Experimental Neurology 287 (2017) 235–242

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Reduced respiratory neural activity elicits a long-lasting decrease in the CO_2 threshold for apnea in anesthetized rats

N.A. Baertsch, T.L. Baker *

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

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 21 July 2016
Accepted 25 July 2016
Available online 26 July 2016

Keywords:
Plasticity
Apnea
Apneic threshold
iPMF
Phrenic
Chemoreflex
Central sleep apnea
CSA
Control of breathing
Reduced respiratory neural activity
Respiratory motor neurons

ABSTRACT

Two critical parameters that influence breathing stability are the levels of arterial pCO_2 at which breathing ceases and subsequently resumes – termed the apneic and recruitment thresholds (AT and RT, respectively). Reduced respiratory neural activity elicits a chemoreflex-independent, long-lasting increase in phrenic burst amplitude, a form of plasticity known as inactivity-induced phrenic motor facilitation (iPMF). The physiological significance of iPMF is unknown. To determine if iPMF and neural apnea have long-lasting physiological effects on breathing, we tested the hypothesis that patterns of neural apnea that induce iPMF also elicit changes in the AT and RT. Phrenic nerve activity and end-tidal CO_2 were recorded in urethane-anesthetized, ventilated rats to quantify phrenic nerve burst amplitude and the AT and RT before and after three patterns of neural apnea that differed in their duration and ability to elicit iPMF: brief intermittent neural apneas, a single brief "massed" neural apnea, or a prolonged neural apnea. Consistent with our hypothesis, we found that patterns of neural apnea that elicited iPMF also resulted in changes in the AT and RT. Specifically, intermittent neural apneas progressively decreased the AT with each subsequent neural apnea, which persisted for at least 60 min. Similarly, a prolonged neural apnea elicited a long-lasting decrease in the AT. In both cases, the magnitude of the AT decrease was proportional to iPMF. In contrast, the RT was transiently decreased following prolonged neural apnea, and was not proportional to iPMF. No changes in the AT or RT were observed following a single brief neural apnea. Our results indicate that the AT and RT are differentially altered by neural apnea and suggest that specific patterns of neural apnea that elicit plasticity may stabilize breathing via a decrease in the AT.

1. Introduction

There is considerable interest in the neural regulation of breathing stability, both under normal conditions, such as during sleep, and in clinical patients with ventilatory control disorders such as apnea of prematurity, central sleep apnea (CSA), chronic heart failure (CHF), spinal cord injury (SCI), and difficulty weaning from mechanical ventilation (Deak and Kirsch, 2014; Hagen, 2015; Javaheri and Dempsey, 2013; MacIntyre, 2013; Martin et al., 2012; Mateika and Syed, 2013; Strey et al., 2013; Sowho et al., 2014; Yue and Guilleminault, 2010). During steady-state conditions, changes in breathing are primarily under chemical control and are dominated by fluctuations in arterial pCO_2. Acting through central and peripheral chemoreceptors, CO_2 drives breathing via a negative feedback loop, and the linear relationship between the ventilatory response to changes in $PaCO_2$ is a major determinant of breathing stability (Dempsey and Skatrud, 1986; Javaheri and Dempsey, 2013; Khoo et al., 1982). Two critical parameters of this relationship are the point at which $PaCO_2$ has been sufficiently reduced for ventilation to cease and the subsequent point at which $PaCO_2$ has been sufficiently elevated for breathing to resume, designated as the apneic (AT) and recruitment (RT) thresholds, respectively. The difference between the level of $PaCO_2$ during eupnea and the $PaCO_2$ at the AT (i.e., the CO_2 reserve) is predictive of breathing stability, with a larger CO_2 reserve promoting stability by increasing the amount by which $PaCO_2$ needs to fall to cause apnea (Javaheri and Dempsey, 2013; Fig. 1).

