High-frequency spinal cord stimulation of inspiratory muscles in dogs: a new method of inspiratory muscle pacing

Anthony F. DiMarco and Krzysztof E. Kowalski

Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio

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DiMarco AF, Kowalski KE. High-frequency spinal cord stimulation of inspiratory muscles in dogs: a new method of inspiratory muscle pacing. J Appl Physiol 107: 662–669, 2009. First published June 11, 2009; doi:10.1152/japplphysiol.00252.2009.—Despite clinically available methods of diaphragm pacing, most patients with ventilator-dependent tetraplegia are still dependent on mechanical ventilation. Given the significant disadvantages of these devices, additional pacing options are needed. The objective of this study was to evaluate a novel and potentially more physiologic method of inspiratory muscle activation, which involves the application of high-frequency (>200 Hz) stimulation to the ventral surface of the spinal cord in the high thoracic region. Studies were performed in 13 anesthetized dogs. High-frequency spinal cord stimulation (HF-SCS) results in the activation of both the diaphragm and inspiratory intercostal muscles, in concert, at physiologic firing frequencies and the generation of large inspired volumes. Mean maximum firing frequencies of motor units in the parasternal (2nd interspace), the external intercostal (3rd interspace), and the diaphragm muscles were 10.6 ± 0.4, 11.7 ± 0.4, and 10.4 ± 0.3 Hz, respectively. These values were not significantly different from those occurring during spontaneous breathing at comparable inspired volumes. Maximum inspired volume was 0.93 ± 0.01 liter, which approximates the inspiratory capacity of these animals. Moreover, ventilation can be maintained on a chronic basis by this method (6 h) without evidence of system fatigue. Our results suggest that HF-SCS results in activation of spinal cord tracts that synapse with the inspiratory motoneuron pools, allowing processing of the stimulus and consequent physiological activation of the inspiratory muscles. HF-SCS has the potential to provide an effective method of inspiratory muscle pacing.

Diaphragm pacing; phrenic nerve pacing

DIAPHRAGM PACING (DP) is a clinically useful modality to provide artificial ventilatory support in patients with ventilator-dependent tetraplegia (14, 32, 34). DP has significant advantages compared with mechanical ventilation, including greater mobility, elimination of fear of ventilator disconnection, elimination of embarrassment and social stigma associated with connection to a respirator, better speech quality, and improved sense of well-being and overall health (4, 13, 32).

Current methods of DP involve the placement of electrodes on each of the phrenic nerves in the thorax (33) or in the body of the diaphragm in the vicinity of the phrenic nerve motor points (18, 19). With both methods, bilateral phrenic nerve stimulation is necessary to maintain adequate ventilation. Despite careful prescreening of patients to ensure phrenic nerve viability, however, many patients (~50%) have inadequate inspired volume generation to maintain full-time ventilatory support (45, 53). There are also many patients who are ineligible for pacing due to inadequate phrenic nerve function. The vast majority of ventilator-dependent tetraplegic persons, therefore, still require the use of mechanical ventilation.

Several factors may account for inadequate inspired volume production during DP. First, peripheral nerve electrodes generally do not achieve complete diaphragm activation due to the high thresholds of some axons (29, 35, 41, 49). Second, this methodology results in a reversed recruitment order of motor units: large-diameter motor axons that innervate large, more fatigue-resistant muscle fibers are activated initially, while smaller, fatigue-resistant muscle fibers are only recruited at higher stimulus levels, if at all (42). With reconditioning, during which DP is applied with low frequencies (<20 Hz), however, the diaphragm is converted from a normally mixed fiber population to one comprised predominantly of slow fibers that have relatively low maximum force-generating capacities (1, 33, 46, 47). Consequently, fiber-type conversion improves endurance but restricts maximum inspired volume generation. Finally, there is lack of coincident inspiratory intercostal/accessory (IC) muscle activation, a muscle group that is responsible for the generation of ~35–40% of the vital capacity (2).

In this paper, we describe a new method of inspiratory muscle activation in which diaphragm activation in concert with inspiratory intercostal muscle activation can be achieved with upper thoracic spinal cord stimulation (SCS) at high stimulus frequencies (>200 Hz). This is a unique method of inspiratory muscle activation since stimulation occurs via spinal pathways that synapse with inspiratory motoneurons, resulting in a more physiological recruitment pattern. High-frequency spinal cord stimulation (HF-SCS), therefore, may provide a more effective means of inspiratory muscle pacing.

