Experimental Neurology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Chemoreception and neuroplasticity in respiratory circuits

William H. Barnett [a], Ana P. Abdala [b], Julian F.R. Paton [b], Ilya A. Rybak [c], Daniel B. Zoccal [d], Yaroslav I. Molkov [a,*]

[a] Georgia State University, Atlanta, GA, United States
[b] School of Physiology, Pharmacology and Neuroscience, University of Bristol, UK
[c] Drexel University College of Medicine, Philadelphia, PA, United States
[d] São Paulo State University, Araraquara, Brazil

ARTICLE INFO

Article history:
Received 30 November 2015
Received in revised form 22 April 2016
Accepted 26 May 2016
Available online xxxx

Keywords:
Respiration
Obstructive sleep apnea
Hypertension
Chronic intermittent hypoxia
Peripheral chemoreception
Plasticity

ABSTRACT

The respiratory central pattern generator must respond to chemosensory cues to maintain oxygen (O_2) and carbon dioxide (CO_2) homeostasis in the blood and tissues. To do this, sensorial cells located in the periphery and central nervous system monitor the arterial partial pressure of O_2 and CO_2 and initiate respiratory and autonomic reflex adjustments in conditions of hypoxia and hypercapnia. In conditions of chronic intermittent hypoxia (CIH), repeated peripheral chemoreceptor input mediated by the nucleus of the solitary tract induces plastic changes in respiratory circuits that alter baseline respiratory and sympathetic motor outputs and result in chemoreflex sensitization, active expiration, and arterial hypertension. Herein, we explored the hypothesis that the CIH-induced neuroplasticity primarily consists of increased excitability of pre-inspiratory/inspiratory neurons in the pre-Bötzinger complex. To evaluate this hypothesis and elucidate neural mechanisms for the emergence of active expiration and sympathetic overactivity in CIH-treated animals, we extended a previously developed computational model of the brainstem respiratory-sympathetic network to reproduce experimental data on peripheral and central chemoreflexes post-CIH. The model incorporated neuronal connections between the 2nd-order NTS neurons and peripheral chemoreceptors afferents, the respiratory pattern generator, and sympathetic neurons in the rostral ventrolateral medulla in order to capture key features of sympathetic and respiratory responses to peripheral chemoreflex stimulation. Our model identifies the potential neuronal groups recruited during peripheral chemoreflex stimulation that may be required for the development of inspiratory, expiratory and sympathetic reflex responses. Moreover, our model predicts that pre-inspiratory neurons in the pre-Bötzinger complex experience plasticity of channel expression due to excessive excitation during peripheral chemoreflex. Simulations also show that, due to positive interactions between pre-inspiratory neurons in the pre-Bötzinger complex and expiratory neurons in the retrotrapezoid nucleus, increased excitability of the former may lead to the emergence of the active expiratory pattern at normal CO_2 levels found after CIH exposure. We conclude that neuronal type specific neuroplasticity in the pre-Bötzinger complex induced by repetitive episodes of peripheral chemoreceptor activation by hypoxia may contribute to the development of sympathetic over-activity and hypertension.

1. Introduction

Hypertension is a highly prevalent public health problem that affects a large proportion of population worldwide (Kearney et al., 2005; Carey, 2013; Go et al., 2014). Accumulating evidence shows that reducing sympathetic nerve activity decreases blood pressure in hypertensive patients, especially in those who are resistant to pharmacologic antihypertensive treatment (Esler, 2009; Fisher and Paton, 2012), suggesting that sympathetic overactivity is a major contributor to the development and maintenance of hypertension. Moreover, experimental data indicate that increased activity of the sympathetic nervous system is pivotal for the development of high blood pressure in rodent models of hypertension (Simms et al., 2009; Malpas, 2010; Briant et al., 2015). This scenario of hypertension and sympathetic overactivity is observed in obstructive sleep apnea (OSA) patients (Narkiewicz et al., 1998). OSA is a condition characterized by recurrent upper airway collapses during sleep and affects approximately 20% of adult population in USA (Konecny and Somers, 2011). Untreated OSA has cumulative effects on the cardiovascular system, leading to augmented baseline sympathetic activity and arterial hypertension that can be refractory to pharmacological therapies (Williams et al., 2010; Pedrosa et al., 2011). Studies estimate that 50–56% of individuals with OSA are hypertensive (Dudenbostel and Calhoun, 2011).

