Respiratory Physiology & Neurobiology 209 (2015) 115–119

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Review

# Neuromolecular mechanisms mediating the effects of chronic intermittent hypoxia on adrenal medulla☆

Ganesh K. Kumar*, Jayasri Nanduri, Ying-Jie Peng, Nanduri R. Prabhakar

*Institute for Integrative Physiology and Center for Systems Biology of $O_2$ Sensing, Biological Sciences Division, University of Chicago, IL, USA*

ARTICLE INFO

*Article history:*
Accepted 1 January 2015
Available online 10 January 2015

*Keywords:*
Catecholamine secretion
Reactive oxygen species
Carotid body chemoreflex
HIF-α isoform dysregulation
Tyrosine hydroxylase activation
Hypertension

ABSTRACT

Sleep disordered breathing (SDB) with recurrent apnea is a major health problem affecting several million adult men and women. Humans with SDB are prone to develop hypertension. Studies on rodents established that exposure to chronic intermittent hypoxia (CIH) alone is sufficient to induce hypertension similar to that seen in patients with SDB. Available evidence from studies on experimental animals suggests that catecholamines secreted from adrenal medulla (AM), an end-organ of the sympathetic nervous system is a major contributor to CIH-induced hypertension. In this article, we present an overview of our current understanding on how CIH reconfigures AM function and highlight recent findings on the underlying cellular and molecular mechanisms.



## 1. Introduction

Sleep disordered breathing (SDB), with recurrent apnea is a major health problem affecting several million adult men and women (Nieto et al., 2000; Shahar et al., 2001). Recurrent apnea can be either due to obstruction of the upper airway (obstructive sleep apnea, OSA) or defective generation of respiratory rhythm by the central nervous system (central apnea). People with SDB are at increased risk to develop hypertension and stroke (Nieto et al., 2000; Shahar et al., 2001; Peppard et al., 2000). Recurrent apneas are associated with intermittent hypoxia, intermittent hypercapnia, and variation in intra-thoracic pressure. Studies on experimental animals established that exposure to chronic intermittent hypoxia (CIH) alone is sufficient to induce hypertension similar to that seen in patients with SDB (Fletcher, 2001). Considerable evidence suggests that catecholamines (CA) secreted from adrenal medulla (AM) is a major contributor to CIH-induced hypertension (Bao et al., 1997; Peng et al., 2014). In this article, we present a brief review of how CIH affects AM function and discuss the underlying cellular and molecular mechanisms.

## 2. Contribution of adrenal medulla (AM) to CIH-induced hypertension

Patients with SDB have a sustained elevation in blood pressure during daytime wherein apneas are absent (Carlson et al., 1993; Somers et al., 1995) and they exhibit a more pronounced raise in blood pressure during apneic episodes than normal subjects (Stoohs and Guilleminault, 1992; Imadojemu et al., 2002). Likewise, rats exposed to CIH, mimicking $O_2$ saturation profiles seen in recurrent apnea patients, also developed hypertension (Fletcher et al., 1992) and displayed a marked increase in blood pressure in response to brief episodes of hypoxia as compared to normoxia exposed controls (Peng et al., 2014) (Fig. 1).

Adrenal medullary chromaffin cells (AMC) synthesize and secrete catecholamines (CA) in response to a variety of stresses including hypoxia. Two studies have examined the role of CA derived from AMC in CIH-induced changes in blood pressures using adrenal medulla ablation as an experimental tool. Bilateral adrenal medullectomy prevented CIH-induced hypertension and increase in plasma catecholamines (Bao et al., 1997) as well as the abnormal elevation in blood pressure induced by acute exposure to hypoxia (Peng et al., 2014) (Fig. 1). These findings suggest that CA secreted from AM is a critical contributor to acute and chronic blood pressure elevation seen in CIH exposed rats.

