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Hypoxia triggers short term potentiation of phrenic motoneuron discharge after chronic cervical spinal cord injury

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ABSTRACT

Repeated exposure to hypoxia can induce spinal neuroplasticity as well as respiratory and somatic motor recovery after spinal cord injury (SCI). The purpose of the present study was twofold: to define the capacity for a single bout of hypoxia to trigger short-term plasticity in phrenic output after cervical SCI and to determine the phrenic motoneuron (PhrMN) bursting and recruitment patterns underlying the response. Hypoxia-induced short term potentiation (STP) of phrenic motor output was quantified in anesthetized rats 11 weeks following lateral spinal cord hemisection at C2 (C2Hx). A 3-min hypoxic episode (12–14% O_2) always triggered STP of inspiratory burst amplitude, the magnitude of which was greater in phrenic bursting ipsilateral vs. contralateral to C2Hx. We next determined if STP could be evoked in recruited (silent) PhrMNs ipsilateral to C2Hx. Individual PhrMN action potentials were recorded during and following hypoxia using a "single fiber" approach. STP of bursting activity did not occur in cells initiating bursting at inspiratory onset, but was robust in recruited PhrMNs as well as previously active cells initiating bursting later in the inspiratory effort. We conclude that following chronic C2Hx, a single bout of hypoxia triggers recruitment of PhrMNs in the ipsilateral spinal cord with bursting that persists beyond the hypoxic exposure. The results provide further support for the use of short bouts of hypoxia as a neurorehabilitative training modality following SCI.

Introduction

Hemilesion of the spinal cord at the second cervical segment (C2Hx) results in paralysis of the ipsilateral diaphragm. However, spontaneous inspiratory phrenic motor activity resumes over a period of weeks to months post-injury (Fuller et al., 2008; Lee et al., 2014a; Nantwi et al., 1999). This recovery appears to be mediated by descending mono- and possibly polysynaptic neuronal pathways that cross the spinal midline caudal to C2 to innervate phrenic motoneurons (PhrMNs) (Goshgarian, 2003, 2009; Hoh et al., 2013; Lane et al., 2009). The activation of ipsilateral PhrMNs after C2Hx is termed the crossed phrenic phenomenon (CPP) and makes a small contribution to inspiratory tidal volume during spontaneous breathing (Dougherty et al., 2012b). The relative strength of the CPP, however, can be considerably enhanced with neurorehabilitation strategies (Alilain et al., 2011; Doperalski and Fuller, 2006; Gransee et al., 2013; Lovett-Barr et al., 2012). Controlled exposure to hypoxia holds promise in this regard since appropriate paradigms can trigger robust spinal neuroplasticity (Baker-Herman et al., 2004) and both somatic motor recovery (Hayes et al., 2014; Trumbower et al., 2012) and respiratory (Tester et al., 2014) motor recovery after SCI in humans. To date, studies involving short-term exposure to hypoxia in SCI models have focused on the persistent increase in phrenic activity that is triggered by repeated exposures. This response, termed phrenic long-term facilitation (LTF), can be evoked following chronic C2Hx in rats, and the response is more robust in the ipsilateral compared to the contralateral motor output (Doperalski and Fuller, 2006). A recent report confirms that LTF of ventilation occurs following intermittent hypoxia in humans with SCI (Tester et al., 2014).

Short term hypoxic exposure can also induce a more transient form of phrenic motor plasticity called short-term potentiation (STP). This response is manifest as a progressive enhancement of phrenic activity that follows the initial carotid-body mediated acute hypoxic response followed by a gradual decline to baseline levels after removal of the hypoxic stimulus (Lee et al., 2009; Powell et al., 1998). To our knowledge, no prior studies have specifically evaluated the capacity of the phrenic motor system to express STP after cervical SCI. The fundamental changes in phrenic motoneuron (PhrMN) regulation that occur after cervical SCI (Lee et al., 2013) likely impact hypoxia induced neuroplasticity (Golder and Mitchell, 2005). For example, in the rat C2Hx model, both serotonin receptor expression and glutamate receptor expression are

Abbreviations: C2Hx, spinal cord hemisection at C2; early-I, early-inspiratory; late-I, late-inspiratory; $P_{ET}CO_2$, end-tidal CO_2 partial pressure; PhrMNs, phrenic motoneurons; SCI, spinal cord injury; T_I, inspiratory duration; T_E, expiratory duration.