A fundamental property of the respiratory neural network that can alter ventilatory responses is plasticity, which is defined as a change in future behavior based on a prior experience (Mitchell and Johnson, 2003; Morris et al., 2003). Importantly, respiratory *plasticity* is distinct from respiratory *modulation* since plasticity does not require the presence of an ongoing stimulus, whereas modulation does. For example, following a period of reduced phrenic motor neuron activity, a long-lasting increase in phrenic motor output to the diaphragm is observed, a behavior that represents a form of respiratory plasticity called inactivity-induced phrenic motor facilitation (iPMF) (Fig. 1). iPMF is pattern

Abbreviations: aCSF, artificial cerebrospinal fluid; AT, apneic threshold; CHF, chronic heart failure; CRSD, Charles River Sprague Dawley; CSA, central sleep apnea; ETCO2, end-tidal CO2; HSD, Harlan Sprague Dawley; iPMF, inactivity-induced phrenic motor facilitation; OSA, obstructive sleep apnea; pLTF, phrenic long-term facilitation; RT, recruitment threshold.

* Corresponding author.
E-mail address: bakert@svm.vetmed.wisc.edu (T.L. Baker).

http://dx.doi.org/10.1016/j.expneurol.2016.07.020
0014-4886/

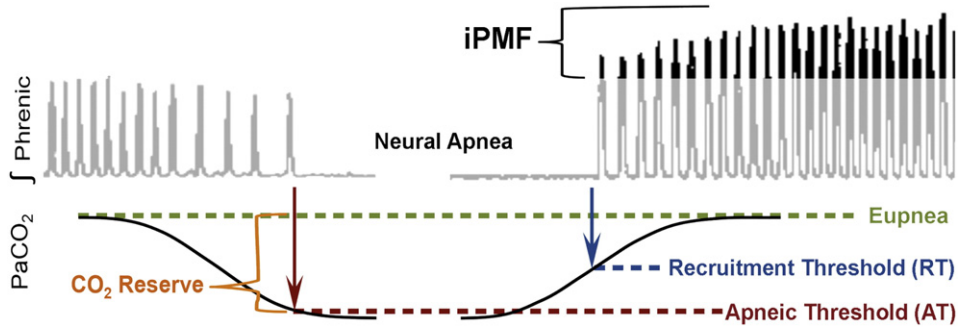

Fig. 1. Schematic defining iPMF and respiratory parameters pertaining to breathing stability. Reduced phrenic neural activity during central neural apnea elicits a rebound increase in drive to the diaphragm via augmentation of phrenic nerve burst amplitude (iPMF). A neural apnea can occur if CO_2 is reduced below the apneic threshold (AT), and breathing will resume once CO_2 has been subsequently elevated above the recruitment threshold (RT). The difference between CO_2 during normal breathing (eupnea) and the level of CO_2 at which the neural drive to breathe is lost (apneic threshold) is defined as the CO_2 reserve.

sensitive since it is more efficiently induced by intermittent versus sustained episodes of neural apnea (Baertsch and Baker-Herman, 2013, 2015). Although iPMF has been most commonly studied following hypocapnia-induced central neural apnea (Baertsch and Baker-Herman, 2013, 2015; Broytman et al., 2013; Streeter and Baker-Herman, 2014b; Strey et al., 2012), iPMF does not require changes in chemoreceptor feedback (O_2 or CO_2; Mahamed et al., 2011). Instead, the primary stimulus for iPMF is reduced synaptic inputs to the phrenic motor pool since C2 axon conduction blockade in descending fiber tracts to phrenic motor neurons is sufficient to elicit iPMF (Streeter and Baker-Herman, 2014a).

Despite our growing understanding of the mechanistic characteristics of inactivity-induced respiratory plasticity, there have been few insights into the long-lasting physiological effects of neural apnea and iPMF on breathing stability. Moreover, although modulation of the AT and RT is well recognized (Altose et al., 1986; Boden et al., 1998; Chowdhuri et al., 2010b; Duffin, 2005; Nakayama et al., 2002; Pleschka et al., 1965; Tanaka et al., 1993), the ability of these important respiratory parameters to undergo plasticity is relatively unknown. Here, we tested the hypothesis that patterns of neural apnea associated with iPMF elicit long-lasting changes in the CO_2-dependent AT and RT for phrenic inspiratory activity. Our results provide the first evidence that plasticity induced by reduced respiratory neural activity (i.e., iPMF) is associated with a long-lasting decrease in the AT, without altering the RT. These findings may have important implications for our understanding of how breathing is stabilized in heathy individuals, as well as inspire novel therapeutic strategies for patients with ventilatory control disorders characterized by breathing instability.

