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CAROTID BODY CHEMO-REFLEX: A DRIVER OF AUTONOMIC ABNORMALITIES IN SLEEP APNEA

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Abstract

Carotid bodies are the principal peripheral chemoreceptors for detecting changes in arterial blood oxygen levels, and the resulting chemo-reflex is a potent regulator of the sympathetic tone, blood pressure, and breathing. Sleep apnea is a disease of the respiratory system affecting several million adult humans. Apneas occur during sleep often due to obstruction of the upper airway (obstructive sleep apnea, OSA) or due to defective respiratory rhythm generation by the central nervous system (central sleep apnea). Patients with sleep apnea exhibit several co-morbidities; most notable among them being the heightened sympathetic nerve activity, and hypertension. Emerging evidence suggests that intermittent hypoxia (IH) resulting from periodic apnea stimulates the carotid body and the ensuing chemo-reflex mediates the increased sympathetic tone and hypertension in sleep apnea patients. Rodent models of IH, simulating the O_2 saturation profiles encountered during sleep apnea have provided important insights into the cellular and molecular mechanisms underlying the heightened carotid body chemo-reflex. This article describes how IH affects the carotid body function, and discusses the cellular, molecular and epigenetic mechanisms underlying the exaggerated chemo-reflex.

Keywords

Sensory long-term facilitation; Gaseous transmitters; Oxidative stress; Sympathetic activation; Hypoxia-inducible factors; DNA methylation

INTRODUCTION

This review article is based on 2016 Michael de Burgh Daly Prize Lecture of the Physiological Society. Professor Daly made seminal contributions to the field of autonomic physiology, especially chemo-reflex regulation of cardiovascular function. I am indeed deeply honored to present this lecture, as my own research in the last couple of decades concerns with the regulation of cardio-respiratory functions by carotid body chemo-reflex in experimental models of sleep apnea.

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Sleep apnea is a disease of the respiratory system affecting nearly 10% of adult human population (Peppard *et al.*, 2013). Apneas occur during sleep often due to obstruction of the upper airway (obstructive sleep apnea, OSA) or due to defective respiratory rhythm generation by the central nervous system (central sleep apnea). In severely affected patients, the frequency of apneas can be as high as 60 per hour and arterial blood O_2 saturations reduce as much as 50% during apneic episodes. Patients with sleep apnea exhibit several co-morbidities most notably a heightened sympathetic nerve activity, and hypertension (Lavie *et al.*, 2000; Nieto *et al.*, 2000; Peppard *et al.*, 2000; Dempsey *et al.*, 2010). In 1980s, Daly suggested that "*a potential mechanism exists therefore whereby the peripheral arterial chemoreceptors could contribute to the neurogenic component of hypertension*" (de Burgh Daly, 1985). This article presents the emerging evidence showing that heightened carotid body chemo-reflex is a major driver of sympathetic activation and hypertension in patients with sleep apnea and then discusses the cellular and molecular mechanisms mediating the exaggerated chemo-reflex in experimental animal models of sleep apnea.

Carotid body chemo-reflex in sleep apnea patients

Patients with OSA exhibit pronounced increases in sympathetic nerve activity and blood pressure during apneic episodes in the night and elevated blood pressure and sympathetic tone during day time, wherein apneas are absent (Peppard *et al.*, 2000; Tamisier *et al.*, 2011). Population based studies showed a clear correlation between the severity of apnea and prevalence of hypertension (Peppard *et al.*, 2000). Obesity is a common co-morbidity in OSA subjects. However, the elevated blood pressures and sympathetic nerve activity are also seen in lean OSA subjects, suggesting that they occur independent of obesity (Narkiewicz & Somers, 1997). OSA patients exhibit elevated circulating and urinary catecholamines (both norepinephrine and epinephrine), which were attributed to increased sympathetic nerve activity (Fletcher *et al.*, 1987; Carlson *et al.*, 1993; Marrone *et al.*, 1993; Somers *et al.*, 1995; Garcia-Rio *et al.*, 2000).

Repetitive apneas lead to periodic hypoxia in the arterial blood. Given that carotid bodies are the major sensory organs for monitoring O_2 levels in the arterial blood (Kumar & Prabhakar, 2012), it was proposed that the carotid body chemo-reflex mediates the sympathetic activation in sleep apnea patients (Cistulli & Sullivan, 1994). Consistent with this notion, sleep apnea patients exhibit augmented carotid body chemo-reflex as evidenced by pronounced activation of the sympathetic nerves, increased blood pressure and ventilatory stimulation in response to acute hypoxia (Hedner *et al.*, 1992; Narkiewicz *et al.*, 1999; Kara *et al.*, 2003). Brief hyperoxia, which decreases carotid body sensory nerve activity leads to a greater depression of breathing (Tafil-Klawe *et al.*, 1991; Kara *et al.*, 2003) and blood pressure (Narkiewicz *et al.*, 1999) in sleep apnea patients as compared with control subjects. These studies suggest that exaggerated carotid body chemo-reflex contributes to increased sympathetic nerve activity and hypertension in sleep apnea patients.

