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ARTERIAL CHEMORECEPTOR ACTIVATION REDUCES THE ACTIVITY OF PARAPYRAMIDAL SEROTONERGIC NEURONS IN RATS

A. C. TAKAKURA [a] **AND T. S. MOREIRA** [b]*

[a] *Department of Pharmacology, Institute of Biomedical Science, University of São Paulo, 05508-000 São Paulo, SP, Brazil*

[b] *Department of Physiology and Biophysics, Institute of Biomedical Science, University of São Paulo, 05508-000 São Paulo, SP, Brazil*

Abstract—The parapyramidal (ppy) region targets primarily the intermediolateral cell column and is probably involved in breathing and thermoregulation. In the present study, we tested whether ppy serotonergic neurons respond to activation of central and peripheral chemoreceptors. Bulbospinal ppy neurons ($n = 30$) were recorded extracellularly along with the phrenic nerve activity in urethane/α-chloralose-anesthetized, paralyzed, intact ($n = 7$) or carotid body denervated ($n = 6$) male Wistar rats. In intact animals, most of the ppy neurons were inhibited by hypoxia ($n = 14$ of 19) (8% O_2, 30 s) (1.5 ± 0.03 vs. control: 2.4 ± 0.2 Hz) or hypercapnia ($n = 15$ of 19) (10% CO_2) (1.7 ± 0.1 vs. control: 2.2 ± 0.2 Hz), although some neurons were insensitive to hypoxia ($n = 3$ of 19) or hypercapnia ($n = 4$ of 19). Very few neurons ($n = 2$ of 19) were activated after hypoxia, but not after hypercapnia. In carotid body denervated rats, all the 5HT-ppy neurons ($n = 11$) were insensitive to hypercapnia (2.1 ± 0.1 vs. control: 2.3 ± 0.09 Hz). Biotinamide-labeled cells that were recovered after histochemistry were located in the ppy region. Most labeled cells (90%) showed strong tryptophan hydroxylase immunocytochemical reactivity, indicating that they were serotonergic. The present data reveal that peripheral chemoreceptors reduce the activity of the serotonergic premotor neurons located in the ppy region. It is plausible that the serotonergic neurons of the ppy region could conceivably regulate breathing automaticity and be involved in autonomic regulation.

Key words: serotonergic neurons, breathing; central respiratory chemoreceptors; raphe.

INTRODUCTION

Chemoreception is a classic physiological process that regulates breathing and arterial pressure (AP) to ensure homeostasis (Feldman et al., 2003; Guyenet, 2008). The chemical sensors are present in the carotid body (peripheral chemoreceptors) and within the central nervous system (CNS) (central chemoreceptors) (Loeschcke, 1982; Ballantyne and Scheid, 2001).

Activation of the arterial chemoreceptor afferents during brief hypoxic episodes increases sympathetic outflow, AP and breathing through excitation of neurons (Guyenet, 2006). The brainstem pathway mediating the reflex sympathoexcitation is well charted and consists of excitation of neurons in the caudal aspect of the nucleus of the solitary tract (cNTS) by primary chemoreceptor afferents (Finley and Katz, 1992; Zhang and Mifflin, 1993; Moreira et al., 2006). Activation of cNTS neurons then leads to excitation, probably directly, of presympathetic neurons of the rostroventrolateral medulla/C1 region (RVLM/C1) (Koshiya and Guyenet, 1996; Guyenet, 2000). Arterial chemoreceptor afferent stimulation also elicits bradycardia and increases in breathing (Guyenet, 2000; Moraes et al., 2011). In addition to the arterial chemoreflex, hypoxia evokes a centrally mediated decrease in body temperature which reduces oxygen consumption (Romanovsky, 2004).

Central chemoreception is the mechanism by which an increase in CO_2 partial pressure (Pa_{CO_2}) stimulates breathing. This mechanism operates in concert with arterial chemoreceptors as a powerful feedback that maintains arterial Pa_{CO_2} within very narrow limits (Feldman et al., 2003; Smith et al., 2006). At this time, there is disagreement as to whether central chemoreception is mediated by a few specialized cell clusters located within the brainstem or on multiple types of acid-sensitive neurons (Guyenet, 2008; Nattie and Li, 2009). Some investigators assume that only a few types of neurons contribute to chemoreception, like the retrotrapezoid nucleus (RTN), locus coeruleus, hypothalamic orexinergic neurons, NTS and medullary raphe neurons (Mulkey et al., 2004; Richerson, 2004; Takakura et al., 2006; Biancardi et al., 2008; Nattie and Li, 2008; Abbott et al., 2009; Dias et al., 2009).

