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REACTIVE OXYGEN RADICALS AND GASEOUS TRANSMITTERS IN THE CAROTID BODY ACTIVATION BY INTERMITTENT HYPOXIA

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

Institute for Integrative Physiology and Center for Systems Biology of O₂ Sensing, Biological Sciences Division, University of Chicago, IL- 60637, USA

Abstract

Sleep apnea is a prevalent respiratory disease characterized by periodic cessation of breathing during sleep causing intermittent hypoxia (IH). Sleep apnea patients and rodents exposed to IH exhibit elevated sympathetic nerve activity and hypertension. A heightened carotid body (CB) chemo reflex has been implicated in causing autonomic abnormalities in IH treated rodents and in sleep apnea patients. The purpose of this article is to review the emerging evidence showing that interactions between reactive oxygen species (ROS) and gaseous transmitters as a mechanism causing hyperactive CB by IH. Rodents treated with IH exhibit markedly elevated ROS in the CB, which is due to transcriptional upregulation of pro-oxidant enzymes by hypoxia-inducible factor (HIF)-1, and insufficient transcriptional regulation of anti-oxidant enzymes by HIF-2. ROS, in turn, increases cystathionine- γ -lyase (CSE)-dependent H₂S production in the CB. Blockade of H₂S synthesis prevents IH-evoked CB activation. However, the effects of ROS on H₂S production is not due to direct effects on CSE enzyme activity, rather due to inactivation of heme oxygenase-2 (HO-2), a carbon monoxide (CO) producing enzyme. CO inhibits H₂S production through inactivation of CSE by PKG-dependent phosphorylation. During IH, reduced CO production resulting from inactivation of HO-2 by ROS releases the inhibition of CO on CSE thereby increases H₂S. Inhibiting H₂S synthesis prevented IH-evoked sympathetic activation and hypertension.

Keywords

Sleep apnea; carbon monoxide; hydrogen sulfide; heme oxygenase-2; cystathionine- γ -lyase

INTRODUCTION

Sleep apnea is a highly prevalent respiratory disorder affecting nearly 10% of adult human population in the USA alone (Peppard, et al., 2013). It is characterized by periodic cessation

Address for Correspondence: Nanduri R. Prabhakar, Institute for Integrative Physiology, The Center for Systems Biology of O₂ Sensing, University of Chicago, MC 5068, 5841 South Maryland Avenue, Chicago, IL 60637, USA, Tel: 773-834-5480, Fax: 773-834-5252, nanduri@uchicago.edu.

Compliance with Ethical Standards

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of breathing movements or airflow during sleep occurring either because of obstruction of the upper airway (obstructive sleep apnea, OSA) or due to disrupted respiratory rhythm generation by the central nervous system (central sleep apnea). Patients with sleep apnea exhibit heightened sympathetic nerve activity, and hypertension (Dempsey, et al., 2010, Lavie, et al., 2000, Nieto, et al., 2000, Peppard, et al., 2000).

Periodic hypoxia or intermittent hypoxia (IH) in the arterial blood is a hallmark manifestation of sleep apnea. Rodents exposed to IH, simulating the periodic hypoxemia seen in sleep apnea patients also show sympathetic activation and hypertension (Prabhakar, 2016). Carotid bodies (CBs) are the major sensing organs for detecting hypoxemia, and the CB chemo reflex is an important regulator of sympathetic tone and blood pressure (Kumar and Prabhakar, 2012). Consequently, it was thought that a heightened CB chemo reflex is the primary mediator of increased sympathetic tone and hypertension in patients with sleep apnea (Cistulli and Sullivan, 1994, Prabhakar, 2016). Studies on rodents showed that IH sensitizes the CB response to hypoxia, and induces sensory long term facilitation (sLTF) manifested as long lasting increase in sensory nerve activity after repetitive hypoxia (Peng, et al., 2003, Peng and Prabhakar, 2004). Selective ablation of the CB prevents IH-evoked sympathetic activation and hypertension in IH treated rodents, supporting the idea that CB chemo reflex is a major mediator of autonomic dysfunction caused by sleep apnea. In this article, we present emerging evidence for interplay between reactive oxygen species (ROS) and gasotransmitters as a major cellular mechanism underlying CB hyperactivity by IH.

IH treated CBs exhibit elevated ROS levels

IH, unlike continuous hypoxia is characterized by periodic re-oxygenations. It was proposed that ROS levels are increased during the re-oxygenations, which in turn impact CB activity (Prabhakar, 2001, Yuan, et al., 2004). Indeed, ROS levels are elevated in CBs of IH-treated rodents as evidenced by decreased aconitase enzyme activity (Peng, et al., 2003), an established biochemical marker of ROS (Gardner, 2002), increased malondialdehyde levels (Yuan, et al., 2016), a marker of oxidized lipids (Ayala, et al., 2014) and serum 8-isoprostane and nitrotyrosine, markers of oxidative stress (Ho, et al., 2013). Although the role of ROS in CB activation by hypoxia under basal conditions is uncertain (Acker, et al., 2006), treating IH-exposed rats with manganese (III) tetrakis(1-methyl-4-pyridyl)porphyrin pentachloride (MnTMPyP), a membrane permeable ROS scavenger (5mg/kg, i.p., every day for ten days) completely prevented IH-induced sensitization of the CB response to hypoxia and sLTF (Peng, et al., 2003, Peng and Prabhakar, 2004). Similar effects of other anti-oxidants on CB activation by IH were also reported (Del Rio, et al., 2010). These findings suggest that ROS is a major contributor to CB excitation by IH.