MATERIALS AND METHODS

Studies were performed on 13 mongrel dogs (mean weight 16.5 ± 0.6 kg) with the approval of the Institutional Animal Care and Use Committee of Case Western Reserve University. Each animal was anesthetized initially with pentobarbital sodium (PB, 25 mg/kg) given intravenously. Additional doses of PB (1–2 mg/kg) were provided, as required.

Auffed endotracheal tube (10-mm ID) was sutured into the trachea in the midcervical region. A catheter was placed in the femoral vein to administer fluids and supplemental anesthesia; a catheter was also placed in the femoral artery to monitor blood pressure (model PT300, Grass Instruments, Quincy, MA) and heart rate (P511 AC Amplifier, Grass Instruments). Body temperature was maintained with a heating blanket (Harvard Apparatus, Cambridge, MA) at 38 ± 0.5°C. End-tidal PCO2 was monitored at the trachea and oxygen saturation from the earlobe (Nellcor N-100, Hayward, CA). Airway pressure was measured at functional residual capacity (FRC) following airway occlusion with a pressure transducer (Validyne, MP54, Northridge, CA) connected to the airway opening. Tidal volume was recorded by electrical integration of the flow signal from a pneumotachograph (Series 3700, Hans Rudolph, Kansas City, MO).

Address for reprint requests and other correspondence: A. F. DiMarco, MetroHealth Medical Center, Rammelkamp Center for Education and Research, 2500 MetroHealth Dr., Cleveland, OH 44109 (e-mail: afd3@case.edu).
A laminectomy was performed at the T4–T5 level for electrode placement. A four-plate electrode with 4-mm contact leads (model 3986A, Medtronic, Minneapolis, MN) was positioned under direct vision onto the ventral surface of the spinal cord and advanced 3 cm to the T3 level. We have ascertained based on many previous postmortem canine studies that this distance results in electrode placement between the T2 and T3 motor units. Based on preliminary trials, this location was found to result in maximal inspired volume generation. An indifferent ground electrode was implanted in the back musculature. A Grass square-wave pulse stimulator (model S88, Grass Instruments) equipped with a stimulus isolation unit (PSIU6, Grass Instruments) was used to provide electrical stimulation over a wide range of stimulus frequencies (0–1.000 Hz) and stimulus amplitudes (0–15 mA). Stimulus train duration was fixed at 1.2 s since a plateau in pressure and volume generation is generally achieved by this time and this duration is in the range of that applied during inspiratory muscle pacing.

Bipolar teflon-coated, stainless steel fine-wire electrodes, uninsulated at their terminal 5 mm, were used to assess multiunit inspiratory muscle EMG recordings of the parasternal (2nd interspace), dorsal portion of the external intercostal muscles (3rd interspace, posterior axillary line), and costal diaphragm (via the 7th interspace). Intercostal recordings were made from muscles over these regions of the rib cage since they are electrically active during resting breathing (10, 21, 22). Inspiratory muscle activation was further characterized by single motor unit (SMU) recordings. SMU recordings were made using teflon-coated stainless steel electrodes with an uninsulated portion of only ~1 mm, to provide greater selectivity. EMG potentials were amplified (1,000–10,000 times) and filtered (50 Hz to 5.0 kHz) (model BMI-830, Charles Ward Enterprises, Ardmore, PA). All parameters were monitored and stored on an eight-channel data-acquisition recorder (model Dash 8X, AstroMed, West Warwick, RI) for subsequent analysis (AstroView X, Data Review Software, AstroMed).

To model patients with spinal cord injury, the cervical spinal cord was sectioned at the C2 level using watchmaker forceps. Complete section was verified by lifting a hook across the area of transaction.

Protocol 1 (n = 4). Following C2 section, changes in airflow pressure and inspired volume were assessed during SCS over a wide range of stimulus currents (0.3–6 mA, 0.2-ms pulse width). Our measurement was limited to 6 mA since higher levels resulted in significant body movement and therefore have no practical application. At each level of stimulus current, the effects of changes in respiratory rate and mean blood pressure were also monitored every 30 min. Forelimb and hindlimb muscle activation was also monitored by extensor carpi radialis and quadriceps EMG, respectively.

Following 5.5 h of chronic stimulation, stimulus parameters were increased for an additional 30 min in each animal to induce hyperventilation. This was performed as an additional test to assess the capacity of the system to sustain artificial ventilation.