Experimental models of sleep apnea

Fletcher and co-workers (Fletcher, 1995) developed a rodent model of intermittent hypoxia (IH) simulating the O_2 profiles encountered during sleep apnea. These investigators exposed adult rats to alternating cycles of hypoxia (12s of 3–5% O_2) and room air (15–18s), 60

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episodes/hour for 6–8 hours during day time, which is the sleep time for rodents. Rats exposed to several days of IH showed hypertension, increased sympathetic nerve activity and elevated urinary catecholamines recapitulating the phenotype reported in sleep apnea patients (Fletcher, 1995). Carotid body chemo-reflex is augmented in IH exposed rodents, as seen by exaggearated sympathetic nerve activity, blood pressure and ventilatory responses to acute hypoxia (Rey *et al.*, 2004; Peng *et al.*, 2006b; Huang *et al.*, 2009). Disrupting the chemo-reflex pathway either by sectioning the carotid sinus nerves (Fletcher *et al.*, 1992; Lesske *et al.*, 1997) or by selective ablation of the carotid body, while preserving the carotid baroreceptor function (Peng *et al.*, 2014b) or by denervating the adrenals, a major sympathetic end organ (Peng *et al.*, 2014b) prevented the development of hypertension and elevated plasma catecholamines in IH treated rodents. The following section describes the effects of IH on major components of the chemo-reflex pathway including the carotid body, brainstem neurons, and the adrenal medulla.

Carotid body (sensor)—IH exposure results in augmented **c**arotid body response to acute hypoxia in rats (Peng & Prabhakar, 2004), mice (Peng *et al.*, 2006b) and cats (Rey *et al.*, 2004). IH also induces a form of carotid body plasticity manifested as sensory long-term facilitation (sensory LTF), which is characterized by long-lasting, progressive increase in baseline sensory nerve activity following repetitive acute hypoxia (Peng *et al.*, 2003). Sensory LTF was observed despite maintaining normal arterial blood gas composition and blood pressure (Peng *et al.*, 2003). The effects of IH on the carotid body were seen after exposure to a minimum of three consecutive days of IH (8 hours per day) but not with a single exposure to IH for 8 hours. The magnitude of the augmented hypoxic sensitivity and sensory LTF increased as the duration of IH extended to 10 days (Peng *et al.*, 2003). Increasing the severity of acute hypoxia used for IH conditioning from 10% to 5% O_2 had no noticeable further impact on the magnitude of the carotid body response to acute hypoxia (Peng *et al.*, 2003). These studies suggest that IH leads to remodeling of the carotid body function manifested as sensitization of the hypoxic sensory response and induction of sensory LTF.

What might be the functional consequences of carotid body remodeling by IH? It was proposed that sensitization of the carotid body response to hypoxia leads to greater sympathetic activation and blood pressures during apneic episodes and the sensory LTF contributes to elevated baseline sympathetic nerve activity and blood pressure (Prabhakar, 2013; Fig. 1). Given that enhanced carotid body activity leads to breathing instability with a greater number of apneas (Longobardo *et al.*, 1982), it was further suggested that the augmented carotid body activity might exacerbate the occurrence of apneas (Prabhakar, 2013, Fig. 1).

Brainstem neurons—Sensory information from the carotid body is conveyed to the nucleus tractus solitarius (nTS) and then to the neurons in the rostral ventrolateral medulla (RVLM), which then translate the carotid body sensory input to changes in sympathetic nerve activity. Kline *et al* reported increased post-synaptic activity of nTS neurons in IH treated animals (Kline *et al.*, 2007). IH treated rodents also showed an upregulation of N-methyl-D-aspartate receptor 1 (NMDA-R1) and Glutamate receptor 2/3 expressions in the

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commissural nTS, which receives sensory input from the carotid body (Reeves *et al.*, 2003; Costa-Silva *et al.*, 2012), and augmented AMPA-and NMDA-mediated neuronal excitability (de Paula *et al.*, 2007).

The sympathetic outflow is modulated by the respiratory rhythm (Haselton & Guyenet, 1989; Dempsey *et al.*, 2002), which in turn contributes to neurogenic vasomotor tone (Bachoo & Polosa, 1985). Coupling between the respiratory and sympathetic nerve activities occurs in part, at the RVLM (Dampney, 1994; Guyenet, 2000). The RVLM contains two major groups of pre-sympathetic bulbo-spinal neurons including those expressing adrenaline (C1 group) and non-catecholaminergic neurons (Stornetta *et al.*, 2002). A majority of the bulbo-spinal RVLM pre-sympathetic neurons exhibit respiratory-related activity (McAllen, 1987; Haselton & Guyenet, 1989; Miyawaki *et al.*, 1995). Recently, Moraes, Machado and co-workers (Moraes *et al.*, 2013) reported that a specific population of non-catecholaminergic respiratory-modulated pre-sympathetic neurons in RVLM of IH exposed rats exhibit augmented excitatory synaptic inputs from the respiratory network. The enhanced excitatory synaptic activity of these neurons by IH was due to altered properties of the ion channels in post-inspiratory neurons (Moraes *et al.*, 2015). Collectively, these studies indicate that altered neuronal coupling between the respiratory and pre-sympathetic neurons in the RVLM is an important central mechanism contributing to activation of the sympathetic nervous system by IH.