Medullary raphe neurons contain a cluster of serotonergic neurons that provide neuromodulatory inputs to the respiratory network, play a major role in central chemoreception and contribute to thermoregulatory control mechanisms (Morrison, 2004; Richerson, 2004; Madden and Morrison, 2005; Hodges et al., 2008).

*Corresponding author. Address: Department of Physiology and Biophysics, Institute of Biomedical Science, University of São Paulo, Av. Prof. Lineu Prestes, 1524, 05508-900 São Paulo, SP, Brazil. Tel: +55-11-3091-7764; fax: +55-11-3091-7285.
E-mail address: tmoreira@icb.usp.br (T. S. Moreira).

Abbreviations: AP, arterial pressure; cNTS, caudal aspect of the nucleus of the solitary tract; CV_{ISI}, coefficient of variation; iPNA, PNA "integration"; MAP, mean arterial pressure; Pa_{CO_2}, CO2 partial pressure; mvPNA, minute volume PNA; ppy, parapyramidal; PNA, phrenic nerve activity; PNA ampl, PNA amplitude; PNA freq, PNA frequency; RTN, retrotrapezoid nucleus.

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However, the exact involvement of the raphe serotonergic neurons to chemosensory control remains unclear. The main focus of the present study is to determine the responses of the serotonergic neurons within the parapyramidal (ppy) region to chemoreflex activation.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed in adult male Wistar rats weighing 250–280 g. Experimental protocols were approved by the Animal Experimentation Ethics Committee of the Institute of Biomedical Science at the University of São Paulo (ICB/USP).

Surgery and anesthesia

General anesthesia was induced with 5% halothane in 100% oxygen. Rats received a tracheostomy and surgery was done under artificial ventilation with 1.4–1.5% halothane in 100% oxygen. All rats were subjected to the following previously described surgical procedures: femoral artery cannulation for AP measurement, femoral vein cannulation for administration of fluids and drugs, removal of the occipital plate for insertion of a recording electrode into the medulla oblongata via a dorsal transcerebellar approach, and skin incision over the lower jaw for placement of a bipolar stimulating electrode next to the mandibular branch of the facial nerve (Moreira et al., 2006; Takakura et al., 2011). The phrenic nerve was accessed by a dorsolateral approach after retraction of the right shoulder blade. All animals were bilaterally vagotomized to prevent any influence of artificial ventilation on phrenic nerve activity (PNA). In one group of rats ($n = 6$) a complete baro- and peripheral chemoreceptor deafferentation was performed by sectioning the vagosympathetic trunks, the superior laryngeal nerves and the glossopharyngeal nerves (proximal to the junction with the carotid sinus nerves). In the remaining rats (baro- and chemoreceptor intact group, $n = 7$), the vagus nerves were carefully separated from the vagosympathetic trunk and selectively transected bilaterally. We presume that this procedure left the aortic depressor nerves intact; however these very small nerves were not identified. The phrenic nerve and wire were embedded in adhesive material (Kwik-Cast Sealant, WPI, USP, Sarasota, FL USP).

Upon completion of surgical procedures, halothane was replaced by a mixture of urethane (1.0 g/kg) and α-chloralose (60 mg/kg) administered slowly intravenously (i.v.). All rats were ventilated with 100% O_2 throughout the experiment except during the hypoxia protocols. The O_2 concentration of the breathing mixture was monitored with a P_{O_2}-sensitive electrode located at the intake of the ventilator. Rectal temperature (maintained at 37 °C) and end tidal-CO_2 were monitored throughout the experiment with a capnometer (CWE, Inc, Ardmore, PA, USA) that was calibrated twice per experiment against a calibrated CO_2/N_2 mix. This instrument provided a reading of $< 0.1\%$ CO_2 during inspiration in animals breathing 100% O_2 and an asymptotic, nearly horizontal reading during expiration. The adequacy of the anesthesia was monitored during a 20-min stabilization period by testing for the absence of withdrawal response, the lack of AP change and the lack of change in PNA rate or amplitude to a firm toe pinch. After these criteria were satisfied, the muscle relaxant pancuronium was administered at an initial dose of 1 mg/kg i.v. and the adequacy of the anesthesia was thereafter gauged solely by the lack of increase in AP and PNA rate or amplitude to a firm toe pinch. Approximately hourly supplements of one-third of the initial dose of urethane were needed to satisfy these criteria during the course of the recording period (4 h).

