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Effects of vagotomy on hypoglossal and phrenic responses to hypercapnia in the decerebrate rat

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ABSTRACT

Hypercapnia characterizes a variety of physiological and pathological states and must be compensated effectively by the respiratory, cardiovascular, renal, and intra- and extracellular pH buffering systems to maintain homeostasis. Several studies have examined the respiratory response to hypercapnia, but contemporaneous changes in respiratory frequency and tidal volume prevent investigating the pure influence on respiratory amplitude. Therefore, we sought to test the effect of hypercapnia on hypoglossal (XII) and phrenic nerve (PN) inspiratory (Insp) and XII pre-inspiratory (pre-I) activities in vagus-intact and vagus-denervated animals. Experiments were performed on six artificially-ventilated unanesthetized pre-collicular decerebrate Sprague-Dawley adult male rats. Vagotomy under normocapnic conditions effected the consistent appearance of significant XII pre-I and a greater increase in XII than PN Insp amplitude. In the vagus-intact state, administration of a hypercapnic (5% CO_2, 95% O_2) gas mixture resulted in a greater increase in XII than PN Insp activity. In the vagotomized state, hypercapnia caused a drastic increase in XII pre-I and significant non-differential increases in both XII and PN Insp activity. The increase in XII pre-I was significantly greater than hypercapnia-induced increases in XII and PN Insp discharges. Following vagotomy, duration and amplitude of XII pre-I are potently modulated by CO_2 tension. Based on our results, we conclude that vagal afferents exert differential inhibition of PN Insp and XII pre-I/Insp motor outputs. The role of vagal control in orchestration and optimization of respiratory response to hypercapnia is discussed.

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1. Introduction

The phrenic motor system controls the main inspiratory muscle in mammals, the diaphragm, while the hypoglossal nerve (XII) controls tongue protrudor and retractor muscles, responsible for maintaining adequate upper airway capacitance in preparation for and during inspiration (Brouillette and Thach, 1980; Dobbins and Feldman, 1995; Fregosi and Fuller, 1997; Lee and Fuller, 2010a). The phrenic nerve (PN) discharges during inspiration (Insp) and occasionally exhibits post-inspiratory (post-I) activity depending on experimental conditions (Richter, 1982; Prabhakar et al., 1986; Takeda and Matsumoto, 1997; Poon and Song, 2014). Based on recordings of phrenic and intercostal nerves, it was proposed that the respiratory cycle contains three phases—of active inspiration, phase 1 of expiration (E1 or post-inspiration), and phase 2 of expiration (E2), during which lung volume is at functional residual capacity (Richter, 1982; Richter and Ballantyne, 1983; Richter and Smith, 2014). Expiration is usually a passive process in mammals, where decrementing post-I activity in crural diaphragm and laryngeal adductor muscles smoothly prolong lung deflation (to prevent rapid loss of tidal volume) and improve alveolar gas exchange. Thus, E1 has been termed 'laryngeal-controlled' expiration (Dutschmann and Dick, 2012).

Hypercapnia is characterized by increases in blood partial pressure of carbon dioxide (CO_2) (e.g., during physical exercise or secondary to respiratory insufficiency due to a variety of pathological conditions), which stimulates peripheral and central chemoreceptors (Loeschcke, 1973; Schlaefke, 1981; Nattie, 1999; Guyenet, 2008). Excitation of chemoreceptors, in turn, activates compensatory cardiorespiratory responses characterized by increases in arterial pressure (i.e., sympathetic chemoreflex), respiratory rate, inspiratory tidal volume (V_T), and initialization of active expiration by abdominal and internal intercostal muscles (Abdala et al., 2009; Pagliardini et al., 2011). In conscious humans (Önal et al., 1981; Finucane and Singh, 2009) and conscious adult animals (Iscoe et al., 1983; Easton et al., 1993; Smith et al., 2006), hypercapnia elicits tachypnea as well as increased inspiratory V_T

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(i.e., respiratory amplitude), both responses of which are linked and interact during compensatory respiratory behaviors; the same has been observed in vagotomized animals (e.g., Kong and Berger, 1986). However, these models, wherein concurrent changes in respiratory frequency and V_T occur, do not permit investigation of the pure influence of hypercapnia on amplitude characteristics of respiratory motor outputs.

A role for vagal afferents in modulation of the response to hypercapnia has been demonstrated across several studies. In anesthetized cats, it has been proposed that *tonic* afferent vagal activity primarily modulates expiratory phase duration (and consequently respiratory frequency) during the response to hypercapnia (Miserocchi and Milic-Emili, 1975; Pisarri et al., 1986; Schelegle et al., 1995; Lee et al., 2007). The important role of *phasic* inspiratory inhibition by vagal afferents of intercostal and hypoglossal activity as manifest in genioglossal and hyoglossal muscle discharge (measured by EMG) during hypercapnia was described in anesthetized rats by Bailey et al. (2001), who proposed that hypercapnia-induced increases in V_T may lead to stronger activity of pulmonary stretch receptors, which in turn may inhibit the pontomedullary respiratory central pattern generator (Pack et al., 1986).