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altered in PhrMNs, and both receptors are involved in the modulation of phrenic bursting and plasticity (Mantilla et al., 2012). In addition, both the magnitude of the acute phrenic response to hypoxia (Fuller et al., 2008) and the ability to induce LTF are altered (Doperalski and Fuller, 2006). Accordingly, our first purpose was to define the temporal characteristics of hypoxia-induced phrenic STP following chronic C2Hx. Since the contralateral phrenic motor pool shows compensatory increases in output after C2Hx and that limit subsequent neuroplasticity (Doperalski and Fuller, 2006), we hypothesized that STP would be more robust in the ipsilateral motor pool.

There are two prior publications of PhrMN discharge patterns after SCI (El-Bohy and Goshgarian, 1999; Lee et al., 2013). The first report from El-Bohy and Goshgarian described ipsilateral PhrMN bursting in rats a few hours after a C2Hx lesion. During induction of the crossed-phrenic phenomenon the discharge frequency of PhrMNs ipsilateral to C2Hx was increased, and previously silent PhrMNs were recruited during intense respiratory stimulation (El-Bohy and Goshgarian, 1999). We recently reported that following chronic C2Hx most active PhrMNs in the ipsilateral spinal cord initiate bursting well after the onset of inspiration, and PhrMNs showed a decrease in discharge duration and burst frequency (Lee et al., 2013). To our knowledge, there is currently no information on how PhrMNs respond to hypoxia following chronic SCI, and PhrMN burst patterns during any form of hypoxia-induced phrenic motor plasticity remain almost completely unexplored (Lee and Fuller, 2011). As mentioned above, several groups are investigating hypoxia as a neurorehabilitative tool following chronic SCI (Hayes et al., 2014; Tester et al., 2014; Trumbower et al., 2012). Accordingly, the second purpose of our investigation was to determine the impact of an acute hypoxic exposure on PhrMN bursting ipsilateral to chronic C2Hx. The majority of PhrMNs in the ipsilateral spinal cord are expected to be inactive following C2Hx (Lee et al., 2013), and accordingly we predicted that hypoxia would cause recruitment of PhrMNs, but more importantly, that the discharge of these recruited cells would persist upon restoration of arterial oxygen levels to baseline values. In other words, the second goal of this work was to describe the capacity for short term hypoxia-induced plasticity in PhrMN bursting following chronic cervical SCI.

Materials and methods

Animals

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida. Male Sprague–Dawley rats (N = 23) were obtained from Harlan Inc. (Indianapolis, IN, USA). The experimental design did not require uninjured animals since our a priori purpose and hypotheses focused on whether or not a phrenic motor response (i.e., hypoxia-induced STP), which has been well documented in spinal-intact animals (Lee et al., 2009), could also be evoked after SCI. Thus, in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the University of Florida IACUC we studied only C2Hx animals, and this served to reduce the number of experimental animals needed to complete this study. The C2Hx lesion was surgically induced at 3 months of age (93 ± 1 day), and all terminal neurophysiology procedures were done approximately 3 months post-injury (11 ± 1 week).

