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Reduced respiratory neural activity elicits phrenic motor facilitation

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ABSTRACT

We hypothesized that reduced respiratory neural activity elicits compensatory mechanisms of plasticity that enhance respiratory motor output. In urethane-anesthetized and ventilated rats, we reversibly reduced respiratory neural activity for 25–30 min using: hypocapnia (end tidal CO_2 = 30 mmHg), isoflurane (~1%) or high frequency ventilation (HFV; ~100 breaths/min). In all cases, increased phrenic burst amplitude was observed following restoration of respiratory neural activity (hypocapnia: 92 ± 22%; isoflurane: 65 ± 22%; HFV: 54 ± 13% baseline), which was significantly greater than time controls receiving the same surgery, but no interruptions in respiratory neural activity (3 ± 5% baseline, $p < 0.05$). Hypocapnia also elicited transient increases in respiratory burst frequency (9 ± 2 *versus* 1 ± 1 bursts/min, $p < 0.05$). Our results suggest that reduced respiratory neural activity elicits a unique form of plasticity in respiratory motor control which we refer to as inactivity-induced phrenic motor facilitation (iPMF). iPMF may prevent catastrophic decreases in respiratory motor output during ventilatory control disorders associated with abnormal respiratory activity.

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1. Introduction

The respiratory control system must produce a regular, rhythmic motor output from birth until death, with only the briefest of pauses. In addition, this motor output must be precisely regulated in order to enable optimal gas exchange, yet maintain sufficient dynamic range to respond to respiratory and metabolic challenges. However, little is known about the neural mechanisms that ensure respiratory neurons continue to function adequately despite changes in neuronal excitability or synaptic inputs that occur throughout life.

Rodent models of spinal injury suggest that reduced respiratory-related inputs elicit compensatory plasticity in the phrenic motor system. For example, while the immediate response to a C_2 hemisection is a complete silencing of ipsilateral phrenic motor output as bulbospinal inputs are severed, over time the ipsilateral phrenic nerve will develop modest respiratory-related neural output (Alilain and Goshgarian, 2008; Fuller et al., 2006, 2008, 2009; Golder et al., 2003; Golder and Mitchell, 2005; Nantwi et al., 1999; Vinit et al., 2007), leading to the suggestion that endogenous mechanisms of compensatory plasticity restored partial function to the previously silent phrenic motor pool (Fuller et al., 2006; Goshgarian, 2009; Lane et al., 2009; Mantilla and Sieck, 2009; Mitchell, 2007). Although neurophysiological evidence of this spontaneous partial phrenic recovery typically takes weeks to months following injury to observe, morphological plasticity within the phrenic motor pool can be observed within minutes to hours following injury (Goshgarian et al., 1989; Hadley et al., 1999a,b; Sperry and Goshgarian, 1993). These morphological changes are likely due to disruption of descending respiratory drive to the phrenic motor pool, rather than the injury itself, since reversible C_2 axon conduction block induces similar morphological changes in the phrenic motor pool within 4 h (Castro-Moure and Goshgarian, 1996, 1997). These morphological changes are accompanied by a functional enhancement of diaphragm EMG activity following restoration of spinal axon conduction (Castro-Moure and Goshgarian, 1996), although it is unknown if enhanced diaphragm activity is due to increased phrenic neural output or increased response at the neuromuscular junction. Nevertheless, these data suggest a robust and rapid response to removal of descending respiratory-related inputs to the phrenic motor pool.

Here, we tested the hypothesis that reduced respiratory-related neural activity elicits a rapid and robust form of plasticity in phrenic motor output. We chose three different methods with different mechanisms of action to induce a reversible, global reduction in respiratory-related activity in separate groups of mechanically ventilated, urethane-anesthetized rats: (1) hyperventilation to lower arterial P_{CO_2} below the apneic threshold (hypocapnia), (2) delivery of a volatile anesthetic to depress respiratory drive (isoflurane) and (3) isocapnic high frequency ventilation (HFV) to induce a vagal-reflex inhibition of breathing. Following 25–30 min of reduced respiratory neural activity, baseline conditions were restored, and changes in phrenic burst amplitude and frequency were measured. We demonstrate for the first time that reduced respiratory neural

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activity elicits a prolonged phrenic motor facilitation (inactivity-induced PMF; iPMF). An understanding of these novel mechanisms may yield insights concerning ventilatory control disorders associated with abnormal respiratory motor activity.

2. Methods

2.1. Animals

All experiments were performed on male Sprague–Dawley rats (Harlan colony 217). All protocols conformed to the requirements of the Institutional Animal Care and Use Committee at the University of Wisconsin, Madison.

2.2. Surgical preparation

Rats were induced with isoflurane (2.5–3.5% in 50% O_2), tracheotomized and pump ventilated (Harvard Apparatus, Rodent Ventilator 683). Rats exposed to hypocapnia or isoflurane were subjected to bilateral vagotomy, whereas the vagi were left intact in rats experiencing high frequency ventilation. The tail vein and femoral artery were catheterized for fluid infusion and blood sampling, respectively. A dorsal approach to the left phrenic nerve was used; the nerve was cut distally, desheathed and submersed in mineral oil. All rats were then converted slowly to urethane anesthesia (1.6–1.8 g/kg, *i.v.*) over 15–25 min, and then paralyzed with pancuronium bromide (2.5 g/kg, *i.v.*). A 1.5–2 mL/h fluid infusion of 1:1 lactated ringers and hetastarch (Hespan, 6% hetastarch in 0.9% sodium chloride) solution was then started, and continued through the experiment. Inspired O_2 was 50% for all experiments.

