Experimental Neurology 256 (2014) 46–56

Contents lists available at ScienceDirect

Experimental Neurology

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

Regular Article

Decreased spinal synaptic inputs to phrenic motor neurons elicit localized inactivity-induced phrenic motor facilitation ☆

K.A. Streeter, T.L. Baker-Herman *

Department of Comparative Biosciences, University of Wisconsin-Madison, 2015 Linden Drive, Madison, WI 53706, USA

ARTICLE INFO

Article history:
Received 17 December 2013
Revised 6 March 2014
Accepted 11 March 2014
Available online 25 March 2014

Keywords:
Phrenic
Plasticity
Inactivity induced phrenic motor facilitation
Inactivity
Motor neurons
Control of breathing
Respiratory
Spinal cord
Diaphragm

ABSTRACT

Phrenic motor neurons receive rhythmic synaptic inputs throughout life. Since even brief disruption in phrenic neural activity is detrimental to life, on-going neural activity may play a key role in shaping phrenic motor output. To test the hypothesis that spinal mechanisms sense and respond to reduced phrenic activity, anesthetized, ventilated rats received micro-injections of procaine in the C2 ventrolateral funiculus (VLF) to transiently (~30 min) block axon conduction in bulbospinal axons from medullary respiratory neurons that innervate one phrenic motor pool; during procaine injections, contralateral phrenic neural activity was maintained. Once axon conduction resumed, a prolonged increase in phrenic burst amplitude was observed in the ipsilateral phrenic nerve, demonstrating inactivity-induced phrenic motor facilitation (iPMF). Inhibition of tumor necrosis factor alpha (TNFα) and atypical PKC (aPKC) activity in spinal segments containing the phrenic motor nucleus impaired ipsilateral iPMF, suggesting a key role for spinal TNFα and aPKC in iPMF following unilateral axon conduction block. A small phrenic burst amplitude facilitation was also observed contralateral to axon conduction block, indicating crossed spinal phrenic motor facilitation (csPMF). csPMF was independent of spinal TNFα and aPKC. Ipsilateral iPMF and csPMF following unilateral withdrawal of phrenic synaptic inputs were associated with proportional increases in phrenic responses to chemoreceptor stimulation (hypercapnia), suggesting iPMF and csPMF increase phrenic dynamic range. These data suggest that local, spinal mechanisms sense and respond to reduced synaptic inputs to phrenic motor neurons. We hypothesize that iPMF and csPMF may represent compensatory mechanisms that assure adequate motor output is maintained in a physiological system in which prolonged inactivity ends life.

© 2014 Elsevier Inc. All rights reserved.

Introduction

From birth until death, phrenic motor neurons must transmit a stable, rhythmic motor output to the diaphragm. This output must be of an appropriate magnitude to enable adequate gas exchange, yet remain dynamic to enable appropriate responses to respiratory challenges or engage in non-respiratory behaviors. However, throughout life, many organisms face physiological or pathophysiological conditions that alter phrenic neural activity (Strey et al., 2013). Mechanisms whereby the respiratory control system maintains stable yet dynamic phrenic motor output despite perturbations in respiratory neural activity are unknown.

An emerging principle of neuroscience is that neural activity is sensed and adjusted locally to assure neurons operate in an optimal range (Turrigiano, 2008); however, little is known about the role of on-going activity in shaping respiratory motor output. We recently demonstrated that reducing central respiratory neural activity in ventilated rats elicits a rebound increase in phrenic motor output, a form of plasticity termed inactivity-induced phrenic motor facilitation (iPMF; Mahamed et al., 2011). Since multiple forms of central apnea with different mechanisms of action elicit a phenotypically similar iPMF, we propose that iPMF is due to a common feature: reduced respiratory neural activity. However, it remains possible that iPMF is induced by factors other than respiratory neural inactivity per se. For example, the most common method to elicit iPMF is hyperventilation (Baertsch and Baker-Herman, 2013; Broytman et al., 2013; Mahamed et al., 2011; Strey et al., 2012), which creates a central neural apnea by lowering arterial CO_2 below the threshold for breathing. However, apart from stopping respiratory neural drive, the attendant hypocapnia and/or alkalosis may decrease cerebral blood flow and reduce oxygen unloading in the CNS (Brian, 1998; Vogel et al., 1996), both of which could lead to brain hypoxia (Nwaigwe et al., 2000; Schneider et al., 1998), a stimulus known to elicit prolonged increases in respiratory motor output (Bavis and Mitchell, 2003; Blitz and Ramirez, 2002). Thus, a direct demonstration that reduced respiratory neural activity elicits iPMF without accompanying changes in arterial blood gases is lacking. Further, since central

☆ The authors declare no conflicts of interest.

