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The role of the crossed phrenic pathway after cervical contusion injury and a new model to evaluate therapeutic interventions

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ABSTRACT

More than 50% of all spinal cord injury (SCI) cases are at the cervical level and usually result in the impaired ability to breathe. This is caused by damage to descending bulbospinal inspiratory tracts and the phrenic motor neurons which innervate the diaphragm. Most investigations have utilized a lateral C2 hemisection model of cervical SCI to study the resulting respiratory motor deficits and potential therapies. However, recent studies have emerged which incorporate experimental contusion injuries at the cervical level of the spinal cord to more closely reflect the type of trauma encountered in humans. Nonetheless, a common deficit observed in these contused animals is the inability to increase diaphragm motor activity in the face of respiratory challenge. In this report we tested the hypothesis that, following cervical contusion, all remaining tracts to the phrenic nucleus are active, including the crossed phrenic pathway (CPP). Additionally, we investigated the potential function these spared tracts might possess after injury. We find that, following a lateral C3/4 contusion injury, not all remaining pathways are actively exciting downstream phrenic motor neurons. However, removing some of these pathways through contralateral hemisection results in a cessation of all activity ipsilateral to the contusion. This suggests an important modulatory role for these pathways. Additionally, we conclude that this dual injury, hemicontusion and post contra-hemisection, is a more effective and relevant model of cervical SCI as it results in a more direct compromise of diaphragmatic motor activity. This model can thus be used to test potential therapies with greater accuracy and clinical relevance than cervical contusion models currently allow.

Introduction

Most spinal cord injuries (SCIs) occur at the cervical level (NSCISC, 2012). Located at this level are the phrenic motor neurons, which innervate the diaphragm; spinal pre-phrenic interneurons; and the excitatory bulbospinal tracts which provide the inspiratory drive to breathe (Boulenguez et al., 2007; Dobbins and Feldman, 1994; Ellenberger and Feldman, 1988; Goshgarian and Rafols, 1984; Goshgarian et al., 1991; Hayashi et al., 2003; Lane et al., 2008, 2009; Lee et al., 2009; Ling et al., 1995; Lipski et al., 1993, 1994; Lu et al., 2004; Porter, 1895; Routal and Pal, 1999; Yamada et al., 1988). As a result, injuries at the cervical level can lead to the inability to inspire air (Inskip et al., 2009; Krassioukov, 2009). A mechanical ventilator is regularly needed to help these patients survive. However, this drastic intervention severely diminishes their quality of life, as well as increases their demands for extensive health care (Berlly and Shem, 2007; Brown et al., 2006; Linn et al., 2000; Strauss et al., 2006). Therefore, exploring ways to restore respiratory motor function following cervical SCI holds great clinical importance and relevance.

For more than a century the model used to study cervical SCI and the resulting respiratory motor deficits is the lateral C2 hemisection (Goshgarian, 2003, 2009; Porter, 1895). A lateral C2 hemisection disrupts bulbospinal inputs from the rostral ventral respiratory group (RVRG) of the medulla to the ipsilateral phrenic motor nucleus resulting in the paralysis of the hemidiaphragm. However, recovery to the paralyzed hemidiaphragm can be accomplished through activation of a spared pathway originating at the RVRG (Goshgarian, 2003, 2009). This pathway has been termed the crossed phrenic pathway (CPP). It descends contralateral to the lesion and then crosses over at the level of the phrenic nucleus, effectively bypassing the hemisection (Goshgarian, 1981, 2003; Goshgarian et al., 1991; O'Hara and Goshgarian, 1991; Porter, 1895). While the strengths of this model are the consistency and reproducibility of both the lesion type and motor deficit, a criticism to these studies is the relative paucity of trauma that is similar to a laceration SCI (Schwab and Bartholdi, 1996). Advancing to a more clinically relevant contusion model has been a recent goal (El-Bohy et al., 1998; Pearse et al., 2005; Schrimsher and Reier, 1992; Young, 2002). Through a cervical contusion model, one can investigate strategies to strengthen respiratory bulbospinal pathways weakened by injury or regeneration of axons that have been severed. Development of this model is important because pathways such as the CPP are not likely to be present after injury. Ironically, hindering the advancement to such a model is the intended

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functional deficit. Severe contusions at the cervical level will result in major respiratory motor compromise, which limits post-contusion survival, and/or requires prolonged ventilation. These effects ensure the resulting model is not viable for logistical and ethical reasons.

However, recently a few studies have been published utilizing a cervical contusion model of SCI (Baussart et al., 2006; Choi et al., 2005; el-Bohy et al., 1998; Golder et al., 2011; Lane et al., 2012; Nicaise et al., 2012; Stamegna et al., 2011). In these studies, the cervical contusions were localized to the midline or to the lateral cord, at various levels through the cervical region, and at different impact forces. While the injuries were not homogenous, a common deficit following injury emerged which was more subtle, or less than ideal, compared to complete abolishment of hemidiaphragmatic activity. These cervically contused rats were unable to augment respiratory motor function, which included both phrenic nerve and diaphragmatic activity (Baussart et al., 2006; Choi et al., 2005; el-Bohy et al., 1998; Golder et al., 2011; Lane et al., 2012; Stamegna et al., 2011). In response to asphyxia, C2 lateral and C4/5 midline contused animals were not able to increase phrenic nerve activity (el-Bohy et al., 1998). Additionally, animals with C4 midline contusions, unlike non-injured animals, could not increase diaphragmatic activity during hypercapnic exposure (Lane et al., 2012). Under similar hypercapnic conditions, C4/5 laterally contused animals could not increase ventilatory output (Choi et al., 2005; Golder et al., 2011).

