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Spinal pathways mediating phrenic activation during high frequency spinal cord stimulation

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ABSTRACT

High frequency spinal cord stimulation (HF-SCS) is a method of inspiratory muscle activation resulting in phrenic motoneuron activation via stimulation of spinal cord pathways. The specific pathways mediating this response, however, are unknown. The aim of this study was to assess the potential role of upper cervical (C1–C4) pre-phrenic interneurons (UCI) and localize the pathways in the thoracic spinal cord mediating activation of phrenic motoneurons during HF-SCS. In 7 anesthetized, spinalized (C1 level) dogs, HF-SCS was applied at the T2 level. Diaphragm EMG, inspired volume and airway pressure generation were monitored before and following sequential spinal cord sections at the C4 and C8 levels. Section at the C4 level and dorsal columns at C8 resulted in no significant changes. However, lateral funiculi section (C8 level) resulted in significant reductions in each parameter. We conclude that during upper thoracic HF-SCS, the phrenic motoneuron pools are activated via spinal pathways located in the lateral funiculus but UCI are not involved.

1. Introduction

Based upon previous animal studies, the application of electrical stimulation in the region of the upper thoracic spinal cord with high stimulus frequencies (~300 Hz) results in the activation of spinal cord pathways that synapse with the inspiratory motoneuron pools (DiMarco and Kowalski, 2009, 2010, 2011). This method results in processing of the stimulus within these neurons and consequent physiologic activation of the inspiratory muscles. Physiologic activation is evidenced by the fact that high frequency spinal cord stimulation (HF-SCS) results in an asynchronous pattern of diaphragm and inspiratory intercostal muscle activation and single motor unit firing frequencies similar to those observed during spontaneous breathing. Consequently, HF-SCS may be a useful method of inspiratory muscle pacing in subjects with ventilator-dependent cervical spinal cord injury (SCI).

Optimal activation of the inspiratory muscles occurred via the application of HF-SCS with a single electrode positioned at the T2 spinal level on the ventral epidural surface (DiMarco et al., 1987; DiMarco and Kowalski, 2009, 2010, 2011). The specific neuronal networks in the cervical spinal cord mediating phrenic motoneuron activation during HF-SCS, however, are unknown. Potential mechanisms include activation of pre-phrenic interneurons, located in the upper cervical spinal cord (C1–C4), which synapse with the phrenic and upper thoracic inspiratory motoneuron pools and/or activation of neuronal circuits either cephalad to, or in close vicinity to the phrenic motoneurons (Dobbins and Feldman, 1994; Duffin and Iscoe, 1996; Hayashi et al., 2003; Hilaire et al., 1986; Lane et al., 2008b, 2009; Lipski and Duffin, 1986; Lois et al., 2009; Nakazono and Aoki, 1994; Palisses and Viala, 1987). If the mechanism of HF-SCS to activate the phrenic motoneurons is dependent upon functional neuronal structures in the upper cervical spinal cord, this method would not be successful in providing inspiratory muscle pacing in ventilator-dependent subjects with SCI. To investigate the potential role of upper cervical pre-phrenic interneurons on diaphragm activation, the degree of phrenic activation was monitored during HF-SCS, before and after sequential section of the spinal cord at the upper C4 level.

The spinal cord pathways in the vicinity of the stimulating electrode, which mediate activation of the phrenic motoneuron pools via HF-SCS, are also unknown. Previous investigators, however, have described afferent inputs from the lower thoracic intercostal and abdominal muscles that reflexly facilitate phrenic motoneuron discharge via spinal cord pathways (intercostal to phrenic reflex) (Decima et al., 1967, 1969; Decima and von Euler, 1969). These pathways have been shown to reside bilaterally in the ventrolateral funiculus of the thoracic spinal cord. We hypothesized that pathways in this region of the spinal cord mediate activation of

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the phrenic motoneurons during HF-SCS. To test this hypothesis, the degree of phrenic activation was monitored before and after sequential section of the spinal cord at the C8 level. The degree of phrenic activation was monitored by measurements of diaphragm EMG, inspired volume and airway pressure generation during HF-SCS.

2. Methods

Studies were performed on 7 mongrel dogs (mean weight 15.6 ± 0.5 kg) with the approval of the Institutional Animal Care and Use Committee of Case Western Reserve University. Animals were anesthetized initially with pentobarbital sodium (PB, 25 mg/kg), administered intravenously. Additional doses of PB (1–2 mg/kg) were provided as needed to maintain an absent corneal reflex and absent response to noxious stimuli.

A size 10 mm ID endotracheal tube was sutured into the trachea in the midcervical region. A catheter was placed in the femoral vein to administer supplemental anesthesia and fluids. Blood pressure and heart rate were monitored from a catheter placed in the femoral artery (Waveline Pro Multi-Function Monitor, DRE Inc., Louisville, KY). Oxygen saturation was monitored from the earlobe and end-tidal PCO_2 at the trachea (Waveline Pro). Body temperature was maintained with a heating blanket (Harvard Apparatus, Cambridge, MA) at 38 ± 0.5 °C. Airway pressure was measured at functional residual capacity (FRC) following airway occlusion with a pressure transducer (Validyne, MP45, Northridge, CA), which was connected to the airway opening. Tidal volume was measured by electrical integration of the flow signal from a pneumotachograph (Series 3700, Hans Rudolph, Kansas City, MO).

