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Vagal afferent control of abdominal expiratory activity in response to hypoxia and hypercapnia in rats

Eduardo V. Lemes [a], Daniel B. Zoccal [a,b,*]

[a] Department of Physiological Sciences, Center of Biological Sciences, Federal University of Santa Catarina (UFSC), Florianópolis, SC, Brazil
[b] Department of Physiology and Pathology, School of Dentistry of Araraquara, São Paulo State University (UNESP), Araraquara, SP, Brazil

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ABSTRACT

In the present study, we tested the hypothesis that vagal afferent information modulates the pattern of expiratory response to hypercapnia and hypoxia. Simultaneous recordings of airflow, diaphragmatic (DIA) and oblique abdominal muscle (ABD) activities were performed in anesthetized (urethane, 1.2 g/kg), tracheostomized, spontaneously breathing male Wistar rats (290–320 g, $n = 12$). The animals were exposed to hypercapnia (7 and 10% CO_2 for 5 min) and hypoxia (7% O_2 for 1 min) before and after bilateral vagotomy. We verified that the percentage increase in DIA burst amplitude elicited by hypercapnia and hypoxia episodes was similar between intact and vagotomized rats ($P > 0.05$). In contrast, hypercapnia and hypoxia promoted a marked increase in ABD activity in vagotomized, but not in intact rats ($P < 0.01$). These amplified expiratory motor changes after vagotomy were associated with enhanced expiratory airflow ($P < 0.01$) and augmented tidal volume responses ($P < 0.01$). Our data indicates that, in anesthetized conditions, the removal of peripheral afferent inputs facilitates the processing of active expiration in response to hypercapnia and hypoxia in rats.

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1. Introduction

In mammals, breathing results from the synchronized activity of cranial and spinal motor nerves that drive periodic contractions of respiratory muscles (Richter and Smith, 2014). In conditions of normoxia and normocapnia, inspiration begins with the contraction of inspiratory muscles whilst expiration occurs passively due to the recoil forces of the lungs and the chest (St-John and Paton, 2003). The inspiratory/expiratory phase switching is determined by the coordinated activity of neurons of the respiratory central pattern generator (CPG) located in the lower brainstem (Richter and Smith, 2014; Rybak et al., 2004a; Smith et al., 2007). In the ventrolateral surface of medulla oblongata, the inspiratory neurons of the pre-Bötzinger complex (pre-BötC) are considered essential for the inspiratory rhythm generation (Smith et al., 1991; Tan et al., 2008). The pre-BötC inspiratory neurons are suggested to interact with the expiratory neurons of the Bötzinger complex (BötC) and form the core of the respiratory CPG (Rybak et al., 2004b; Smith et al., 2007). This core establishes reciprocal synaptic connections with other pontine and medullary respiratory compartments, including the dorsolateral pons (Dobbins and Feldman, 1994; Molkov et al., 2013), dorsal respiratory group (Alheid et al., 2011; de Castro et al., 1994) and chemosensitive nuclei (Biancardi et al., 2008; Rosin et al., 2006), whose integrity is essential for the generation of the eupneic breathing pattern (Costa-Silva et al., 2010; Richter, 1982; Richter and Spyer, 2001; Smith et al., 2007).

Sensory afferents located in the lungs, airways and carotid bodies provide a powerful feedback information to respiratory CPG and contribute to shape the breathing pattern (Hayashi et al., 1996; Janczewski et al., 2013; Kubin et al., 2006; Moraes et al., 2012a). Afferent inputs from pulmonary stretch receptors, mainly from the slowly adapting receptors (SARs), modulate central respiratory activity according to the lung volume (Kubin et al., 2006). The activation of SARs vagal afferents suppresses the inspiratory motor activity and prolongs the expiratory phase – representing the so-called Hering-Breuer reflex (Backman et al., 1984; Bonham et al., 1993). The removal of pulmonary afferents, by bilateral vagotomy, promotes a marked increase in baseline inspiratory amplitude as well as in the inspiratory and expiratory phase durations, supporting the concept that the activation of SARs vagal afferents importantly contributes to inspiratory/expiratory phase transition during eupneic breathing (Molkov et al., 2013; Mörschel and Dutschmann, 2009).

* Corresponding author at: Department of Physiology and Pathology, School of Dentistry of Araraquara, São Paulo State University (UNESP), Rua Humaitá, 1680, Araraquara, SP, CEP: 14801-903, Brazil. Tel.: +55 16 3301 6555; fax: +55 16 33016488.
E-mail addresses: zoccal@foar.unesp.br, daniel.zoccal@gmail.com (D.B. Zoccal).

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Peripheral and central chemoreceptors adjust the breathing pattern according to the levels of oxygen (O_2) and carbon dioxide (CO_2) in the arterial blood, respectively (Costa-Silva et al., 2010; Powell et al., 1998; Takakura et al., 2014). In conditions of hypoxia and hypercapnia, increases in the inspiratory motor activity occur to enhance respiratory frequency and tidal volume (Powell et al., 1998). In addition, the stimulation of peripheral and central chemoreceptors also transforms expiration into an active process, promoting the emergence of periodic contractions in the abdominal expiratory muscles (Abdala et al., 2009a; Moraes et al., 2012a). Therefore, the ventilatory responses to hypoxia and hypercapnia are associated with increments in both inspiratory and expiratory motor activities. However, the experimental evidence supporting this notion was obtained in anesthetized-vagotomized ventilated rats (Marina et al., 2010) and in the decerebrated in situ preparations (Abdala et al., 2009a; Moraes et al., 2012a), in which pulmonary vagal afferents are absent. Previous studies performed in anesthetized cats reported that abdominal expiratory muscle contractions are engaged when lung deflation is hindered (Marek et al., 2008), suggesting that interactions between pulmonary afferent pathways and the mechanisms responsible for the generation of active expiration may occur. Based on that, in the present study we hypothesized that the pattern of ventilatory response to hypercapnia and hypoxia may modify after the removal of vagal feedback information. Specifically, we considered the possibility that the activation of vagal afferents during hypoxic and hypercapnic episodes modulates the amplitude of evoked expiratory motor activity. Therefore, herein we investigated the effects of bilateral vagotomy on the pattern of inspiratory and expiratory motor responses to hypercapnia and hypoxia.