IH-evoked ROS generation in the CB is due to activation of pro-oxidants like NADPH-oxidase-2 (Nox2) (Lam, et al., 2012, Peng, et al., 2009, Peng, et al., 2014, Zhan, et al., 2005) and xanthine oxidoreductase (XO) (Nanduri, et al., 2013). IH also inhibits 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). In addition, IH reduces the activity of anti-oxidant enzymes such as superoxide dismutase 2 (Sod-2) in the CB (Nanduri, et al., 2009).

Recent studies showed that transcriptional regulation by hypoxia-inducible factors (HIFs) is a major molecular mechanism underlying the increased ROS generation by IH. HIF-1 and HIF-2 are the two best studied members of the HIF family, which are heterodimers, comprised of an O₂-regulated α subunits and a constitutively expressed HIF-1 β subunit (Prabhakar and Semenza, 2012). IH increases HIF-1 α protein in the CB (Peng, et al., 2014) through Ca²⁺-dependent activation of protein kinase C (PKC) and the resulting increase in protein synthesis by the mammalian-target of rapamycin (mTOR) (Yuan, et al., 2008). HIF-1 mediates the transcriptional activation of Nox2, a major pro-oxidant enzyme by IH (Yuan, et al., 2011). Mice with partial deficiency of HIF-1 α exhibit absence of IH-induced increase in ROS and activation of the CB (Peng, et al., 2006). In striking contrast, IH decreases HIF-2 α protein in the CB via Ca²⁺-dependent protease, calpain (Nanduri, et al., 2009). The IH-induced decrease in HIF-2 α protein leads to insufficient transcription of mRNA encoding several anti-oxidant enzymes in the CB (Nanduri, et al., 2009). HIF-2 α heterozygous mice, like IH exposed mice, exhibit augmented hypoxic sensitivity of the CB, increased ROS and these effects were blocked by anti-oxidant treatment (Peng, et al., 2011). These findings suggest that transcriptional dysregulation of pro-and anti-oxidant enzymes resulting from imbalanced expression of HIF-1 α and HIF-2 α is an important molecular mechanism contributing to increased ROS generation in CB by IH.

Established species of reactive O₂ radicals include hydrogen peroxide (H₂O₂), superoxide anion (O₂^{•-}), and hydroxyl radicals (•OH). Peng et al (Peng, et al., 2009) found that CB activation by IH can be prevented by treating CBs with polyethylene glycol (PEG)-catalase, a scavenger of H₂O₂ but not scavenger of O₂^{•-}. They further found that challenging control rat CBs with nanomolar concentrations of H₂O₂, like IH treated CBs, augments the hypoxic sensory response and induces sensory LTF by repetitive hypoxia. These findings suggest that H₂O₂ is a major O₂-derived radical that contributes to CB activation by IH.

ROS-dependent H₂S production mediates CB activation by IH

Emerging evidence suggest that H₂S is an important gasotransmitter for CB stimulation by hypoxia. Cystathionine γ -lyase (CSE) and cystathionine β -synthase (CBS) are two major enzymes that catalyze the formation of endogenous H₂S. CSE-like immunoreactivity was seen in glomus cells of carotid bodies from mice and rats as evidenced by co-localization with tyrosine hydroxylase (TH), an established marker of glomus cells (Peng, et al., 2010). CBS expression was also reported in mice (Li, et al., 2010) and cat carotid bodies (Fitzgerald, et al., 2011). However, mice lacking CSE display remarkable reduction in basal H₂S levels in the CB as compared to wild type mice (Peng, et al., 2010). Either genetic ablation or pharmacological inhibition of CSE markedly attenuate CB sensory response to hypoxia (Peng, et al., 2010), hypoxia-evoked [Ca²⁺]_i elevation in glomus cells (Makarenko, et al., 2012), and stimulation of breathing by hypoxia, a hallmark of the CB chemo reflex (Kumar and Prabhakar, 2012). Exogenous H₂S, like hypoxia, stimulates the CB sensory nerve activity in rats, mice, rabbits and cats (Jiao, et al., 2015, Li, et al., 2010, Peng, et al., 2010). However, CB glomus cell responses to anoxia (PO₂ ~ 0 mmHg), as opposed to hypoxia (PO₂ ~ 40mmHg) were found to be unaffected by putative inhibitors of CSE (Kim, et al., 2015). These studies suggest that CSE-derived H₂S is an important mediator of CB sensory response to hypoxia but not to anoxia.

