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Carotid Body Chemoreflex Mediates Intermittent Hypoxia-Induced Oxidative Stress in the Adrenal Medulla

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Abstract

Intermittent hypoxia (IH) increases reactive oxygen species generation resulting in oxidative stress in the adrenal medulla (AM), a major end-organ of the sympathetic nervous system which facilitates catecholamine secretion by hypoxia. Here, we show that carotid body chemoreflex contributes to IH-induced oxidative stress in the AM. Carotid bodies were ablated by cryocoagulation of glomus cells, the putative O_2 sensing cells. Carotid body ablated (CBA) and control rats were exposed to IH and the redox state of the AM was assessed biochemically. We found that IH raised reactive oxygen species levels along with an increase in NADPH oxidase (Nox), a pro-oxidant enzyme and a decrease in superoxide dismutase-2 (SOD2), an anti-oxidant enzyme. Further, IH increased hypoxia-inducible factor (HIF)-1α, whereas decreased HIF-2α, the transcriptional regulator of Nox and SOD-2, respectively. These IH-induced changes in the AM were absent in CBA rats. Moreover, IH increased splanchnic nerve activity and facilitated hypoxia-evoked catecholamine efflux from the AM and CBA prevented these effects. These findings suggest that IH-induced oxidative stress and catecholamine efflux in the AM occurs via carotid body chemoreflex involving HIF α isoform mediated imbalance in pro-, and anti-oxidant enzymes.

Keywords

Reactive oxygen species; sleep disordered breathing; hypoxia-inducible factors; carotid body ablation; pro-oxidant and anti-oxidant

1. Introduction

Sleep disordered breathing (SDB), a highly prevalent health problem in adult men and infants born prematurely, is associated with intermittent hypoxia (IH). Humans with SDB are prone to develop cardiovascular abnormalities including hypertension. Studies in rodents exposed to IH showed that reactive oxygen species (ROS) generation is increased in peripheral and central nervous tissues and pretreatment with anti-oxidant prevented not only the elevation in ROS but also the cardiovascular abnormalities evoked by IH (*for references see* Prabhakar et al. 2007). Since ROS emerges as a critical mediator of IH-induced patho-physiology, it is of considerable importance to determine whether ROS generation is due to a direct effect of IH on various tissues or is mediated by an indirect mechanism. The pO_2 of most tissues under basal, normoxic condition is in the range of 30–60 mmHg (Carreau et al.

2011) which is much lower than the arterial pO_2 of ~ 100 mmHg. In each cycle of IH, the arterial O_2 saturation of rats decreases from 97% to 80% (Peng et al. 2014). Consequently, most tissues may not be able to sense this modest level of hypoxia during IH. On the other hand, carotid bodies, due to their very high blood flow and exquisite sensitivity to hypoxia, are capable of sensing and responding to modest changes in pO_2 during each cycle of IH and the resulting augmented chemoreflex may, in turn, transmit the changes in pO_2 to other tissues. These considerations led us to *hypothesize* that activation of carotid body chemoreflex contributes to IH-induced ROS generation in various tissues. In a recent study, we tested this hypothesis by examining ROS generation in the adrenal medulla (AM), an end-organ of the sympathetic nervous system activated by the carotid body chemoreflex (Peng et al. 2014).

2. Methods

Animals

Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Chicago. Experiments were performed on adult, male Sprague-Dawley rats. At the termination of the experiment, rats were killed with an overdose of anesthesia. Carotid body ablation (CBA) was performed bilaterally using cryocoagulation with liquid nitrogen as described previously (Verna et al. 1975). Sham-operated rats served as controls.

Exposure to IH

Control (sham-operated) and CBA rats were allowed to recover from surgery for 1 week. Rats were exposed to alternating cycles of hypoxia (5% O_2 for 15 s) and normoxia (21% O_2 for 5 min) between 09.00 and 17.00 h for 10 days. Rats exposed to alternating cycles of normoxia served as controls.

Measurements of ROS, NADPH oxidase (Nox) and Superoxide Dismutase (SOD)

The cytosolic and mitochondrial aconitase activities were measured as an index of ROS generation as described (Peng et al., 2014). Enzyme activities of Nox and SOD were determined in the membrane and mitochondrial fractions as described (Peng et al. 2014).

Western blot analysis of Hypoxia-Inducible Factor (HIF) α isoforms

The expression of HIF-1α and HIF-2α proteins was analyzed by immunoblot analysis as described (Peng et al. 2014).

Splanchnic nerve activity

The branch of the left splanchnic nerve was isolated and cut above the coeliac ganglion. The central cut ends of the nerves were placed on bipolar platinum-iridium electrode for recording electrical activity as described (Peng et al. 2014).

Data analysis

All data are presented as means ± SEM. Statistical significance was assessed by one-way ANOVA followed by unpaired t-test. P value less than 0.05 was considered significant.