* Corresponding author at: Department of Comparative Biosciences, University of Wisconsin, 2015 Linden Drive, Madison, WI, USA.
E-mail address: bakert@svm.vetmed.wisc.edu (T.L. Baker-Herman).

http://dx.doi.org/10.1016/j.expneurol.2014.03.007
0014-4886/© 2014 Elsevier Inc. All rights reserved.

apnea reduces respiratory neural activity throughout the neuraxis, it is unknown if global (brainstem) versus local (spinal) mechanisms give rise to iPMF. Indeed, central apnea elicits facilitation in multiple respiratory-related motor pools, including phrenic (iPMF; Mahamed et al., 2011), hypoglossal (iHMF; Baker-Herman and Strey, 2011) and intercostal (iIMF; Strey et al., 2013), suggesting an input common to all signals (i.e., brainstem respiratory neurons) could give rise to inactivity-induced plasticity.

We have begun to understand iPMF mechanisms following a central neural apnea. Our working model suggests that tumor necrosis factor alpha (TNFα) in or near the phrenic motor nucleus plays a critical role in inducing iPMF (Broytman et al., 2013), consistent with other reports suggesting that TNFα plays an essential role in the healthy CNS by increasing synaptic strength following reduced neural activity (Steinmetz and Turrigiano, 2010; Stellwagen and Malenka, 2006). Mechanisms by which TNFα increases phrenic motor output involve the activation of atypical protein kinase C isoforms (aPKCs), since TNFα induced phrenic motor facilitation requires aPKC activity (Broytman et al., 2013). Indeed, iPMF induced by a central neural apnea requires activation of aPKC isoforms PKCζ and/or PKCι/λ to transition from an early, labile form of plasticity to long-lasting iPMF. It is unknown if similar mechanisms give rise to iPMF following local (spinal) disruptions in respiratory neural activity.

Here, we tested the hypotheses that: 1) reduced respiratory neural activity elicits iPMF independent of changes in arterial blood gases, 2) local mechanisms in or near the phrenic motor pool sense and respond to reduced respiratory neural activity and 3) mechanisms similar to those that give rise to iPMF following central neural apnea are also required for iPMF following localized axon conduction block. To test these hypotheses, ventilated rats received spinal (C2) micro-injections of procaine to locally disrupt bulbospinal inputs to phrenic motor neurons on one side of the spinal cord, while contralateral synaptic inputs remained unaffected.

Methods

Animals

Experiments were performed on adult (3–5 months), male Sprague–Dawley rats (Harlan Laboratories; colony 211a and 217). Rats were housed two per cage in a controlled environment (12 h light/dark cycle), with food and water ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison.

Electrophysiology preparation

Rats were anesthetized with isoflurane in a closed chamber and transferred to a heated table where anesthesia (3% isoflurane, in 50% O_2:N_2 balance) was continued through a nose cone. Core body temperature was maintained at 37.0 ± 1.0 °C. The trachea was exposed, cannulated and immediately connected to a pump ventilator (Harvard Apparatus, Rodent Ventilator 683). A flow through carbon dioxide analyzer was used to monitor end-tidal PCO_2 ($ETCO_2$; Capnogard, Novametrix); $ETCO_2$ was maintained at ~45 mm Hg during surgery by adding CO_2 to the inspired gas mix. A bilateral vagotomy was performed to prevent ventilator entrainment. Tracheal pressure was monitored to verify that rats continued to generate respiratory efforts throughout the surgery (i.e. did not inadvertently experience neural apnea due to anesthetic-induced respiratory depression; Mahamed et al., 2011). A femoral arterial catheter was placed to monitor blood pressure and sample arterial blood gases throughout the protocol (ABL-500; Radiometer). The left tail vein was catheterized (Surflo *i.v.* catheter and injection plug) and rats were slowly converted to urethane anesthesia (1.7–1.8 g/kg *i.v.*) while inspired isoflurane was withdrawn. Using a dorsal approach, both phrenic nerves were isolated, cut distally and de-sheathed. A partial laminectomy and durotomy were performed at cervical spinal segment 2 (C2) to expose the left dorsal rootlets. An intrathecal catheter (2 French, Access Technologies) connected to a Hamilton syringe was placed underneath the dura and advanced caudally to spinal segment C4. Following surgery, rats were paralyzed with pancuronium bromide (2.5 mg/kg, *i.v.*), followed by a slow infusion (1–3 mL/h) of a bicarbonate/lactated ringers (1:4) solution to maintain fluid and acid base homeostasis.