Adrenal medulla—The adrenal medulla is a major end organ of the sympathetic nervous system which contributes to circulating catecholamines. IH exposed rats exhibit markedly augmented acute hypoxia-induced catecholamine secretion from the adrenal medulla (Kumar *et al.*, 2006).

Are the effects of IH direct or indirect?

IH produces a modest drop in arterial blood O_2 saturation during each episode of hypoxia, which approximates to a reduction in arterial PO_2 from ~100 to 80 mmHg i.e., ~20 mmHg reduction (Peng *et al.*, 2014b). This small drop in arterial PO_2 is likely to be directly sensed by the carotid body because of its exquisite sensitivity to O_2 (Kumar & Prabhakar, 2012) and highest blood flow (de Burgh Daly *et al.*, 1954). On the other hand, this drop in PO_2 to ~80 mmHg may not be sufficient to directly affect many central and peripheral tissues, because PO_2 of most of these tissues ranges between 30 and 60 mmHg under normoxia (Carreau *et al.*, 2011). However, neural activity is a potent stimulant for eliciting the cellular responses (Fields *et al.*, 2005; Carulli *et al.*, 2011; Ganguly & Poo, 2013). It is conceivable that the effects of IH on tissues such as nTS, RVLM and adrenal medulla are mediated indirectly through enhanced neural input from the carotid body. Consistent with this possibility, selective ablation of the carotid body prevents IH-induced changes in the nTS and RVLM (Peng *et al.*, 2014b). Likewise, the effects of IH on adrenal medulla could be blocked either by carotid body ablation or by sympathetic denervation (Peng *et al.*, 2014b). A recent study (Moraes *et al.*, 2015) also reported that carotid body neural input is required for IH-induced changes on ion channel properties of post-inspiratory neurons. Collectively, these findings suggest that IH directly affects the carotid body function, whereas the effects of IH on

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brainstem neurons and the adrenal medulla are indirect, and mediated by sensory input from the carotid body (Fig. 2).

Cellular mechanisms underlying chemo-reflex activation by IH

Reactive oxygen species (ROS) were proposed to mediate the exaggerated carotid body chemo-reflex by IH (Prabhakar, 2001; Yuan *et al.*, 2004). Supporting this possibility, rodents exposed to IH showed elevated ROS levels in the three major components of the chemo-reflex pathway including the carotid body (Peng *et al.*, 2003; Peng *et al.*, 2014b), nTS and RVLM (Zhan *et al.*, 2005; Peng *et al.*, 2014b), and adrenal medulla (Kumar *et al.*, 2006; Peng *et al.*, 2014b).

Multiple sources contribute to IH-induced increase in ROS. These include: a) NADPH-oxidase-2 (Nox2) (Zhan *et al.*, 2005; Peng *et al.*, 2009; Lam *et al.*, 2012; Peng *et al.*, 2014b), b) Xanthine oxidoreductase (XO) (Nanduri *et al.*, 2013), and c) inhibition of mitochondrial electron transport chain (ETC) at the complex I (Peng *et al.*, 2003; Yuan *et al.*, 2004), which is known to increase ROS generation (Ambrosio *et al.*, 1993). The inhibitory effects of IH on mitochondrial complex I are indirect and require ROS generated by Nox2 (Khan *et al.*, 2011). Cellular ROS levels also depend on their rate of degradation by anti-oxidant enzymes. IH decreases the enzyme activity of superoxide dismutase 2 (Sod-2), a major anti-oxidant enzyme in the carotid body (Nanduri *et al.*, 2009b), nTS, RVLM and adrenal medulla (Peng *et al.*, 2014b). These studies suggest that elevated ROS generation by IH in the chemo-reflex pathway is due to increased activity of pro-oxidant enzymes and decreased activity of anti-oxidant enzymes (Fig. 2).

The importance of ROS signaling was assessed by treating IH exposed rats with anti-oxidants. Anti-oxidant treatment during IH exposure prevented the elevated ROS levels, blocked the exaggerated carotid body activity, enhanced adrenal medullary catecholamine secretion, elevated plasma catecholamine levels, and hypertension (Peng *et al.*, 2003; Peng & Prabhakar, 2004; Peng *et al.*, 2006b; Peng *et al.*, 2014b). In contrast, a single application of anti-oxidant on the last day of IH treatment was found to be ineffective in preventing the carotid body hypersensitivity to hypoxia (Peng *et al.*, 2003), suggesting that ROS-mediated signaling cascade rather than ROS generation *per se* is critical for evoking heightened chemo-reflex by IH.

Molecular determinants of ROS generation by IH

The effects of IH on ROS levels develop over time and require transcriptional regulation of genes encoding the pro-and anti-oxidant enzymes (Pawar *et al.*, 2008; Nanduri *et al.*, 2009a; Peng *et al.*, 2009). Recent studies showed that the hypoxia-inducible factor (HIF) family of transcriptional activators is critical for IH-induced ROS generation. HIF-1 and HIF-2 are the two best studied members of the HIF family, which are heterodimers, comprised of an O_2-regulated α subunits and a constitutively expressed HIF-1β subunit (Prabhakar & Semenza, 2012).