## 3. CIH facilitates catecholamine secretion from AM

The effect of CIH on CA secretion from AM was examined in age-matched adult male rats exposed to either CIH (alternating

☆ This paper is part of a special issue entitled "Molecular basis of ventilatory disorders" guest-edited by Dr. Mietek Pokorski.

* Corresponding author at: Institute for Integrative Physiology, The Center for Systems Biology of $O_2$ Sensing, Department of Medicine, MC 5068, 5841 South Maryland Avenue, Chicago, IL 60637, USA. Tel.: +1 773 834 7652; fax: +1 773 834 5252.
*E-mail address:* gkumar@bsd.uchicago.edu (G.K. Kumar).

http://dx.doi.org/10.1016/j.resp.2015.01.001
1569-9048/

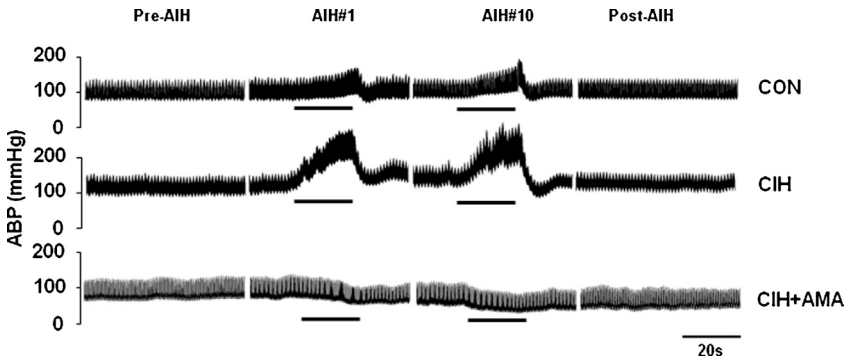


**Fig. 1.** Evidence for the involvement of adrenal medulla in CIH-induced increases in arterial blood pressure (ABP) in response to acute intermittent hypoxia (AIH). Three groups of rats were studied: Sham-operated and normoxia-exposed (CON); Sham-operated and exposed to CIH for 10 days (CIH) and bilateral adrenal medulla ablated (AMA) and CIH-exposed (CIH + AMA). ABP was monitored before, during and after AIH challenges. Each AIH episode consists of 12% $O_2$ for 15 s followed by 21% $O_2$ for 5 min. Representative changes in ABP during pre-AIH, first and 10th AIH, and post-AIH periods are shown. The black bar indicates the duration of hypoxic challenge.

cycles of 5% $O_2$ for 15 s and 21% $O_2$ for 5 min, 8 h/day) or normoxia for 10 days (Kumar et al., 2006). Noradrenaline (NA) and adrenaline (A) effluxes were monitored from freshly prepared AM slices that are harvested from CIH and normoxia exposed rats. In CIH exposed rats, acute hypoxia evoked a robust efflux of NA and A from AM in a time-dependent manner wherein three days of CIH was ineffective whereas ten days of CIH produced a robust efflux. However, hypoxia-evoked CA efflux was absent in control rats. Unlike hypoxia, hypercapnia-evoked CA efflux was unaffected by CIH, suggesting that CIH selectively augments hypoxia-evoked CA secretion from AM. A similar CIH-induced facilitation of CA secretion by hypoxia was also observed in single AMC isolated from adult mice (Kuri et al., 2007). It is noteworthy that unlike CIH, continuous hypoxia was ineffective in facilitating CA efflux from rat AM. Collectively, these findings demonstrate that CIH exposure leads to selective facilitation of hypoxia-evoked CA release from AM of rats and mice.

### 3.1. Signaling mechanisms mediating the effects of CIH on AM

The magnitude of CA secretion from AMC is, in part, set by regulating the number of release-competent granules, a population that comprises the 'readily-releasable pool' (Heinemann et al., 1993). Further, increase in intracellular $Ca^{2+}$ levels is a pre-requisite for catecholamine secretion from AMC. In the following sections, the effects of CIH on readily releasable pool of secretory vesicles and calcium signaling in the AM will be presented.