Adult rats and mice exposed to IH show markedly elevated H₂S concentrations in the CB and this effect was absent in rats treated with L-propargylglycine (L-PAG), an inhibitor of CSE, and in CSE deficient mice (Yuan, et al., 2016), suggesting that IH increases CSE-dependent H₂S production. A recent study examined whether ROS contributes to enhanced H₂S production by IH (Yuan, et al., 2016). Treating rats with MnTMPyP, a membrane-permeable ROS scavenger (Gardner, et al., 1996), during the ten days of IH exposure blocked the IH-induced increase in H₂S concentrations in the CB. Rats treated with inhibitor of H₂S synthesis or CSE deficient mice exhibit remarkable absence of IH-induced increase in baseline sensory activity, augmented hypoxic sensitivity, and sensory LTF (Yuan, et al., 2016). These findings indicate that ROS-dependent increase in CSE-derived H₂S mediates CB activation by IH.

How do ROS increase H₂S production?

CSE is the major H₂S synthesizing enzyme in the CB (Peng, et al., 2010). Studies on human embryonic kidney (HEK)-293 cells expressing CSE showed absence of direct effects of IH-evoked ROS on CSE activity and H₂S production (Yuan, et al., 2016). CBs express heme oxygenase-2 (HO-2), an enzyme that catalyzes the synthesis of another gaseous messenger carbon monoxide (CO) (Prabhakar, et al., 1995). CO inhibits H₂S production by inactivating CSE through protein kinase G (PKG)-dependent phosphorylation (Yuan, et al., 2015). CBs of IH treated rats showed reduced CO levels, PKG activity and CSE phosphorylation, and all these effects were absent following administration of the ROS scavenger MnTMPyP (Yuan, et al., 2016). These findings suggest that ROS generated during IH inhibits HO-2- CO production leading to an increase in H₂S production by CSE.

Analysis of underlying mechanisms showed that ROS generated by IH had no impact on the apparent K_m of HO-2 for heme, but reduced maximal CO synthesis (V_{max}), and this effect was prevented by ROS scavenger (Yuan, et al., 2016), suggesting redox regulation of CO synthesis by HO-2. Redox regulation of proteins/enzymes, in general, involve oxidation and reduction of cysteine thiols (McCoubrey, et al., 1997). The C-terminal region of HO-2 contains three heme regulatory motifs (HRM) at cysteine residues (Cys¹²⁷, Cys²⁶⁵, and Cys²⁸²) (McCoubrey, et al., 1997). Mutational analysis revealed that ROS generated by IH targets Cys²⁶⁵ in the HRM, thereby decrease V_{max} and CO generation leading to increased H₂S production by CSE (Yuan, et al., 2016). Furthermore, H₂O₂ was identified as the major O₂ radical species inhibiting HO-2-CO production by IH (Yuan, et al., 2016). The signaling pathways involving ROS and gaseous transmitters in CB activation by IH are summarized in fig. 1.

Functional significance of IH-induced ROS-gasotransmitter signaling in the CB

Chemo reflex arising from the CB is a major regulator of sympathetic tone and blood pressure (Prabhakar, et al., 2015). Like sleep apnea patients, IH exposed rats exhibit markedly elevated baseline sympathetic nerve activity and hypertension (Prabhakar, et al., 2015). To assess whether H₂S signaling in the CB contributes to IH-induced sympathetic activation, and hypertension, rats were treated with L-PAG, an inhibitor of CSE, during the

last two days of ten day IH exposure. Blood pressure and splanchnic sympathetic nerve activity (SNA) were elevated in IH-exposed rats, as compared to control rats, and these effects were absent in IH exposed rats treated with L-PAG. Furthermore, unlike wild-type, CSE null mice treated with IH displayed no changes in blood pressure and absence of sympathetic activation as evidenced by absence of elevated plasma catecholamines (Yuan, et al., 2016). These findings suggest that interaction between ROS and gaseous transmitters in the CB plays an important role in evoking autonomic morbidities by IH.

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Abbreviations

CB	carotid body
IH	intermittent hypoxia
OSA	obstructive sleep apnea
CSE	central sleep apnea
ROS	reactive oxygen species
CSE	Cystathionine γ -lyase
CBS	cystathionine β -synthase
HO-2	heme oxygenase-2
CO	carbon monoxide
H₂S	hydrogen sulfide
H₂O₂	hydrogen peroxide

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Figure 1.

Intermittent hypoxia (IH) leads to carotid body (CB) activation through inactivation of heme oxygenase (HO)-2 by reactive oxygen species (ROS, H₂O₂) leading to reduced carbon monoxide (CO) and protein kinase G (PKG) activity, which in turn increase cystathionine- γ -lyase (CSE)-derived H₂S resulting in chemo reflex-dependent activation of the sympathetic nervous system and hypertension.