IH increases HIF-1α protein in the carotid body, nTS and RVLM as well as adrenal medulla (Peng *et al.*, 2014b). The increased HIF-1α protein expression by IH requires Ca^{2+}-dependent activation of protein kinase C (PKC) and the ensuing increase in protein synthesis

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by the mammalian-target of rapamycin (mTOR) (Yuan *et al.*, 2008). Blockade of HIF-1α activation either by pharmacological or by genetic approaches completely abolishes IH-induced increase in ROS generation (Yuan *et al.*, 2011). Further studies revealed that HIF-1 mediates the transcriptional activation of Nox2, a major pro-oxidant enzyme by IH (Yuan *et al.*, 2011). Mice with heterozygous deficiency of HIF-1α exhibit remarkable absence of IH-induced increase in ROS, activation of the carotid body, hypertension and elevated plasma catecholamines (Peng *et al.*, 2006b).

HIF-2α (also known as endothelial PAS domain protein-1, EPAS-1) shares ~50% sequence homology to HIF-1α and also interacts with HIF-1β (Ema *et al.*, 1997; Tian *et al.*, 1997). In striking contrast to HIF-1α, IH decreases HIF-2α protein in the carotid body, adrenal medulla (Nanduri *et al.*, 2009b) and in nTS and RVLM (Peng *et al.*, 2014b). The HIF-2α protein degradation by IH is mediated by Ca^{2+}-dependent protease, calpain (Nanduri *et al.*, 2009b). The reduced HIF-2α expression by IH results in transcriptional down regulation of mRNAs encoding several anti-oxidant enzymes including the Sod-2 in the carotid bodies (Nanduri *et al.*, 2009b). Mice partially deficient in HIF-2α display several phenotypic characteristics of IH exposed mice including the augmented hypoxic sensitivity of the carotid body, elevated blood pressures, increased plasma catecholamines, disrupted breathing with apneas, and oxidative stress and all these effects were blocked by anti-oxidant treatment (Peng *et al.*, 2011a).

The above described studies suggest that an imbalance between HIF-1 and HIF-2 and the resulting alteration in transcriptional regulation of pro-oxidant (e.g., Nox2) and anti-oxidant enzymes (e.g., Sod-2) is a major molecular mechanism underlying increased ROS generation in the chemo-reflex pathway by IH. IH-evoked imbalance in HIFα isoforms and the ensuing dysregulated pro-and anti-oxidant enzyme genes in the nTS, RVLM and adrenal medulla requires sensory input from the carotid body demonstrating a hitherto uncharacterized role for sensory signals from the carotid body in regulating the redox state through HIF-dependent transcriptional changes in pro-and anti-oxidant enzymes (Fig. 2).

How ROS augments chemo-reflex?

Carotid body is composed of two major cell types: the type I (also called glomus) cells and type II cells. A substantial body of evidence suggests that type I cells are the initial sites of hypoxic sensing in the carotid body and these cells work in concert with the nearby afferent nerve ending as a ‘sensory unit’ (Kumar & Prabhakar, 2012). Carotid body activation by IH was not associated with changes in morphology of the chemoreceptor tissue (Peng *et al.*, 2003; Del Rio *et al.*, 2011), and was seen in *ex vivo* carotid bodies (Peng *et al.*, 2003; Peng *et al.*, 2009), suggesting that the altered carotid body function is due to direct effects of IH on hypoxia sensing by the glomus cells.

Emerging evidence suggests that hypoxia sensing by the carotid body requires carbon monoxide (CO) generation by heme oxygenase (HO)-2 and hydrogen sulfide (H_2S) synthesis by cystathionine-γ-lyase (CSE) in glomus cells (Prabhakar & Semenza, 2015). O_2 is a potent regulator of CO production from HO-2. Under normoxia, CO production is high whereas CO production is markedly decreased during hypoxia in the carotid body (Peng *et al.*, 2010; Peng *et al.*, 2014a; Yuan *et al.*, 2015). The reduced CO generation, in turn activates

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CSE-dependent H_2S synthesis through inhibition of protein kinase G-dependent phosphorylation of CSE (Yuan *et al.*, 2015). Increased H_2S levels, in turn stimulate the carotid body neural activity during hypoxia (Li *et al.*, 2010; Peng *et al.*, 2010; Telezhkin *et al.*, 2010; Peng *et al.*, 2014a; Yuan *et al.*, 2015). However, a recent study reported that inhibitors of H_2S synthesis do not prevent anoxia (zero percent oxygen)-induced elevation of $[Ca^{2+}]_i$ and inhibition of TASK-like K^+ channel of rat glomus cells (Kim *et al.*, 2015). These findings suggest that H_2S mediates carotid body response to "physiological" hypoxia.

The role of CO-H_2S signaling in IH-evoked carotid body activation was examined (Yuan *et al.*, 2016). This study showed that ROS generated during IH inhibits HO-2-dependent CO generation and this effect requires Cys^{265} in the heme regulatory motif of HO-2. The decreased CO in turn increases H_2S production by activating CSE. Inhibiting CSE-derived H_2S synthesis either by genetic knock down of CSE or by pharmacological blockade prevents the chemo- reflex activation and hypertension in IH treated rats (Yuan *et al.*, 2016). These findings demonstrate that ROS is epistatic to H_2S in driving chemo-reflex-dependent hypertension by IH (Fig. 3).