#### 3.1.1. Readily releasable pool

The effect of CIH on the readily releasable pool of secretory granules was studied in adult mice AMC using a dual-pulse protocol (Kuri et al., 2007). The results from this study showed that CIH significantly increases the number of readily releasable pool of secretory granules as compared to normoxic control. Further, CIH activated protein kinase C (PKC) in AM as evidenced by the increased phosphorylation of PKC at Thr-514 and a PKC inhibitor prevented CIH-induced increase in the number of readily releasable pool of secretory granules (Kuri et al., 2007).

#### 3.1.2. $Ca^{2+}$ signaling

AMC from CIH exposed rats exhibited elevated basal $[Ca^{2+}]_i$ and this effect was attributed to calcium mobilization via activation of ryanodine receptors (RyRs) by S-glutathionylation (Souvannakitti et al., 2010). In addition, CIH increased RyR 2 and 3 mRNA levels in rat AM. Blockade of RyRs prevented the baseline $[Ca^{2+}]_i$ elevation suggesting that S-glutathionylation-mediated activation of RyRs contributes to augmented intracellular $Ca^{2+}$ stores in CIH exposed AMC.

Acute hypoxia-evoked increase in $[Ca^{2+}]_i$ was markedly enhanced in CIH treated neonatal AMC and this effect was mediated by increases in both voltage-gated $Ca^{2+}$ flux and intracellular $Ca^{2+}$ stores (Souvannakitti et al., 2009). CIH up regulated $Ca_v3.1$ and $Ca_v3.2$ T-type $Ca^{2+}$ channel mRNA levels and augmented T-type $Ca^{2+}$ currents in neonatal rat AMC. Mibefradil, a blocker of T-type $Ca^{2+}$ channels not only attenuated hypoxia-evoked $[Ca^{2+}]_i$ elevation but also the enhanced CA secretion from AMC of CIH-treated rats (Souvannakitti et al., 2010). These findings, taken together, suggest that PKC-mediated increase in the number of readily releasable pool of secretory granules and up regulation of $Ca^{2+}$ signaling pathways contributes to CIH-induced enhanced CA secretion in AM (Fig. 2).

### 3.2. CIH increases CA levels via activation of tyrosine hydroxylase

Kumar et al. (2006) investigated the effect of CIH on catecholamine levels in rat AM and found that 10 days of CIH significantly increased NA and A levels as compared to normoxic controls. The mechanism(s) underlying CIH-induced increase in CA levels was investigated in pheochromocytoma 12 (PC12) cell cultures which express dopamine (DA) as the major catecholamine. IH (consisting of 1% $O_2$ for 15 s followed by 21% $O_2$ for 4 min; 60 cycles) increased DA levels in PC12 cells with a concomitant increase in tyrosine hydroxylase (TH) enzyme activity, the rate-limiting enzyme in CA biosynthesis without altering TH protein levels (Kumar et al., 2003). IH-induced increase in TH activity was in part due to increased phosphorylation of serine 40, which is located at the N-terminal regulatory domain of TH. The increases in enzyme activity and serine phosphorylation of TH caused by IH were significantly attenuated in PC12 cells that are pre-treated with protein kinase inhibitors (Kumar et al., 2003). Taken together, these findings suggest that IH-induced raise in CA levels is in part due to increased CA synthesis via activation of TH involving serine phosphorylation.

## 4. Involvement of reactive oxygen species (ROS)

Considerable evidence suggests that ROS signaling is an important cellular mechanism mediating the systemic responses to CIH (for references see Prabhakar et al., 2007). Consistent with this notion, CIH increased ROS levels in AM of adult (Kumar et al., 2006) and neonatal rats (Souvannakitti et al., 2009) as well as in mice (Kuri et al., 2007). The functional significance of increased ROS levels on CIH-induced changes in AM was assessed by treating rats with manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride (MnTMPyP; 5 mg/kg, IP), a potent scavenger of ROS every day prior