14 Therapeutic Targeting of the Carotid Body for Treating Sleep Apnea in a Pre-clinical Mouse Model

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Abstract

Sleep apnea with periodic cessation of breathing during sleep is a highly prevalent respiratory disorder affecting an estimated 10% of adults. Patients with sleep apnea exhibit several co-morbidities including hypertension, stroke, disrupted sleep, and neurocognitive and metabolic complications. Emerging evidence suggests that a hyperactive carotid body (CB) chemo reflex is an important driver of apneas in sleep apnea patients. Gasotransmitters carbon monoxide (CO) and hydrogen sulfide (H_2S) play important roles in oxygen sensing by the CB. We tested the hypothesis that an augmented CB chemo reflex stemming from disrupted CO-H_2S signaling may lead to sleep apnea. This possibility was tested in mice deficient in hemeoxygenase-2 (HO-2), an enzyme involved in CO synthesis, which were shown to exhibit hyperactive CB activity due to high H_2S levels. We found that HO-$2^{-/-}$ mice exhibit a high incidence of apneas during sleep compared to wild type mice. Blocking the CB hyperactivity with L-propargylglycine, an inhibitor of cystathionine-γ-lyase (CSE), which catalyzes H_2S synthesis, prevented apneas in HO-$2^{-/-}$ mice. These findings suggest that targeting CB with inhibitors of CSE might be a novel therapeutic strategy for preventing sleep apnea.

Keywords

Hemeoxygenase-2 · Carbon monoxide · Cystathionine-γ-lyase · Hydrogen sulfide · Obstructive · Central sleep apnea

14.1 Introduction

Sleep disordered breathing (SDB) with obstructive (OSA) and central apnea (CSA) is a clinically prevalent respiratory disorder affecting an estimated 10% of the adult human population (Peppard et al. 2013). Patients with SDB exhibit a wide spectrum of pathologies including hypertension, stroke, neurocognitive and metabolic dysfunctions and disrupted sleep (St-Onge et al. 2016). Continuous positive airway pressure (CPAP) is the current treatment of choice for OSA (Combs et al. 2014; Ip et al. 2012; Veasey

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et al. 2006) and adaptive servo-ventilation is considered beneficial for CSA patients (Javaheri et al. 2016; Yamauchi et al. 2016). However, a substantial number of OSA patients do not respond to CPAP therapy (Antic et al. 2015; Gilmartin et al. 2005; Quan et al. 2012). Adaptive ventilation in heart failure patients exhibiting CSA showed 30% mortality with no demonstrable benefits for CSA (Cowie et al. 2015). These findings suggest that current treatment strategies are suboptimal for normalizing breathing in SDB patients. The estimated cost burden in unmanaged sleep apnea patients is exorbitantly high and ranges between ~$65 and 165 billion per year in the U.S. (Frost and Sullivan 2016; Harvard Medical School Division of Sleep Medicine 2010), highlighting a need for developing alternative therapeutic strategies for preventing SDB. However, development of additional therapeutic strategies critically depends on the availability of suitable animal model(s) exhibiting SDB. SDB patients often exhibit a mixed phenotype of OSA and CSA (Javaheri et al. 2009; Morgenthaler et al. 2006). Therefore, identifying animal model(s) exhibiting both OSA and CSA will facilitate the development of alternative therapeutic strategies.

Reflex arising from the carotid body (CB), the primary sensory organ for detecting hypoxia, is a major regulator of breathing (Kumar and Prabhakar 2012). Clinical studies have implicated abnormal CB chemo reflex in both genesis and downstream pathologies caused by sleep apnea (Dempsey et al. 2012; Kara et al. 2003; Mansukhani et al. 2015; Xie et al. 1995). Emerging evidence suggests that gasotransmitters play an important role in oxygen sensing by the CB (Makarenko et al. 2012; Peng et al. 2010, 2014; Prabhakar and Peers 2014; Yuan et al. 2015). Mice lacking hemeoxygenase-2 (HO-2), an enzyme responsible for the generation of endogenous carbon monoxide (CO), exhibit exaggerated CB response to hypoxia, due to increased cystathionine-γ-lyase (CSE)-derived H_2S synthesis (Yuan et al. 2015). These findings led us to hypothesize that HO-2 null mice exhibit high incidence of apnea, and pharmacologic blockade of H_2S synthesis prevents apnea. We tested this hypothesis by monitoring breathing under normoxia with whole body plethysmography continuously from 10 AM to 4 PM (which is the sleep time for rodents) in adult wild type and HO-$2^{-/-}$ mice of both genders.