In vivo recordings of physiological variables and neuronal activity

Mean arterial pressure (MAP), the activity of the phrenic nerve (PNA), tracheal CO_2 and single units were recorded as previously described (Mulkey et al., 2004; Takakura et al., 2011).

As in prior work, the caudal and ventral boundaries of the facial motor nucleus were identified in each rat by the large (up to 5 mV) negative antidromic field potential generated in the facial motor nucleus by stimulating the mandibular branch of the facial nerve (for details see Brown and Guyenet, 1985). Before starting the experiments, ventilation was adjusted to lower end-expiratory CO_2 to 4% at steady-state (60–80 cycles/s; tidal volume 1–1.2 ml/100 g). Variable amounts of pure CO_2 were then added to the breathing mixture to adjust end-expiratory CO_2 to the desired level.

All analog data (end-expiratory CO_2, PNA, MAP and units) were stored on a microcomputer via a micro-1401 digitizer from Cambridge Electronics Design (CED, Cambridge, UK) and processed off-line using version 6 of the Spike 2 software (CED) as described previously (Takakura et al., 2011). Process included action potential discrimination and binning, neuronal discharge rate measurement, and PNA ''integration'' (iPNA). Neural minute × volume (mvPNA, a measure of the total PNA per unit of time) was determined by averaging iPNA over 50 s in vagotomized rats and normalizing the result by assigning a value of 0 to the dependent variable recorded at low levels of end-expiratory CO_2 (below PNA threshold) and a value of 1 at the highest level of PCO_2 investigated (between 9.5% and 10%). Integrated PNA was obtained after rectification and smoothing ($\tau = 0.015$ s) of the original signal, which was acquired with a 30–300-Hz bandpass filter. PNA amplitude (PNA ampl) and PNA frequency (PNA freq) were normalized in each experiment by assigning to each of the two variables a value of 100 at saturation of the chemoreflex (high CO_2) and a value of 0 to periods of apnea. The carotid chemoreceptors were stimulated by switching the breathing mixture from 100% O_2 to 8–10% O_2 balanced with N_2 for 30 s using an electronic valve. Evidence that the stimulus activated brainstem neurons via stimulation of carotid chemoreceptors were obtained by demonstrating that denervation of these receptors eliminated the excitatory effect of the stimulus on PNA (Takakura et al., 2006).

The CED software was also used for acquisition of peri-event histograms of neuronal activity and peri-event averages of iPNA. The peri-event histograms of neuronal single-unit activity were triggered on iPNA trace and represented the summation of at least 100 respiratory cycles (350–800 action potentials per histogram).

The steady-state relationship between ppy neuronal activity and end-expiratory CO_2 was obtained by stepping the inspired CO_2 level to various values for a minimum of 3 min and up to 5 min. The mean discharge rate of the neuron was measured during the last 30 s of each step at which time end-expiratory CO_2 and the discharge of the neuron appeared to have reached equilibrium. End-expiratory CO_2 was measured by averaging the maximum values recorded from 10 consecutive breaths at the midpoint of the time interval sampled.

Extracellular recording and juxtacellular labeling of neurons in the ppy region

Single-unit recording experiments were performed in 13 urethane/α-chloralose-anesthetized and artificially ventilated rats. A concentric bipolar stimulating electrode was placed in the dorsolateral funiculus of the spinal cord at T2-T4 for antidromic activation of ppy neurons (Mason, 1997; Mulkey et al., 2004). The exposed surface of the spinal cord was immersed in warm mineral oil. The activity of neurons in the ppy region was recorded extracellularly as previously described