Previous studies have implicated endothelin-1 (ET-1), in mediating augmented hypoxic sensitivity of the carotid body by IH (Rey *et al.*, 2006; Iturriaga, 2013; Peng *et al.*, 2013). 5-hydroxytryptamine (5-HT) and angiotensin II (Ang II) were proposed to mediate sensory LTF of IH carotid bodies (Peng *et al.*, 2009; Peng *et al.*, 2011b). ET-1, 5-HT and Ang II also increase ROS generation (Peng *et al.*, 2006a; Gorąca *et al.*, 2016). It is likely that the effects of ET-1, 5-HT and Ang II are mediated through ROS-dependent activation of H_2S production, which might explain why blocking H_2S production alone was sufficient to prevent the augmented hypoxic sensitivity, sensory LTF and the ensuing chemosensory reflex-mediated hypertension.

Although IH has been shown to increase ROS levels in the nTS and RVLM, how ROS affects the neuronal activity at these sites has not yet been delineated. IH-induced augmented catecholamine secretion from the adrenal medulla (Kumar *et al.*, 2006; Souvannakitti *et al.*, 2009; Souvannakitti *et al.*, 2010) was shown to be mediated in part by ROS-dependent activation of protein kinase C and the resulting recruitment of readily releasable pool of secretory vesicles (Kuri *et al.*, 2007). ROS has also been shown to facilitate Ca^{2+} influx via low threshold T-type Ca^{2+} channels as well as mobilization of intracellular Ca^{2+} stores by ryanodine receptor (RyR) activation in adrenal medullary chromaffin cells of IH treated rats (Souvannakitti *et al.*, 2010).

Are the effects of IH on chemo-reflex reversible?

Continuous positive airway pressure (CPAP) is the treatment of choice for OSA. However, CPAP is ineffective in normalizing blood pressures in a subset of OSA patients (Mulgrew *et al.*, 2010; Dudenbostel & Calhoun, 2012). The efficacy of CPAP treatment might depend on the duration of OSA. A recent study examined the recovery of blood pressure and chemo-reflex in adult rats exposed to either short-term (10 days) or long-term (30 days) IH (Nanduri *et al.*, 2016). Short-term IH-induced hypertension and the exaggerated chemo-reflex completely recovered after terminating IH (Pawar *et al.*, 2008; Nanduri *et al.*, 2016). In contrast, the effects elicited by long-term IH persisted 30 days after terminating IH

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(Nanduri *et al.*, 2016). These findings suggest that the ineffectiveness of CPAP treatment may be a consequence of long-term IH associated with chronically undiagnosed and untreated OSA, leading to persistent activation of the chemo-reflex.

In addition to adults, recurrent apnea is also a major clinical problem in infants born pre-term. Pre-term infants with recurrent apneas often exhibit an enhanced hypoxic ventilatory response (Nock *et al.*, 2004), a hallmark of heightened carotid body chemo-reflex. Exposing neonatal rat pups to IH simulating the apnea of prematurity also activates the chemo-reflex (Peng *et al.*, 2004; Pawar *et al.*, 2008). Remarkably, adult rats exposed to IH in the neonatal period exhibit augmented carotid body sensitivity to hypoxia, hypertension and irregular breathing with apnea (Pawar *et al.*, 2008; Nanduri *et al.*, 2012). Recent poulation based studies showed that young adults who were born preterm exhibit sleep-disordered breathing with apneas and increased incidence of hypertension (Paavonen *et al.*, 2007; Hibbs *et al.*, 2008). Long-lasting activation of the chemo-reflex by neonatal IH might explain the early onset of cardio-respiratory dysfunction in young adults born preterm.

The persistent hypertension elicited by long-term and neonatal IH was associated with elevated ROS levels in the carotid bodies and adrenal medulla (Nanduri *et al.*, 2012; Nanduri *et al.*, 2016). The increased ROS levels were in part due to markedly reduced anti-oxidant enzyme (AOE) genes expression in the chemo-reflex pathway (Nanduri *et al.*, 2012; Nanduri *et al.*, 2016).

Epigenetic regulation of redox state by long-term IH

How might long-term and neonatal IH result in persistent down regulation of anti-oxidant enzyme genes? Emerging evidence suggests that epigenetic regulation by DNA methylation results in long-lasting suppression of gene expression (Feinberg, 2007). AOE genes that are downregulated by long-term and neonatal IH displayed DNA hypermethylation, and increased DNA methyl transferase (Dnmt) enzyme activity (Nanduri *et al.*, 2012; Nanduri *et al.*, 2016). DNA methylation occurs at cytosine residues located immediately 5′ to a guanine residue, which are known as CpG dinucleotides referred to as “CpG islands” (Illingworth & Bird, 2009). Further studies identified hypermethylation of a single CpG dinucleotide in the region close to the transcription start site of *Sod2* gene in response to long-term and neonatal IH (Nanduri *et al.*, 2012; Nanduri *et al.*, 2016). Treating rats with decitabine, a DNA hypomethylating agent during long-term and neonatal IH exposures restored AOE gene expression, normalized ROS levels in the chemo-reflex pathway, along with normalization of blood pressures and breathing (Nanduri *et al.*, 2012; Nanduri *et al.*, 2016). These studies suggest that DNA methylation of AOE genes and the ensuing long-lasting oxidative stress in the chemo-reflex pathway contribute to persistent hypertension caused by long-term and neonatal IH (Fig. 4). However, the mechanism(s) by which long-term and neonatal IH activates DNA methylation remain to be investigated.