Clinical and experimental evidence suggests that chronic exposure to intermittent hypoxia (CIH) is a main factor leading to cardiovascular dysfunction in OSA patients (Fletcher, 2001; Caples et al., 2005). In rats, CIH promotes hypertension linked to elevated baseline sympathetic vasomotor tone and higher noradrenaline plasma levels (Braga et al.,

* Corresponding author at: Department of Mathematics and Statistics, Georgia State University, 30 Pryor St SW, 750-COE, Atlanta, GA 30303, United States.
E-mail address: ymolkov@gsu.edu (Y.I. Molkov).

http://dx.doi.org/10.1016/j.expneurol.2016.05.036
0014-4886/

2006; Zoccal et al., 2007; Zoccal et al., 2008; Zoccal et al., 2009) highlighting a relationship among CIH, sympathetic overactivity and hypertension. Importantly, the high levels of sympathetic activity of CIH rats were associated with a strengthened coupling between respiratory and sympathetic networks. Indeed, we originally reported (Zoccal et al., 2008) that CIH exposure promotes an increase in sympathetic activity during the expiratory phase, specifically during the late part of expiration (late-E). These additional expiratory bursts in sympathetic activity of CIH rats were coupled to the late-E bursts emerging in the abdominal expiratory motor output. Moreover, the late-E activity was present at rest in eucapnia in CIH-treated animals but never in untreated control animals, and was eliminated by a reduction of CO_2 content in the perfusate (Molkov et al., 2011). The involvement of respiratory-sympathetic interactions in the development of hypertension in CIH rats is further supported by recent findings that late-E modulation in the pre-sympathetic neurons of rostral ventrolateral medulla (RVLM) depends on synaptic inputs from bulbar respiratory neurons rather than on changes in their intrinsic properties (Moraes et al., 2013; Moraes et al., 2014). All together these data indicate that CIH-induced sympathetic overactivity is linked to the transition of expiration from a passive to an active process at rest. These findings represent novel and unexplored aspects of central mechanisms underpinning arterial hypertension in CIH rats (Moraes et al., 2012b).

The development of arterial hypertension in rats exposed to CIH is fully prevented by previous ablation of carotid body peripheral chemoreceptors (Fletcher et al., 1992), indicating that the plasticity in the neural circuitries of the peripheral chemoreflex, elicited by repeated stimulation during CIH (Moraes et al., 2015), may underpin the development of the observed respiratory and sympathetic changes. Therefore, it is important to understand the neural pathways engaged during peripheral chemoreceptor stimulation in order to identify potential neural mechanisms triggering active expiration and sympathetic overactivity in CIH rats. Here, we discuss the hypothesis that central plasticity accounts for the facilitation of sympathetic and respiratory response to peripheral chemoreflex in CIH conditioned rats. Accordingly, the objectives of this study were (i) to model the neural pathways required for the adjustments in the respiratory and sympathetic motor outputs during peripheral chemoreflex activation, (ii) to understand the functional implications of their repetitive activation during CIH conditioning, and (iii) to shed light on where within the network the neuronal plasticity occurs that is responsible for the sustained active expiration and sympathoactivation following CIH exposure.

2. Methods

In the present study, we combined recent published studies (Braga et al., 2006; Zoccal et al., 2008; Molkov et al., 2011; Moraes et al., 2012a; McBryde et al., 2013; Moraes et al., 2014) and new experimental data obtained in the *in situ* arterially perfused preparation of decerebrate rats, as described in details below.

2.1. Experimental data

2.1.1. Animals and ethical approval

Experiments were performed on male Holtzman rats, weighing 70–90 g, obtained from the Animal Care Unit of the São Paulo State University, Araraquara, and kept at 22 ± 1 °C on a 12-h light/dark cycle (lights on 06:00–lights off 18:00), with access to food and water *ad libitum*. All experimental approaches followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23 revised 1996) and by the Brazilian National Council for Animal Experimentation Control (CONCEA), and was approved by the Local Ethical Committee in Animal Experimentation (protocol 18/2014).