14.2 Mice with Deficiency in HO-2 Exhibit Sleep Apnea

14.2.1 HO-$2^{-/-}$ Mice Exhibit High Incidence of Apnea and Hypopnea

Figure 14.1 shows examples of tracings of breathing in a wild type and a HO-$2^{-/-}$ mouse. Wild type mouse displayed relatively regular breathing during the 6 h period of recording. However, HO-$2^{-/-}$ mouse exhibited irregular breathing with apnea and hypopnea. We defined apnea as cessation of breathing for >2.5 breaths duration, excluding post sigh apnea, and hypopnea as

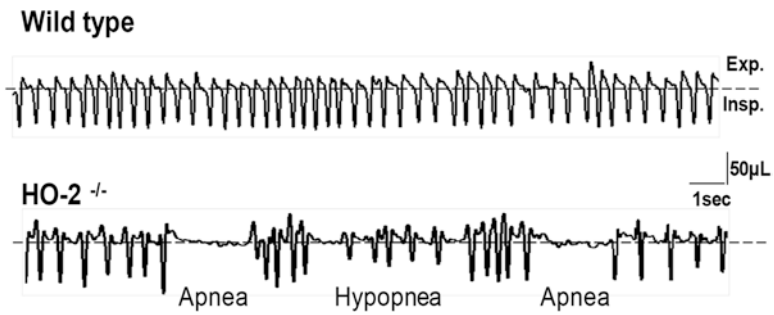

Fig. 14.1 Apnea and hypopnea in HO-2 null mice

Table 14.1 Incidence of apnea and hypopnea in wild type and HO-2$^{-/-}$ mice

Index (events/hr)	wild type (n = 40)	HO-2$^{-/-}$ (n = 70)
Apnea:		
<5	20	13
5~9	11	4
10~19	7	13
≥20	2	40
Hypopnea:		
<20	22	4
20~39	12	13
40~79	5	14
≥80	1	39

Table 14.2 Incidence of apnea and hypopnea during each state (events/stage hr) in wild type and HO-2$^{-/-}$ mice (n = 7 each)

	wild type	HO-2$^{-/-}$
Apnea:		
Wake	3 ± 2	5 ± 3
NREM	10 ± 4	29 ± 4*
REM	13 ± 7	60 ± 15**
Hypopnea:		
Wake	9 ± 3	17 ± 8
NREM	16 ± 7	55 ± 11*
REM	16 ± 5	70 ± 14**

*$P < 0.05$; **$P < 0.01$ (vs WT), data presented as mean ± SEM

breathing events with ≥30% reduction in tidal volume. As shown in Table 14.1, a majority of HO-2$^{-/-}$ mice showed greater than 20 events of apnea per hour (apnea index), ranging from 20 to 168 apneas/hour, whereas only 5% wild type mice exhibited such severe apneas. Likewise, 56% HO-2$^{-/-}$ mice showed greater than 80 events of hypopnea per hour (hypopnea index, Table 14.1), whereas only 2.5% wild type mice displayed similar hypopnea index. Chi-squared test showed that the distributions of apnea and hypopnea were significantly different between wild type and HO-2$^{-/-}$ mice ($P < 0.01$). These observations suggest that HO-2$^{-/-}$ mice exhibit high incidence of apnea and hypopnea.

14.2.2 HO-2$^{-/-}$ Mice Exhibit Sleep Apnea

Sleep-wake states significantly influence breathing. To examine the impact of sleep-wake states on the occurrence of apneas, we simultaneously monitored electroencephalogram (EEG), electromyogram of neck muscles (EMG) and breathing in un-sedated wild type and HO-2$^{-/-}$ mice. Sleep-wake states are determined based on analysis of combination of EEG spectrum and neck muscle tone. Wake state is characterized by EEG activity of mixed frequency with low amplitude and high muscle tone. Rapid eye movement (REM) sleep is identified by both the frequency of theta waves (6–9 Hz) and muscle atonia. Non-REM (NREM) sleep is characterized by high amplitude slow waves in the delta frequency range (1–4 Hz) and low muscle tone. Incidence of apnea and hypopnea during wake state were low and comparable between wild type and HO-2$^{-/-}$ mice (Table 14.2). NREM or REM sleep had little impact on apnea or hypopnea index in wild type mice. In striking contrast, apnea and hypopnea indices significantly increased during NREM sleep, and further increased during REM sleep in HO-2$^{-/-}$ mice (Table 14.2). These results demonstrate that HO-2$^{-/-}$ mice exhibit sleep apnea.