Concluding remarks

The studies summarized in this review primarily focused on the effects of IH on chemo-reflex activation and its relevance to autonomic morbidities associated with sleep apnea. Less information is currently available on the contribution of other changes associated with

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sleep apnea such as mild hypercapnia, changes in intra thoracic pressure and sleep fragmentation to cardio-respiratory pathologies. Much need to be studied as to the role of other epigenetic mechanisms including histone modifications and small ineterfering RNAs in chemo-reflex driven hypertension by sleep apnea.

Central sleep apnea (CSA) patients, like OSA subjects also exhibit exaggerated chemo-reflex and are greatly at increased risk of hypertension (Solin *et al.*, 2000). In both OSA and CSA, the acute elevations in blood pressures occurring during apneic episodes may predispose patients to hemorrhagic stroke, while chronic hypertension increases the risk of heart failure. Thus, controlling hypertension in sleep apnea patients is a major clinical problem. CPAP, which is the present treatment of choice for OSA, is not always effective in a subset of patients (Thomas *et al.*, 2004), and treatment options for CSA are even more limited. Thus, there is an absolute need for alternative therapeutic strategies for controlling blood pressure in patients with sleep apnea. IH, which occurs in both OSA and CSA produces hypertension and marked elevations in blood pressures during simulated apneic episodes in rodents, and these effects are prevented by carotid body ablation (Fletcher *et al.*, 1992; Peng *et al.*, 2014b). However, removal of the carotid bodies as a therapeutic strategy in sleep apnea patients has serious limitations. Carotid body removal adversely affects many of the physiological functions including high altitude adaptation, maintenance of arterial blood gases during exercise, and cardio-respiratory responses to acute hypoxia. On the other hand, reducing the IH-induced heightened carotid body activity by pharmacological approaches might be a viable therapeutic option for normalizing blood pressures in sleep apnea patients. Although ROS signaling is central to IH-evoked carotid body hyperexcitability and the ensuing chemo-reflex, anti-oxidants have limited therapeutic value. Anti-oxidants must be administered during the entire duration of IH exposure in order to normalize carotid body function and hypertension, and when given after the onset of heightened chemo-reflex they are totally ineffective in normalizing carotid body function (Peng *et al.*, 2003). A recent study by Yuan *et al.* (2016) showed that systemic administration of L-propargyl glycine (L-PAG, 30 mg/kg; IP), an inhibitor of CSE-dependent H_2S synthesis given after the onset of IH-induced hypertension normalizes the carotid body activity, sympathetic nerve activity and blood pressure. Thus, inhibitors of CSE-derived H_2S synthesis may be a novel approach to treat chemo-reflex driven hypertension in patients with sleep apnea.

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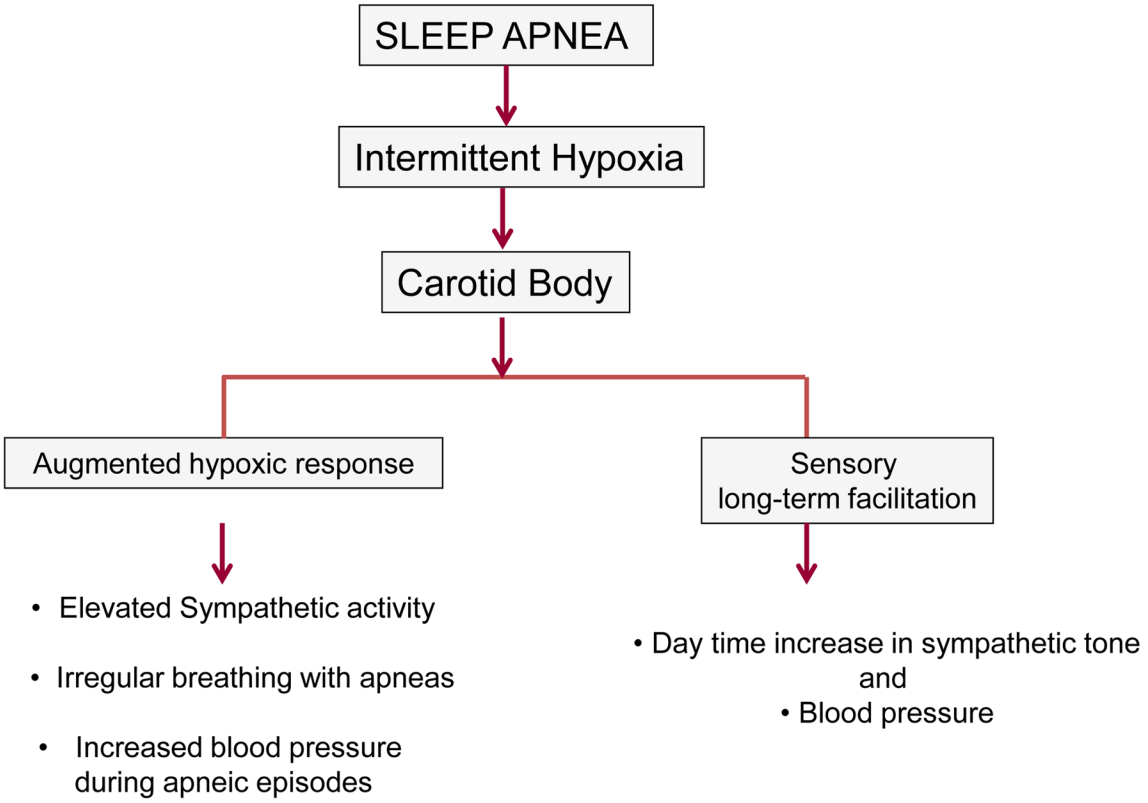