In the present study we tested the hypothesis that high cervical contusion injuries result in the maximal activation of all spared pathways to the phrenic nucleus. Such activation would make further augmentation of diaphragmatic activity impossible. To accomplish this we examined the activation state of a specific bulbospinal pathway, the CPP, to the phrenic nucleus following cervical contusion. Specifically, we laterally contused animals at the C3/4 level followed by an ipsilateral C2 hemisection one week later, leaving only the CPP intact. If this specific pathway was functional, ipsilateral hemidiaphragmatic activity would still be present. Additionally, we performed a C2 hemisection contralateral to the contusion to examine the role, if any, the CPP played in mediating activity to the hemidiaphragm ipsilateral to the contusion. What we present is a potential anatomical substrate for returning the ability to respond to respiratory challenge. Further to this, our data represents a new and stronger clinically relevant model of cervical spinal cord contusion with a robust respiratory motor deficit to investigate and evaluate therapeutic interventions.

Materials and methods

C3/4 spinal cord hemi-contusion

Adult male Sprague Dawley rats (retired breeders, 250–300 g, Harlan) were anesthetized with a ketamine (70 mg/kg) and xylazine (7 mg/kg) solution administered intraperitoneally. After the animals reached the appropriate surgical plane of anesthesia, the animals were prepared for surgery by shaving the dorsal surface of the neck area and scrubbing with betadine and 70% ethyl alcohol. A midline incision was performed starting from the base of the skull and extended to the fourth cervical segment. Paravertebral muscles were incised and retracted laterally. The muscles attached to the transverse processes were detached on both sides of the spine in order to fit the impactor clamps underneath the transverse processes of C2 and C3.

Following a full and wide C2 vertebrae laminectomy and exposure of C3 and partial C4 spinal cord (SC) segments, a left C3/4 spinal hemicontusion was made using the Infinite Horizon pneumatic impactor (Precision Systems & Instrumentation, Lexington, KY). Specifically, the impactor probe (1.5 mm diameter tip) was raised approximately 5 mm above the intact dura, and the cord contused at a pre-set force of 150 kilodynes (KD) with zero dwell time. Please note that the clamp needed to stabilize the C2 vertebra should be tilted cranially to free enough space for the impactor probe during the injury. The impact of the tip on the SC usually produced some local subdural hemorrhage. Following this, the muscle layers were drawn back together with 3-0 vicryl sutures and the skin stapled together with wound clips.

After the contusion, the animals were allowed to recover on a circulating warm water heating pad until awake and then returned to their home cages. In total, twenty-four animals were contused, of which, eighteen animals survived the initial contusion and were included in this study. These animals received marcaine and buprenorphine (both intramuscularly) for analgesic purposes. 5–10 mL of saline was administered subcutaneously post-operatively. All animals were monitored for seven days post injury, and measures were taken to avoid dehydration and to minimize any potential pain or discomfort. Signs of bladder or bowel dysfunction were monitored daily. All animal procedures were approved by Case Western Reserve University's Institutional Animal Care and Use Committee.

Animal groups

At 7 days after performing the left C3/4 hemi-contusion, all rats were anesthetized with urethane (1.6 g/kg, i.p., Sigma Aldridge) and bilateral diaphragm electromyographic recordings (diaEMGs) were taken (see below). Rats were then randomly sub-grouped into three groups. The first group underwent a left C2 hemisection (LC2H) followed by contralateral phrenicotomy ($n = 6$) (Fig. 1A). The second group underwent contralateral phrenicotomy first, followed by LC2H ($n = 6$) (Fig. 1B). The third group received a right C2 hemisection (RC2H) ($n = 6$) (Fig. 1C). These procedures are outlined further detail below.

Physiological recordings

Animals were anesthetized with urethane and an 8 cm incision at the base of the ribcage was made to expose the abdominal surface of the diaphragm. Following exposure of the diaphragm, bi-polar electrodes, connected to an amplifier and data acquisition system and program (CED Power 1401/Spike 2, Cambridge Electronic Designs, Cambridge, UK), were inserted bilaterally into the crural area of the diaphragm and EMG muscle activity recorded. The EMG signal was band passed filtered between 30 and 3000 Hz (Grass Instruments Amplifier Model P511, West Warwick, RI). During recording, raw diaphragmatic EMG signal was rectified and integrated using Spike2 software. Peak amplitude values were standardized to a 10 or 100 μV calibration pulse.

C2 hemisection

While recording diaEMGs, the spinal cord was re-exposed. The dura was then opened, and with a microblade, a hemisection (either right or left) was made on the animal's spinal cord caudal to the C2 dorsal roots. The hemisection started at the most lateral extent of C2 spinal cord segment and gradually extended to reach the midline.

Contralateral phrenicotomy

A contralateral phrenicotomy was performed either before or after the C2 hemisection (depending on group assignment). This involved a midline incision on the ventral surface of the neck from the angle of the mandible to the sternum. The skin and cervical fascia were excised, and the submaxillary glands were retracted rostrally. The sternohyoideus and omohyoideus muscles overlying the trachea were incised close to the sterno-clavicular junction and retracted rostrally. The right sternomastoideus muscle was detached from the clavicle and retracted laterally. The phrenic nerve was exposed along its course from lateral to medial and perpendicular to the brachial plexus. While recording diaEMGs, a blunted microhook was used to dissect and lift the nerve before complete transection.