2.3. Nerve recordings

The phrenic nerve was placed on a bipolar silver electrode. Phrenic activity was amplified (10kX), band-pass filtered (0.1–5 kHz) and integrated (time constant: 50 ms; CWE, MA-821RSP). Raw and integrated traces were passed to a data acquisition system (WinDAQ, DATAQ Instruments or PowerLab, AD Instruments) for digitization.

2.4. Physiological measurements

End tidal CO_2 was monitored from the expired limb of the ventilator (Novametrix), and used as an index of arterial P_{CO_2} to enable maintenance of isocapnia. These values were confirmed by blood gas analysis (Radiometer Copenhagen, ABL 500) of 0.2 mL arterial blood samples at key points in the protocol. Blood pressure was monitored and used as an indicator of stable conditions and depth of anesthesia by pressor responses to paw pad pinch.

2.5. Protocols

Baseline: Approximately 1 h after conversion to urethane anesthesia, protocols began by determining the apneic and recruitment CO_2 thresholds for phrenic activity. The apneic threshold was determined by monitoring end tidal CO_2 and slowly lowering inspired CO_2 and/or increasing ventilator rate until rhythmic activity ceased. The recruitment threshold was determined by slowly raising end tidal CO_2 and/or lowering ventilator rate and noting the value at which phrenic activity resumed. End tidal CO_2 was raised a further 1–3 mmHg to establish baseline conditions. After nerve signals were steady for >15 min (typically 25–40 min after threshold determination), an arterial blood sample was drawn to establish baseline arterial P_{CO_2}.

Experimental groups: Six experimental groups were studied. Three groups experienced reduced respiratory neural activity (see below) with: (1) hypocapnia ($n = 11$); (2) isoflurane ($n = 7$) or (3) isocapnic high frequency ventilation (HFV; $n = 11$). A separate group of HFV rats were vagotomized to control for non-vagally mediated changes in phrenic output. Results were compared to “time controls” that received similar surgery and experimental duration, but no neural activity deprivation to control for any time dependent changes in phrenic motor output. One group of time controls were vagotomized ($n = 10$), while a separate group were vagi-intact ($n = 6$).

Hypocapnia: Following establishment of baseline, reduced respiratory neural activity was induced by decreasing inspired CO_2 and/or increasing ventilator rate until end tidal CO_2 was 5–10 mmHg below the apneic threshold. Hypocapnia-induced reduced respiratory neural activity was maintained for 25 min, and then phrenic neural activity was resumed by returning arterial P_{CO_2} to baseline values.

Isoflurane: Following establishment of baseline in a separate group of rats, isoflurane was slowly delivered through the inspired gas mixture until respiratory-related neural activity was reduced (final isoflurane ∼1%). Isocapnia was maintained by increasing inspired CO_2 and/or decreasing ventilator rate; arterial blood samples confirmed that CO_2 remained within ∼2mmHg of baseline. Following 30 min of reduced respiratory neural activity, baseline conditions were restored by removing isoflurane from the inspired gas line, while reducing inspired CO_2 and/or increasing ventilator rate to maintain isocapnia.

High frequency ventilation (*HFV*): In a subgroup of rats the vagi were left intact and allowed to entrain to the ventilator, which was set at 50 breaths/min with a constant tidal volume (2.5 mL). Following establishment of baseline, ventilator frequency was increased (∼100 breaths/min) to elicit a reflex inhibition of respiratory neural activity. Isocapnia was maintained by increasing inspired CO_2 to maintain end-tidal P_{CO_2} at its baseline value; arterial blood samples confirmed that CO_2 remained within ∼2 mmHg of baseline. Following 25 min of reduced respiratory neural activity, baseline conditions were restored by returning ventilator frequency to 50 breaths/min, while reducing inspired CO_2 to maintain isocapnia. A separate group of rats were vagotomized prior to HFV to control for non-vagally mediated effects of high ventilation frequencies on phrenic motor output.

Post-stimulus monitoring: Arterial blood samples were analyzed at 15, 30 and 60 min after each treatment protocol to ensure adequate maintenance of baseline arterial P_{CO_2}, P_{O_2}, SBE and pH. At the end of each protocol, a maximal CO_2 response ($90 < PET_{CO_2} < 100$) was assessed to ensure that observed responses were not influenced by deterioration of the preparation. To be included in the analysis, rats had to meet the following criteria: arterial P_{CO_2} maintained within 1.5 mmHg of baseline, arterial $P_{O_2} > 180$ mmHg and base excess within 3 mEq/L of baseline values following restoration of respiratory neural activity.

2.6. Statistical analysis

Integrated phrenic burst amplitude and frequency were analyzed in 30–60 s bins before (baseline), and 15, 30 and 60 min following reduced respiratory neural activity or an equivalent duration in time controls. Phrenic burst amplitude was expressed as percent change from baseline, which was set at 0%. There were no differences in phrenic burst amplitude at any time point between the two sets of time controls; thus, burst amplitude data from these groups were combined. Phrenic burst frequency (vagotomized rats only) was expressed as an absolute change from baseline. A two-way repeated measures ANOVA was used to detect significant differences (Prism 5, GraphPad Software). Specific group differences were then determined by Bonferroni post hoc tests at a significance level of 0.05. All data are presented as means ± SEM.

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