Laminectomies were performed at the C1, C4 and C8 levels to allow spinal cord section at these locations. Based upon previously described techniques, a final laminectomy was performed at the T4–T5 level for placement of an 8 plate stimulation lead with 4 mm contacts (model AD-TEDH Medical Instrument Corp., Racine, WI) at the T2 level on the ventral surface. The lead was positioned under direct vision on the ventral surface of the spinal cord and advanced to the T2 level (as previously described: DiMarco and Kowalski, 2009, 2010, 2011). An indifferent ground electrode was implanted in the back musculature. A grass square-wave pulse stimulator (model S88, Grass Technologies, West Harwick, RI) equipped with a stimulus isolation unit (PSIU6, Grass Technologies) was used to provide monopolar electrical stimulation at 300 Hz over a range of stimulus amplitudes (0–6 mA). Stimulus train duration was fixed at 1.2 s since a plateau in pressure and volume generation is generally achieved by this time. Electrical stimulation was provided over a wide range of stimulus amplitudes to evaluate potential stimulus current related effects.

Inspiratory muscle EMG recordings of the parasternal (2nd interspace), and costal diaphragm (via the 7th interspace) were assessed with the use of bipolar teflon-coated, stainless steel fine-wire electrodes, uninsulated at their terminal ~5 mm. Inspiratory electrical activity was quantified by measuring the peak amplitudes of the moving average EMG.

In each animal, the dura mater was opened and spinal cord sectioned at the high C1 level using watchmaker forceps under microscopic control. Complete section was verified by lifting a hook across the area of transection. Following C1 section, diaphragm EMG, airway pressure and inspired volume were assessed during HF-SCS (0.75–6 mA, 0.2 ms pulse width, 300 Hz) at the T2 level on the ventral surface.

Sequential section of the spinal cord (dorsal columns, lateral funiculi and then ventral funiculi) was performed at the upper C4 level and subsequently at the C8 level (see the schematic diagrams of transverse spinal cord section on the top of each figure). Prior to re-assessment of physiologic parameters during HF-SCS, at least 30 min was allowed to elapse after each spinal cord section procedure, at which time the animal was hemodynamically stable.

All recordings were monitored and stored on a computer utilizing a data acquisition and analysis system (Spike 2 with 1401 interface, Cambridge Electronic Design, Cambridge, UK).

2.1. Data analysis

Mean peak integrated diaphragm and parasternal EMG, inspired volumes and airway pressures under control conditions were compared with those obtained following sequential sectioning of the spinal cord at the C4 and C8 levels, in separate trials. Statistical analysis was performed by using a one-way analysis of variance and the Newman–Keuls test. A p value of <0.05 was accepted as reflective of statistical significance. Results are presented as means ± SE.

3. Results

The effects of sequential section of the spinal cord at the C4 and C8 spinal levels on diaphragm and parasternal EMG activation and inspired volume generation during HF-SCS (2 mA, 300 Hz, 0.2 ms pulse width) is shown for one animal in Fig. 1. Following sequential section of the dorsal columns, lateral funiculi and complete spinal cord at the C4 level, there were no apparent changes in the degree of inspiratory muscle EMG activity or inspired volume compared to control values. Similarly, following section of the dorsal columns at the C8 level, there were no changes in inspired volume or EMG activity. However, following section of the lateral funiculi at this level, there was a marked reduction in the degree of diaphragm activation and associated marked reduction in inspired volume generation. There were no further changes in these parameters following complete section. In contrast, there were no apparent changes in the degree of parasternal activation during spinal cord section at the C8 level.

The mean changes, expressed as % of control, in peak integrated parasternal and diaphragm EMG activity during HF-SCS are provided in Table 1 (2 mA, 300 Hz, and 0.2 ms pulse width). Compared to control values, there were no significant changes in parasternal and diaphragm EMG activity during sequential spinal cord section at the C4 level. Following section of the dorsal columns at the C8 level, these parameters were also not significantly different than control values. However, section of the lateral funiculi at the C8 level resulted in a significant decrease in diaphragm EMG to 8% of control values ($p < 0.01$).

Mean inspired volume generation during HF-SCS at different levels of stimulation under control conditions and following sequential section of the spinal cord is shown in Fig. 2A. Mean inspired volumes during stimulation with 0.75, 2 and 6 mA were 260 ± 71, 703 ± 37 and 682 ± 16 ml under control conditions. There were no significant changes in mean inspired volume generation following sequential section at the C4 level or subsequent section of the dorsal columns at the C8 level. However, following section of the lateral funiculi at the C8 level, inspired volume fell to 184 ± 24, 238 ± 40 and 262 ± 41 ml ($p < 0.05$ for each) during HF-SCS with 0.75, 2 and 6 mA, respectively. There were no further significant changes in inspired volume following complete spinal cord section at this level.

The effects of sequential section of the spinal cord at the C4 and C8 spinal levels on airway pressure generation under conditions of airway occlusion at FRC are shown for one animal in Fig. 3 (same animal as Fig. 1). Following sequential section of the spinal cord at the C4 level and also section of the dorsal columns at the C8 level, airway pressure generation remained unchanged. However, following section of the lateral funiculi at the C8 level, there was a