2.1.2. Chronic intermittent hypoxia (CIH)

The rats were exposed to CIH as previously described (Zoccal et al., 2008). Briefly, the animals were housed in collective cages (maximum of 5 animals per cage) and maintained inside chambers equipped with gas injectors as well as sensors of O_2, CO_2, humidity and temperature, at controlled conditions of temperature (22 ± 1 °C) and humidity (55 ± 10%). The CIH protocol consisted of 5 min of normoxia (FiO_2 of 20.8%) followed by 4 min of pure N_2 injection into the chamber in order to reduce the fraction of inspired O_2 (FiO_2) to 6%, remaining at this level for 40 seconds. After this hypoxic period, pure O_2 was injected to return the FiO_2 back to 20.8%. This 9-minute cycle was repeated 8 h a day (from 9:30 am to 5:30 pm) for 10 days. During the remaining 16 h, the animals were maintained at a FiO_2 of 20.8%. The injections of N_2 and O_2 (White Martins, São Carlos, Brazil) were regulated by a solenoid valve system whose opening-closing control was performed by a computerized system (Oxycycler, Biospherix, USA). In an identical chamber in the same room, the control group was exposed to a FiO_2 of 20.8% 24 h a day for 10 days. The control rats were also exposed to a similar valve noise due to the frequent injection of O_2 to maintain the FiO_2 at 20.8%. In both CIH and control chambers, the gas injections were performed at the upper level of the chamber in order to avoid direct jets of gas impacting on the animals, which could cause stress.

2.1.3. In situ arterially perfused preparation of decerebrate rats

Arterially perfused *in situ* preparations (Paton, 1996) of control and CIH rats were surgically prepared, as previously described (Zoccal et al., 2008). The rats were deeply anesthetized with halothane (AstraZeneca, Cotia, SP, Brazil) until loss of paw withdrawal reflex, transected caudal to the diaphragm, submerged in a chilled Ringer solution (in mM: NaCl, 125; $NaHCO_3$, 24; KCl, 3; $CaCl_2$, 2.5; $MgSO_4$, 1.25; KH_2PO_4, 1.25; dextrose, 10) and decerebrated at the precollicular level. Lungs were removed. Preparations were then transferred to a recording chamber, the descending aorta was cannulated and perfused retrogradely with Ringer solution containing 1.25% Polyethylene glycol (an oncotic agent, Sigma, St Louis, USA) and a neuromuscular blocker (vecuronium bromide, 3–4 $\mu g \cdot ml^{-1}$, Cristália Produtos Químicos Farmacêuticos Ltda., São Paulo, Brazil), using a roller pump (Watson-Marlow 502s, Falmouth, Cornwall, UK) via a double-lumen cannula. The perfusion pressure was maintained in the range of 50–70 mm Hg by adjusting the flow rate to 21–25 $ml \cdot min^{-1}$ and by adding vasopressin to the perfusate (0.6–1.2 nM, Sigma, St. Louis, MO, USA). The perfusate was gassed continuously with 5% CO_2–95% O_2, warmed to 31–32 °C and filtered using a nylon mesh (pore size: 25 μm, Millipore, Billirica, MA, USA). Sympathetic and respiratory nerves were isolated and their activity recorded simultaneously using bipolar glass suction electrodes held in micromanipulators (Narishige, Tokyo, Japan). Left phrenic nerve (PN) discharges were recorded from its central end and its rhythmic ramping activity was used to monitor preparation viability. Left cervical vagus (cVN) and hypoglossal nerves (HN) as well as right thoracic/lumbar abdominal nerves (AbN; T13-L1) were isolated, cut distally and their central activity recorded. Thoracic sympathetic activity was recorded from the left sympathetic chain (tSN) at T8–T12 level. All the signals were amplified, band-pass filtered (0.1–3 kHz; P511, Grass Technologies, Middleton, USA) and acquired in an A/D converter (CED micro 1401, Cambridge Electronic Design, CED, Cambridge, UK) to a computer using Spike 2 software (5 KHz, CED, Cambridge, UK). At the end of the experiments, the perfusion pump was turned off to determine the electrical noise (after the death of the preparations).

All analyses were carried out on rectified and integrated signals (time constant of 50 ms) and performed off-line using Spike 2 software (CED, Cambridge, UK) after noise subtraction. PN burst frequency was determined from the time interval between consecutive integrated phrenic peak bursts and expressed in bursts per minute (bpm). tSN activity was measured as the mean values (in μV) of integrated signals. The changes in the PN burst frequency and tSN in response to peripheral

Please cite this article as: Barnett, W.H., et al., Chemoreception and neuroplasticity in respiratory circuits, Exp. Neurol. (2016), http://dx.doi.org/10.1016/j.expneurol.2016.05.036