Intrathecal compounds

The following compounds were dissolved in artificial CSF (aCSF; in mM: 120 NaCl, 3 KCl, 2 CaCl, 2 MgCl, 23 $NaHCO_3$, 10 glucose bubbled with 95% O_2/5% CO_2 pH 7.4): myristoylated ζ-pseudosubstrate inhibitory peptide (PKCζ-PS; 2 mg/mL; Tocris Bioscience), myristoylated scrambled ζ-pseudosubstrate peptide (scrPKCζ-PS; 2 mg/mL; Tocris Bioscience), soluble TNFα receptor 1 (sTNFR1; .1 μg/μL; R&D Systems), and procaine (20%; Sigma-Aldrich). For all intrathecal compounds, the total injection volume was 10 μL, delivered in 1–2 μL boluses over 2 min. Vehicle treated rats received equivalent volumes of intrathecal aCSF.

Protocols

Following surgery, the left and right phrenic nerves were placed on bipolar silver electrodes and each cavity was filled with mineral oil. Compound action potentials were amplified, band-pass filtered (300–10,000 Hz), and integrated (time constant 50 msec). Raw and integrated signals were digitized and recorded with PowerLab 7 data acquisition system (AD Instruments). One hour after isoflurane was discontinued, baseline phrenic nerve activity was established by manipulating inspired CO_2 until phrenic burst frequency was ~45 bursts/min. Following a 20 min baseline recording period, an arterial blood sample was drawn; arterial $PaCO_2$ and phrenic burst activity at this time point was considered to be "baseline" for all subsequent measurements. Separate groups of rats were subjected to one of the following protocols: 1) C2 axon conduction block with procaine or 2) time controls (see below).

To reversibly block axon conduction unilaterally at C2, a micropipette (tip diameter ~18 μm) filled with procaine was positioned over the left hemi-cord rostral to the C2 dorsal rootlets ~1–1.25 mm lateral to the midline; the micropipette was then advanced ~1.5–1.75 mm into the spinal cord to target bulbospinal axons in the ventrolateral funiculus providing descending respiratory drive to phrenic motor neurons (Fig. 1; Fuller et al., 2003). Using a pneumatic pico-injector (~1–2 psi; Harvard apparatus, PLI-100), ~200 nL of procaine was injected into the C2 VLF while monitoring bilateral phrenic motor output. In some procaine-injected rats, phrenic burst amplitude began to recover after only ~10–15 min of reduced phrenic burst amplitude; thus, additional injections of procaine were necessary to maintain reduced phrenic burst amplitude for 30 min. The following experimental groups were included: 1) intraspinal procaine (n = 7); 2) intrathecal vehicle 20 min prior to intraspinal procaine (n = 6); 3) intrathecal sTNFR1 20 min prior to intraspinal procaine (n = 7), 4) intrathecal PKCζ-PS 20 min prior to intraspinal procaine (n = 7) or 5) intrathecal scrPKCζ-PS 20 min prior to intraspinal procaine (n = 7).

To control for any time-dependent effects of surgery, intraspinal injections or pharmacological treatments, a subgroup of rats received intraspinal injections of aCSF into the VLF ("time controls") and no axon conduction block. The following time controls were included (each group n = 3): 1) intraspinal aCSF; 2) intrathecal vehicle prior to intraspinal aCSF; 3) intrathecal sTNFR1 20 min prior to intraspinal aCSF and 4) intrathecal PKCζ-PS 20 min prior to intraspinal aCSF.

In all experiments, bilateral phrenic motor output was monitored continuously before, during and for 60 min following recovery of axon

Download English Version:

https://daneshyari.com/en/article/3055535

Download Persian Version:

https://daneshyari.com/article/3055535

Daneshyari.com