2. Materials and methods

2.1. Animals and ethical approval

Experiments were performed on male Wistar rats ($n = 18$), weighing 290–320 g, obtained from the Federal University of Santa Catarina Animal Breeding Center and kept at $22 \pm 1\,^{\circ}C$ 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 procedures 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 were approved by the Local Ethical Committee in Animal Experimentation (protocol PP00543).

2.2. Experimental preparation

Surgical procedures and experiments were performed under anesthesia with urethane (1.2 g/kg, i.p.). The level of anesthesia was constantly assessed by the absence of corneal and toe-pinch withdrawal reflexes. Additional doses of urethane were administrated (10–20% of initial dose), when necessary, to maintain anesthesia in adequate levels. Body temperature was maintained at 36–38 °C. Animals were positioned supine and a cervical incision was made to expose the trachea and isolate right and left vagus nerves. The rats were then tracheostomised and the tracheal cannula was connected to a 3-way stopcock, which allowed the simultaneous monitoring of airflow and the administration of gas mixtures. Animals breathed spontaneously and were maintained at 100% oxygen (O_2) during surgery and experimental protocols, except when exposed to hypercapnic or hypoxic episodes. Polyethylene catheters (PE-50 connected to PE-10; Clay Adams, Parsippany, NJ, USA) were inserted into the right femoral artery and vein for arterial pressure measurements and systemic administration of fluids, respectively. Bipolar stainless steel electrodes were implanted in the diaphragm (DIA) and in the oblique abdominal muscles (ABD) to perform electromyographic (EMG) recordings of inspiratory and expiratory motor activities, respectively. To minimize deviations in blood pH and maintain fluid balance, slow intravenous administration of Ringer's solution (in mM: 125 NaCl, 24 $NaHCO_3$, 3 KCl, 2.5 $CaCl_2$, 1.25 $MgSO_4$, 1.25 KH_2PO_4, 10 dextrose) containing lactate (2 mM) was performed (3–4 ml/kg/h) during the experiments (MacFarlane and Mitchell, 2009; Xing and Pilowsky, 2010). Before starting the experimental protocols, a period of at least 30 min was allowed for stabilization.

2.3. Recordings of cardiorespiratory parameters

Inspiratory and expiratory airflows were evaluated using a differential pressure transducer (ML141, ADInstruments, Bella Vista, NSW, Australia) connected to the tracheal cannula. Pulsatile arterial pressure (PAP) was measured by connecting the arterial catheter to a pressure transducer (MLT0380, ADInstruments) that, in turn, was connected to an amplifier (ML221 Bridge Amp, ADInstruments). Airflow and PAP signals were acquired by a data acquisition system (Powerlab, ADInstruments) and recorded on a computer (sampling rate of 1 kHz per channel) using appropriated software (LabChart, ADInstruments). Values of mean arterial pressure (MAP, mmHg) and heart rate (HR, bpm) were derived from PAP signals. DIA and ABD signals were amplified (Bioamplifier, Insight, Ribeirão Preto, SP, Brazil), band-pass filtered (0.1–2 kHz) and acquired at a sampling rate of 2 kHz per channel (LabChart, ADInstruments). Respiratory motor outputs and cardiovascular parameters were recorded simultaneously.

2.4. Experimental protocol

Baseline cardiorespiratory parameters were recorded initially for 15–20 min. The animals were then exposed to hypercapnic (7% and 10% of inspired carbon dioxide, CO_2, balanced in O_2, for 5 min) and hypoxic episodes (7% of inspired O_2, balanced in nitrogen, N_2, for 1 min) and the cardiorespiratory reflex responses were recorded. The hypercapnic and hypoxic gases were administrated to the animals through the tracheal cannula, using a gas mixture device (AVS Projetos, São Carlos, Brazil) coupled to cylinders of 100% of O_2, CO_2 and N_2 (White Martins, Florianópolis, Brazil) and to a gas analyzer (ML206 Gas Analyzer, ADInstruments). The hypercapnic and hypoxic stimuli were applied randomly and a minimum period of 10 min was given between consecutive stimuli. After these procedures, left and right vagus nerves were cut at the cervical level (below the carotid bifurcation) and a 15–20 min period was allowed for stabilization. Baseline and evoked cardiorespiratory parameters were then recorded as aforementioned. At the end of the experiments, animals were euthanized with intravenous injections of KCl 10%.

2.5. Data analysis

The EMG signals were rectified and smoothed (50 ms) for analysis. DIA motor activity was evaluated by its burst amplitude (mV), frequency (referred as respiratory frequency, fR, and expressed in cycles per minute, cpm) and duration (time of inspiration, ms). The time between consecutive bursts was also determined (time of expiration, ms). ABD motor activity was measured by its burst amplitude (mV). Tidal volume (V_T) was calculated from the inspiratory and expiratory flows (ml/s) and normalized by the animal weight (ml/kg). The effects of bilateral vagotomy on baseline inspiratory and expiratory flows, MAP, HR, DIA and ABD activities were assessed by the comparison of average values measured before

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