Previous investigators have demonstrated increases in PN and XII activities in response to hypercapnia, as well as differential effects of hypoxia on XII pre-I and Insp activities in vagotomized animals (Bruce et al., 1982; Fukuda and Honda, 1982a; Lee and Fuller, 2010a; Mitra et al., 1986; Mitra and Cherniack, 1983; St.-John et al., 2004). In anesthetized cats, there exist differential responses of XII vs. PN to chemostimulation (Bruce et al., 1982), while in decerebrate cats, differential XII and PN responses to hypercapnia were not observed (St.-John et al., 2004). In this study, we sought to investigate the influence of hypercapnia on characteristics of PN and XII Insp and XII pre-I activities in the vagus-intact and vagotomized states.

2. Methods

2.1. General surgical preparation

All procedures were approved by the Drexel University Institutional Animal Care and Use Committee, which oversees Drexel University's AAALAC International-accredited animal program. Six spontaneously-breathing, Sprague-Dawley adult male rats (350–420 g) were anesthetized with isoflurane vaporized in O_2 (Matrix; 4–5% induction, 2.0–2.5% maintenance) via a snout mask. Anesthetic depth was maintained at a level such that withdrawal reflexes and changes in heart rate and blood pressure in response to pinches of the distal hind limbs were absent. Following tracheotomy with an atraumatic glass tube, animals were artificially ventilated with the same gas mixture (55–60 cycles/min, V_T 2.6–3.2 ml; Columbus Apparatus). The electrocardiogram (EKG) was measured via three small subcutaneous electrodes using conventional amplification (5000) and band-pass filtering (range: 1–1000 Hz) (UFI, Morro Bay, CA, USA) and monitored using an audio amplifier (model AM10; Grass Instruments) and oscilloscope (Tektronix, Beaverton, OR, USA). One femoral artery and vein were cannulated for measurement of arterial pressure and infusion of drugs/fluids, respectively. During initial surgical preparation and recordings, rectal temperature was maintained at $37.0 \pm 0.1\,^{\circ}C$ via a servocontrolled heating blanket coupled to a rectal thermometer (Harvard Apparatus). Using a ventral approach, the phrenic nerve and medial branch of XII were dissected and transected. The internal carotid artery was exposed and ligated securely with suture.

2.2. Decerebration

After the initial surgical preparation, animals were placed prone in a stereotaxic device and arterial and tracheal cannulae were connected to pressure transducers (CDXII; Argon Medical Devices, Athens, TX) for monitoring arterial blood pressure and lung inflation pressure, respectively, using conventional amplifiers (Gould amplifier, Cleveland, Ohio). Biparietal craniectomies were made using a variable-speed surgical drill (Foredom Electric), the dura was opened widely using iridectomy scissors, and the superior sagittal sinus ligated proximally and distally using suture and divided. The neuraxis was carefully transected at the rostral border of the superior colliculus using a blunt-edged microspatula to avoid severing the posterior communicating arteries at the cranial base. Brain tissue rostral to the transection was removed by suction and residual bleeding was prevented/arrested by packing the anterior and middle cranial fossae with small pieces of gelfoam (USP; Pharmacia) soaked in cold thrombin solution (50 U/ml USP, dissolved in physiological saline).

2.3. Post-surgical stabilization

One hour following decerebration, anesthesia was slowly withdrawn and animals paralyzed by an intravenous bolus (2 mg/kg) followed by continuous infusion (3–4 mg kg^{-1} h^{-1}) of vecuronium bromide (Abbott Labs) dissolved (0.4 mg ml^{-1}) in Ringer-Locke solution in order to maintain a stable mean arterial pressure of 80–90 mmHg. End-tidal CO_2 was maintained between 4.5 and 5.0% (Capstar CWE) by adjusting ventilation *rate*. Animals were resituated in a supine position and nerve recordings initiated $\geq$1 h after decerebration. The central ends of the PNs and XIIs were placed on bipolar silver electrodes (AM Systems; for PN – Cat# 787000, diameter 0.01"; for XII – Cat# 787500, diameter 0.015"; interelectrode distance: 5 mm) and immersed in a mineral oil pool formed by skin flaps.

2.4. Recordings

Bilateral recordings (band-pass filter: 10–5000 Hz; Neurolog, Digitimer) of PN and XII efferent activity were obtained from the peripheral ends of each nerve. The electrical activity of the two PNs and XIIs, expiratory CO_2 level, arterial blood pressure, and tracheal pressure were recorded onto the hard disk of a personal computer at 10,000 samples/s, using a 16-bit A/D converter with visualization software (ADInstruments).

2.5. Interventions

During vagus-intact conditions, following a 10 min control recording under normocapnic conditions 100% O_2 was changed to 5% hypercapnia (5% CO_2, 95% O_2) for 5 min prior to returning to 100% O_2. The animal was allowed to recover for 30 min, after which vagotomy was performed bilaterally. This was followed by an additional 30 min rest period for the vagotomy intervention. Following a 10 min control recording, the ventilated gas mixture (100% O_2) was changed again to 5% hypercapnia (5% CO_2, 95% O_2) for 5 min prior to returning to 100% O_2.

2.6. Data analysis

Data analysis was performed using custom-written scripts for measuring respiratory parameters for Spike2 (version 5, Cambridge Electronic Design) and MATLAB (version R2015a, The MathWorks). The variables of interest in this set of studies was XII-pre-I, XII-Insp, and PN-Insp amplitude. In order to avoid overestimation of changes