3. Results and Discussion

Effect of CBA on ROS generation by IH

The activity of aconitase, an enzyme in the tricarboxylic acid cycle, is sensitive to and inhibited by ROS (Gardner et al. 2002). The enzyme occurs both in the cytosol and in the mitochondria and measuring the inhibition of aconitase activity in both fractions will provide a measure of ROS generation. As shown in Table 1, IH decreased aconitase activity by 60% and 50% in the cytosolic and mitochondrial fractions of the AM, respectively. IH-induced decrease in aconitase activity was absent in CBA treated rats suggesting that oxidative stress in the AM of IH treated rats is mediated by the carotid body chemoreflex.

Effect of CBA on IH-induced changes in Nox and SOD

The cellular level of ROS is tightly regulated by the balance between the activities of the pro-, and anti-oxidant enzymes. NADPH oxidase (Nox) and superoxide dismutase-2 (SOD-2) are the major pro-, and anti-oxidant enzymes, respectively. Therefore, we determined whether carotid body chemoreflex contributes to IH-induced changes in the mRNA levels and enzyme activities of Nox and SOD-2. IH increased mRNA levels of Nox2 by ~ 2.5-fold with a concomitant increase in Nox activity in the AM (Table 2). On the other hand, mRNA levels of SOD-2 decreased by ~ 50% and SOD activity was significantly lower in IH-treated AM than in controls (~ 50%; $p < 0.01$; Table 2). IH-induced changes in Nox and SOD in the AM were absent in CBA rats.

Effect of CBA on IH-induced changes in HIF-α isoform expression

HIF-1α is known to regulate gene expression of pro-oxidant enzymes including Nox whereas that of anti-oxidant enzymes such as SOD is under the control of HIF-2α. Therefore, we determined whether carotid body chemoreflex via altering HIF-α isoform expression in the adrenal medulla contributes to IH-induced changes in Nox2 and SOD-2 mRNA levels. In IH-treated AM, HIF-1α protein expression is significantly higher whereas that of HIF-2α is lower than normoxia-treated control AM (Table 3). However, in CBA rats, IH-induced changes in HIF-α isoform expression were absent.

Effect of CBA on IH-induced changes in adrenal sympathetic nerve activity

A branch of the splanchnic nerve provides the sympathetic input to the AM. We tested whether carotid body chemoreflex mediates the increased sympathetic flow to the AM in IH treated rats. Sham-operated and IH exposed rats exhibit elevated adrenal sympathetic nerve activity both during normoxia and hypoxia and these IH-induced effects were absent in CBA rats.

Effect of CBA on IH-induced functional changes in the adrenal medulla

Previous studies have shown that IH facilitated hypoxia-evoked catecholamine (CA) efflux from the adrenal medulla in a ROS-dependent manner (Kumar et al. 2006; Kuri et al. 2007). Since CBA prevented IH-induced generation of ROS, we tested whether carotid body chemoreflex mediates IH-induced augmented CA efflux in the AM. Consistent with our previous reports, hypoxia markedly enhanced both norepinephrine and epinephrine efflux

from AM slices in IH exposed, sham-operated rats and IH-induced augmented CA efflux by hypoxia was absent in CBA rats (Table 4).

Our results demonstrated that IH-induced increase in ROS in the AM, an end-organ of the sympathetic nervous system is primarily mediated by the chemoreflex arising from the carotid body but not due to a direct effect of hypoxia. At first, IH activates the carotid body and the ensuing chemoreflex induces an imbalance between HIF-1α and HIF-2α that transcriptionally regulate Nox, a pro-oxidant and SOD, an anti-oxidant leading to increased generation of ROS. The carotid body chemoreflex-mediated oxidative stress in the AM facilitates enhanced CA efflux which may contribute to hypertension associated with apneas.

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Table 1

Effect of Carotid Body Ablation (CBA) on IH-induced Oxidative Stress in the Rat Adrenal Medulla

Samples*	**Aconitase ($nmol.min^{-1}.mg^{-1}$)**	
	Cytosol	Mitochondria
Control	6.6 ± 0.4	4.1 ± 0.5
IH-treated	2.6 ± 0.3	2.1 ± 0.2
CBA + IH-treated	5.5 ± 0.7	3.5 ± 0.4

* n = 6–7 rats per group

Table 2

Effect of Carotid Body Ablation (CBA) on IH-induced Alteration in Nox and SOD-2 Activities

Samples*	**Nox activity ($nmol.min^{-1}.mg^{-1}$)**	**SOD-2 activity (% of Control)**
Control	0.42 ± 0.02	100
IH-treated	1.13 ± 0.04	50 ± 2
CBA+IH-treated	0.46 ± 0.02	98 ± 4

* n = 6–7 rats per group

Table 3

Effect of Carotid Body Ablation (CBA) on IH-induced Alteration in HIF-α Isoform Expression

Samples*	HIF-1α protein (% of control)	HIF-2α protein (% of control)
Control	100	100
IH-treated	320 ± 5	52 ± 4
CBA + IH-treated	98 ± 2	97 ± 3

* n = 6–7 rats per group

Table 4

Effect of CBA on IH-induced Augmented Catecholamine Efflux by Hypoxia in the AM

Samples*	Norepinephrine (ng / 100ml)	Epinephrine (ng / 100ml)
Control	20 ± 4	88 ± 6
IH-treated	75 ± 3	190 ± 7
CBA + IH-treated	22 ± 2	105 ± 2

* n = 6–7 rats per group