Figure 1.
Effects of intermittent hypoxia associated with sleep apnea on the carotid body sensory nerve activity and its consequences on cardio-respiratory functions.

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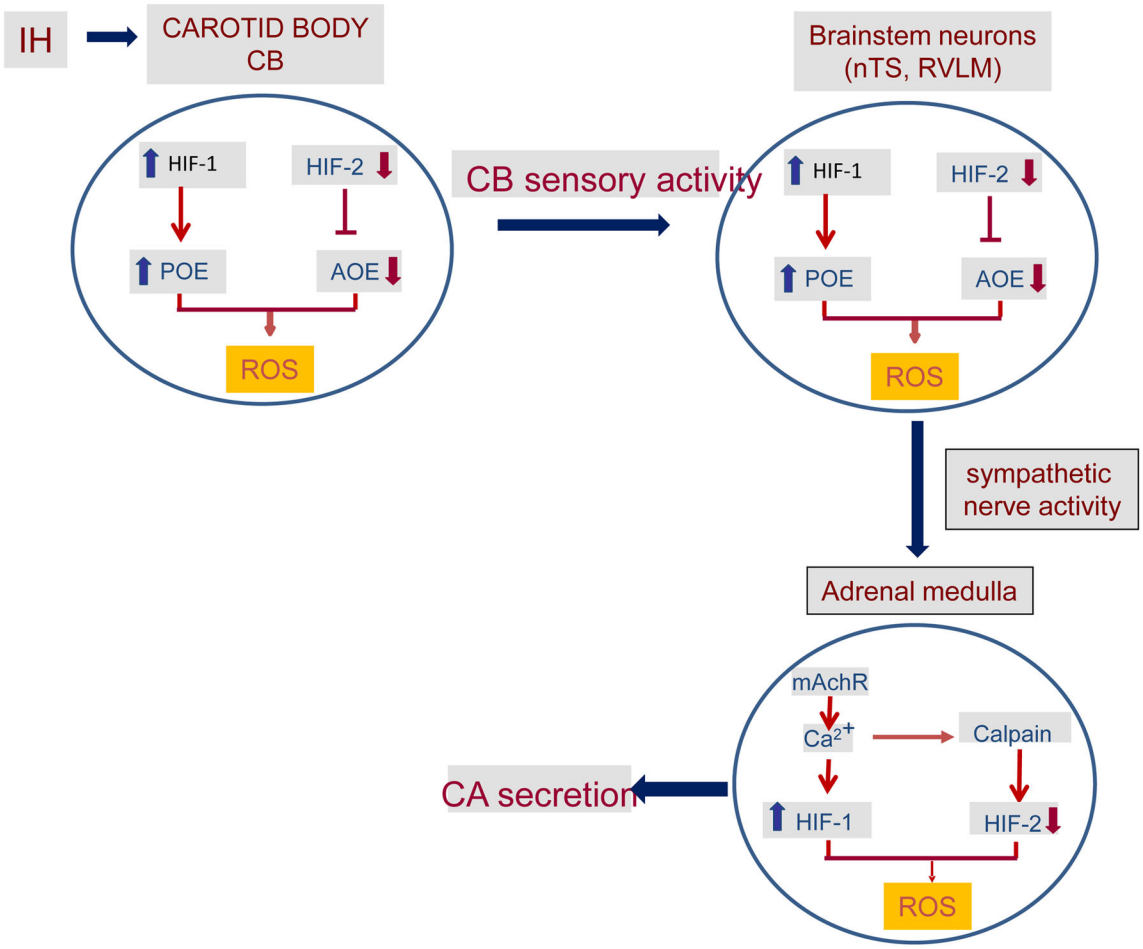

Figure 2.

Direct and indirect effects of intermittent hypoxia (IH) on the chemo-reflex pathway including the carotid body (the sensor), neurons of nucleus of the tractus solitarious (nTS) and rostral ventrolateral medulla (RVLM) and adrenal medulla (sympathetic end organ). HIF-1 and HIF-2= Hypoxia-inducible factors 1 and 2, POE= pro-oxidant enzymes, AOE= anti-oxidant enzymes, ROS= reactive oxygen species, mAChR= muscarinic acetylcholine receptor, CA secretion= catecholamine secretion, CSN= carotid sinus nerve.