14.2.3 HO-2$^{-/-}$ Mice Exhibit Both Central and Obstructive Apnea

Apneas are classified as central and obstructive in nature. To characterize the apnea phenotype, we monitored inspiratory intercostal muscle electromyography activity with chronically implanted electrodes along with breathing in un-sedated HO-2$^{-/-}$ mice (n = 12). There were incidences of apnea with absence of both breathing movements and respiratory muscle activity, which were considered as central apneas. On other hand, some of the apneic events were associated with increased inspiratory muscle activity, which were considered as obstructive apneas. We found that a given HO-2$^{-/-}$ mouse exhibited both central and obstructive apnea during 6 h of recording. Average data showed that 59% of apnea events

Table 14.3 Incidence of obstructive versus central apnea in HO-2$^{-/-}$ mice

	Obstructive Apnea	Central Apnea
Apneas (% of Total)	59 ± 5	41 ± 5*

*$P < 0.05$, data presented as mean ± SEM

Table 14.4 Effect of hyperoxia and hypoxia on incidence of apnea in HO-2$^{-/-}$ mice

	21% O_2	90% O_2	15% O_2
Apnea index (% of 21% O_2)	100 ± 13	34 ± 5**	350 ± 62**

**$P < 0.01$ (vs 21% O_2), data presented as mean ± SEM

are of obstructive type, and 41% are of central type (Table 14.3).

14.3 Enhanced Carotid Body (CB) Chemo Reflex Contributes to Apnea in HO-2 Null Mice

We determined whether an exaggerated CB reflex contributes to high incidence of apnea in HO-2$^{-/-}$ mice. Hyperoxia (90% O_2), which is known to inhibit the CB activity, markedly reduced the number of apneas (Table 14.4). In contrast, hypoxia (15% O_2), a physiological activator of the CB, significantly increased the incidence of apnea (Table 14.4). Further experiments showed that hyperoxia reduced the incidence of both obstructive and central apneas. In contrast, hypoxic breathing increased the incidence of obstructive and central apnea by 3 and 2 fold, respectively. Although apneas could result from reduced chemosensitivity to CO_2 (Kumar et al. 2015), we found that stimulation of central CO_2 chemoreceptors with 2% CO_2 caused only a modest reduction (~32%) in the apnea index in HO-2$^{-/-}$ mice, which was due to reduced incidence of central apnea. These observations suggest that the exaggerated CB reflex is a major contributor to apneas in HO-2$^{-/-}$ mice.

14.4 Pharmacologic Blockade of H_2S Synthesis Prevents Apneas in HO-2 Mice

HO-2$^{-/-}$ mice exhibit increased CSE-derived H_2S production in the CB, and lowering H_2S production by genetic removal of CSE prevents the exaggerated CB response to hypoxia in HO-2$^{-/-}$ mice (Yuan et al. 2015). We found that apnea and hypopneas were absent in HO-2/CSE double knockout mice, suggesting that lowering H_2S production may prevent apnea. While genetic removal of CSE might not be a practical therapeutic strategy to treat sleep apnea clinically, we explored whether pharmacologic blockade of CSE-derived H_2S synthesis stabilizes breathing in HO-2$^{-/-}$ mice. Intra-peritoneal administration of L-propargylglycine (L-PAG), an inhibitor of CSE, reduced the incidence of apnea in a dose-dependent manner (1 ~ 150 mg/kg), with a median effective dose at 30 mg/kg (Table 14.5). L-PAG reduced both central and obstructive apneas. The effect of L-PAG at a dose of 30 mg. kg on apneas was seen within 2 h after administration and completely reversed after 24 h. More importantly, L-PAG (30 mg/kg) administration via oral route was equally effective in reducing the number of apneas and hypopneas. No sign of toxicity was observed with any of the doses of L-PAG tested.

14.5 Summary and Perspective

Our study establishes that HO-2 null mice exhibit a high incidence of apnea and hypopnea during sleep and might serve as a pre-clinical murine model of sleep apnea. Like patients with sleep apnea, HO-2 null mice display both central and obstructive apneas. The enhanced CB chemo reflex mediated by CSE-produced H_2S contributes to high incidence of apneas. Lowing H_2S production by pharmacologic blockade of CSE significantly prevent apneas in HO-2 null mice. Since sleep apnea is a multi-factorial respiratory disease, it would be important to determine whether CSE inhibitors prevent apneas in patients

Table 14.5 Effect of intraperitoneal administration of L-PAG on incidence of apnea in HO-2$^{-/-}$ mice

		L-PAG (mg/kg)					
	Vehicle	1	3	10	30	90	150
Apnea index	100 ± 12	88 ± 9	70 ± 9	68 ± 6*	49 ± 5*	36 ± 5*	20 ± 6*
(% of Vehicle)							

*$P < 0.05$; (vs Vehicle), data presented as mean ± SEM

with sleep apnea caused by other etiologies, such as heart failure, renal insufficiency and stroke.

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