2. Methods

2.1. Animals

Data were collected from 2.5–3.5 month old male rats (n = 50) from Harlan (colony 217; n = 22; HSD) and Charles River (colony P09; n = 28; CRSD) Sprague Dawley rat substrains. Animals were housed 2 per cage with 12 h light/dark cycles and food and water ad libitum. All procedures and experimental protocols were approved by the Animal Care and Use Committee at the University of Wisconsin, Madison.

2.2. Surgical procedures

All rats underwent similar anesthesia and surgical preparation as described by Baertsch and Baker-Herman (2013, 2015). Briefly, isoflurane anesthesia was induced in a closed container and then continued via a nose cone with 2.5–3.5% isoflurane (50% O_2, N_2 balance) flowing from a vaporizer. A custom heated surgery table was used to maintain body temperature near 37.0 °C, which was measured via a rectal thermometer (physitemp, model 700 1H). The tail vein was catheterized for delivery of fluids (1–3 ml/h; lactated Ringer's solution, 0–20% sodium bicarbonate as necessary) to maintain blood pressure and pH throughout experimental protocols. A tracheostomy was performed to enable mechanical ventilation (Harvard Apparatus, Model 683; ~70 br/min, TV: 1 ml/100 g body weight; 50% O_2, N_2 balance). To prevent entrainment of respiratory neural activity with the ventilator, the vagus nerves were cut bilaterally at the cervical level. End-tidal CO_2 ($ETCO_2$) was measured continuously from the expired line immediately adjacent to the bifurcation of the ventilator circuit as an index of arterial pCO_2 with a flow-through capnograph (Capnogard, Respironics). Tracheal pressure was monitored and the fraction of inspired CO_2 was adjusted to ensure that respiratory efforts were present throughout the surgery and to prevent unintended neural apnea. A catheter was placed in the femoral artery to monitor blood pressure and draw blood samples (~0.3 ml) for pH and blood-gas analysis (ABL800; Radiometer, Copenhagen, Denmark). Rats were transferred to urethane anesthesia (1 ml/100 g of 0.175 g/ml urethane infused at 6 ml/h i.v.) and isoflurane was gradually withdrawn. Pressor responses to toe-pinch and/or corneal reflex were tested to assess depth of anesthesia, and supplemental urethane was administered i.v. if a response was observed. The left phrenic nerve was isolated, cut distally, de-sheathed, submerged in mineral oil, and placed on bipolar silver electrodes for electrophysiological recording. Once respiratory neural output could be monitored to ensure continued respiratory effort, pancuronium bromide was infused (1 mg/kg, i.v.) to induce neuromuscular paralysis.

2.3. Experimental protocols

HSD and CRSD rats underwent the same experimental protocols. Rats were slightly hyperventilated to enable rapid induction of a neural apnea without changing ventilator settings (see below), so a small amount of inspired CO_2 was added to the inspired gas mixture to obtain an $ETCO_2$ of ~45 mm Hg (Baertsch and Baker-Herman, 2015). 15–20 min of "baseline" phrenic nerve burst amplitude and frequency was established at least 1 h following transfer to urethane anesthesia to allow sufficient washout of isoflurane. An arterial blood sample was drawn to obtain baseline pCO_2, pO_2, and pH measurements (temperature corrected). Neural apnea was then induced in one of three different patterns: five brief intermittent neural apneas (~1.25 min each, separated by 5 min), a single brief massed neural apnea of an equal cumulative duration (~6.25 min), or a single prolonged neural apnea (30 min). To induce neural apnea, the fraction of CO_2 in the inspired gas mixture was reduced by clamping the CO_2 line upstream of the rotameter and ventilator. The $ETCO_2$ at the point where all rhythmic phrenic activity ceased was noted and designated as the apneic threshold (AT) for phrenic inspiratory activity. In rats receiving brief intermittent neural apnea, the flow of CO_2 was then immediately restored by removing the clamp, and the $ETCO_2$ at which respiratory neural activity resumed was noted and designated as the recruitment threshold (RT) for phrenic