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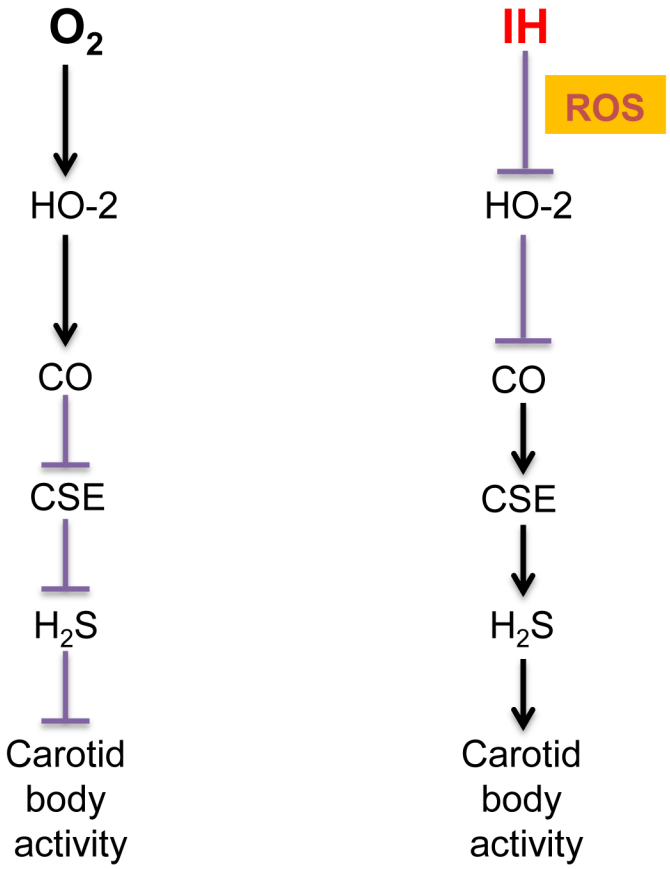

Figure 3.
Schematic illustration of cellular mechanisms underlying carotid body (CB) activation by intermittent hypoxia (IH). ROS= reactive oxygen species, HO-2= heme oxygenase-2, CO= carbon monoxide, CSE= cystathionine-γ-lyase, H_2S = hydrogen sulfide.

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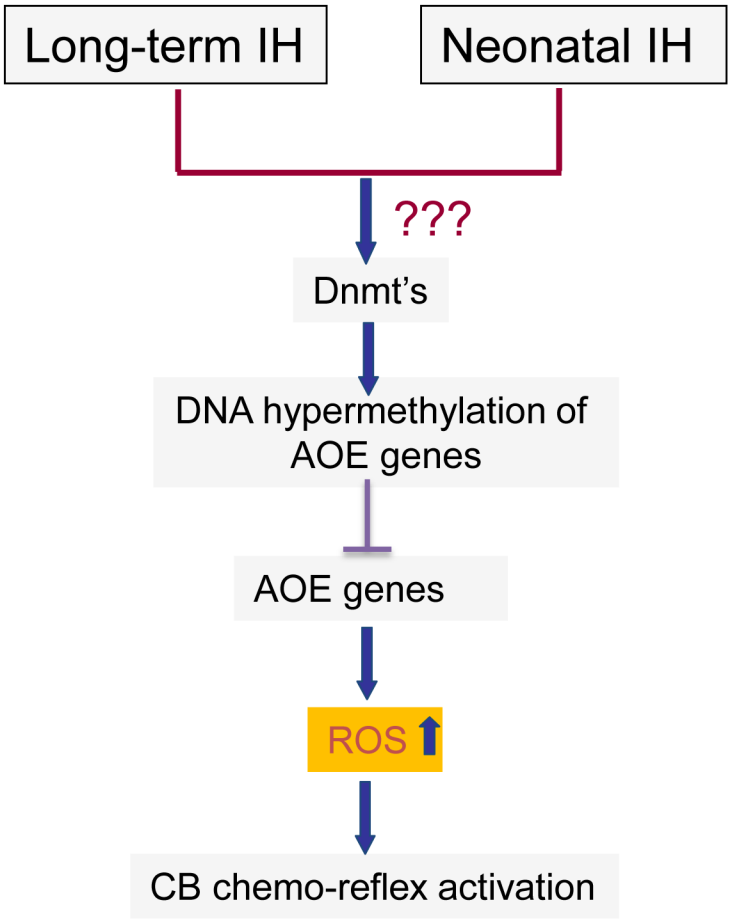

Figure 4.

Schematic illustration of epigenetic mechanisms (e.g., DNA hypermethylation) contributing to persistent elevation of reactive oxygen species (ROS) and activation of carotid body (CB) chemo reflex by long-term intermittent hypoxia (IH) in adults and neonatal IH. Dnmts= DNA methyl transferases; AOE= anti-oxidant enzymes. CB= Carotid body.