Spinal cord injury

These procedures were adapted from our prior reports (Dougherty et al., 2012a, 2012b). Animals were anesthetized with xylazine (10 mg/kg, s.c.) and ketamine (140 mg/kg, i.p., Fort Dodge Animal Health, USA), and then placed in the prone position. A dorsal cervical incision was made from the base of the skull to the C3 spinal segment followed by laminectomy and durotomy at C2. A C2Hx lesion was induced on the left side using a micro-scalpel followed by aspiration. The dura and overlying muscles were sutured with 9-0 suture (Ethicon, USA) and 4-0 polyglycolic acid suture (Webster Veterinary, USA), respectively. The skin was then closed with stainless steel wound clips (Stoelting, USA). Following surgery, yohimbine (1.2 mg/kg, s.c., Lloyd, USA) was given to reverse the effect of xylazine. Animals were then given sterile lactated Ringers solution (5 ml, s.c.) and an analgesic (buprenorphine, 0.03 mg/kg, s.c., Hospira, USA). The post-surgical care protocol included daily injection of lactated Ringers solution (5 ml, s.c.) and a high calorie oral supplement (Nutri-cal, 1–3 ml, Webster Veterinary, USA) until adequate volitional drinking and eating resumed.

Neurophysiology preparation

Isoflurane anesthesia (3–4%) was induced in a closed chamber and then maintained via a nose cone (2–3%). Rats were tracheotomized and mechanically ventilated (model 683; Harvard Apparatus, Inc., USA) with a hyperoxic gas mixture (50–60% O_2, balance N_2). The tidal volume was set at 7 ml/kg and frequency was maintained at 60–70 per minute. Rectal temperature was monitored by an electrical thermometer and maintained at 37.5 ± 1 °C by a servo-controlled heating pad (model TC-1000, CWE Inc., USA). The femoral vein was catheterized (PE-50) to enable conversion to urethane anesthesia (1.6 g/kg, i.v., Sigma, USA) and delivery of a neuromuscular blocking agent (pancuronium bromide, 2.5 mg/kg, i.v., Hospira, USA). Another catheter was inserted into the femoral artery for blood pressure measurement (Statham P-10EZ pressure transducer, CP122 AC/DC strain gauge amplifier, Grass Instruments, USA). The vagus nerves were isolated in the mid-cervical region and sectioned to prevent entrainment of phrenic bursting with the rate of the ventilator. The vagotomy procedure has been used extensively in studies of respiratory neuroplasticity and also crossed phrenic activity after C2Hx (Doperalski and Fuller, 2006; Lee et al., 2010). This procedure helps to ensure standardized conditions across animals and within each experiment, which is an important consideration for basic studies of hypoxia-induced plasticity. However, activation of vagal afferent neurons has an inhibitory influence on ipsilateral phrenic motor output after C2Hx (Lee et al., 2010), and accordingly the vagotomy is likely to have increased the relative activity of ipsilateral PhrMNs. The end-tidal CO_2 partial pressure ($P_{ET}CO_2$) was monitored throughout the experiment using a Capnogard neonatal CO_2 monitor placed on the expired line of the ventilator circuit (Novametrix Medical Systems, Wallingford, USA).

The phrenic nerves were isolated in the cervical region via a ventral approach and sectioned distally (Lee et al., 2009, 2010). The activities of both phrenic nerves were recorded by silver hook electrodes, and then amplified (1000×, Model 1700, A-M Systems, Carlsborg, WA, USA), band-pass filtered (0.3–10 kHz), full-wave rectified and integrated (time constant 100 ms; model MA-1000; CWE Inc., USA). In some of the experiments, the phrenic nerve ipsilateral to C2Hx (i.e. left side) was stripped of connective tissue, desheathed and then separated into small filaments to enable extracellular recording of PhrMN action potentials (Lee et al., 2009, 2013). All signals were digitized using the Cambridge Electronic Design (CED) Power 1401 data acquisition interface and sampling frequencies of 10 kHz for the raw neural signals and 100 Hz for the blood pressure and integrated neural signals. These data were recorded on a PC and analyzed using Spike 2 software (CED Limited, UK).

Experimental protocols

In the first protocol, bilateral phrenic nerve activity was recorded to investigate hypoxia-induced phrenic STP following chronic C2Hx (N = 12). After establishing stable phrenic nerve recordings, $P_{ET}CO_2$ was gradually reduced by increasing the rate of the mechanical ventilator until rhythmic bursting in the right phrenic nerve (i.e. contralateral to C2Hx) ceased for at least 2 min. The ventilator rate was then gradually decreased until inspiratory phrenic bursting reappeared, and this