-2* null mice
Abbreviations: CSNA, carotid body sensory nerve activity; L-PAG, L-propargyl glycine (90 mg/kg; P.O.).

we have attempted to summarize the impact of IH on the carotid body and the contribution of the ensuing chemoreflex to sympathetic activation and hypertension, major co-morbidities associated with OSA in experimental models. It appears that ROS generation by non-transcriptional and transcriptional mechanisms involving dysregulated HIF-1 and HIF-2 and epigenetic mechanisms are crucial for carotid body activation by IH and the ensuing SNA and hypertension in rodent models of IH, providing a much needed cellular and molecular framework for cardiovascular morbidities. However, the extent to which these mechanism(s) contribute to cardiovascular morbidities in OSA patients remains to be established, which is likely to provide new therapeutic strategies for the treatment of cardiovascular co-morbidities of OSA. Besides cardiovascular changes, several other co-morbidities, most notably metabolic dysfunction (type 2 diabetes) and cognitive deficits, are also associated with OSA. It remains to be investigated whether the signalling pathways identified with cardiovascular abnormalities also contribute to metabolic and cognitive abnormalities.

In the second part of the review, we presented evidence for a hyperactive carotid body chemoreflex as a cause of OSA in a murine model. A hyperactive carotid body chemoreflex contributing to OSA in *HO-2* null mice is consistent with a recent study on human subjects implicating the carotid body in causing OSA (Wellman et al., 2008). How might a hyperactive carotid body cause OSA? It is likely that the hyperactive chemoreflex, by stimulating breathing, decreases arterial P_{CO_2}, thereby lowering the excitability of hypoglossal motoneurons, leading to airway collapse and causing OSA. However, establishing this possibility requires further studies. A recent study reported that *HO-2* null mice exhibit reduced hypoglossal motoneuron excitability in brain slices from neonatal mice (Browe et al., 2023). Further studies are necessary to establish reduced hypoglossal tone in adult mice. Although the data from inspiratory intercostal muscles indicate OSA, additional studies with airflow measurements are necessary to establish OSA in *HO-2* null mice. Obstructive sleep apnoea in humans is associated with sleep fragmentation and periodic arousals. Whether *HO-2* null mice also exhibit sleep fragmentation and arousals remains to be established.

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Additional information

Competing interests

None.

Author contributions

N.P., Y.-J.P. and J.N.: conception or design of the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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