Data analysis. Curves were constructed relating stimulus current to inspired volume and airway pressure generation at fixed stimulus frequencies of 50, 100, 200, 300, and 400 Hz. SMU analysis included determination of mean peak discharge frequencies. Comparisons were made, where applicable, using repeated-measures ANOVA and post hoc Newman–Keuls tests. A P value < 0.05 was accepted as statistically significant. Data are reported as means ± SE.

RESULTS

Protocol 1. The effects of SCS on inspired volume and negative airway pressure generation during stimulation at 50 Hz and 300 Hz at various stimulus amplitudes are shown for a representative animal in Fig. 1. At any given level of stimulus current, inspired volume and airway pressure generation were substantially larger at 300 Hz compared with 50 Hz. For example, with stimulation at 2 mA, inspired volume and negative airway pressure were 970 ml and 68 cmH2O compared with 360 ml and 18 cmH2O, with 300 and 50 Hz, respectively. Stimulation currents greater than 3 mA were associated with mild flexion of the forepaws; however, no visible movement was observed with lower stimulus currents.

Mean inspired volume and negative airway pressure generation during electrical stimulation over a wide range of stimulus frequencies and amplitudes are presented in Fig. 2. There were progressive increases in the magnitude of inspired volume and airway pressure generation with increasing stimulus...
frequency from 50 to 300 Hz. With conventional stimulus frequencies (50 Hz), inspired volumes and airway pressures were substantially smaller than those achieved with higher frequencies ($P < 0.05$ compared with 100 Hz and $P < 0.001$ compared with higher stimulus frequencies). Values at 200 Hz were also significantly smaller than those at 300 Hz ($P < 0.01$). Values at 400 Hz, however, were not significantly different from those obtained at 300 Hz. In addition, maximum values of inspired volume and airway pressure were achieved at much lower levels of current during high- compared with low-frequency stimulation (i.e., leftward shift of these curves with increasing stimulus frequency). Mean maximum inspired volume and negative airway pressure were $0.93 \pm 0.01$ liter and $67 \pm 4 \text{cmH}_{2}\text{O}$, respectively (300 Hz, 3 mA). The inspiratory capacity of these animals was $1.04 \pm 0.02$ liter, which was significantly larger than maximum values achieved with HF-SCS ($P < 0.05$).

Protocol 2. Multiunit EMGs of the parasternal and external intercostal muscles and costal diaphragm are shown for one animal in Fig. 3. EMGs obtained during spontaneous breathing are shown in the left panel and those obtained at a comparable inspired volume during HF-SCS are shown in the right panel.

As with spontaneous breathing, HF-SCS generated an asynchronous EMG pattern (i.e., the time when each spike is fired by one motoneuron is not correlated with the time when the spikes of the other motoneurons are discharged) in each of these inspiratory muscles. In contrast, direct phrenic nerve stimulation, as with all motor nerve stimulation techniques, results in synchronous activation of all axons, as reflected in the single action potential following each stimulus artifact spike.

Specific characterization of single motor units in the diaphragm is shown for one animal during HF-SCS in Fig. 4. In response to HF-SCS (300 Hz, 0.5 mA), three separate motor units are readily identified. Each of the three SMUs is shown superimposed in the right portion of Fig. 4, confirming their similar morphology. In this instance, firing frequencies ranged between 7.8 and 14.9 Hz. A representative example of SMU recordings from the parasternal and external intercostal muscles and diaphragm during spontaneous breathing and HF-SCS (300 Hz, 0.5 mA) is shown in Fig. 5. The raw tracings are displayed above a plot of instantaneous firing frequencies of a SMU from each muscle. Superimpositions of the SMU recordings are also provided. The responses observed during HF-SCS appear qualitatively similar to those occurring during spontaneous breathing. Firing frequencies gradually increase during the breath until a plateau is reached in the mid to latter portion of the breath. Similar response patterns during HF-SCS were observed in each animal.

During spontaneous breathing, 31, 34, and 42 single motor units were evaluated for the parasternal and external intercostal muscles and diaphragm, respectively. Mean firing frequencies
were 12.3 ± 0.6, 12.1 ± 0.5, and 10.8 ± 0.3 Hz, respectively. During HF-SCS, 56, 58, and 80 motor units were evaluated for the parasternal and external intercostal muscles and diaphragm, respectively. Mean firing frequencies were 10.6 ± 0.4, 11.7 ± 0.4, and 10.4 ± 0.3 Hz, respectively [nonsignificant (NS) for each comparison during spontaneous breathing vs. HF-SCS]. Mean inspired volumes during spontaneous breathing and HF-SCS were not significantly different at 0.29 ± 0.03 and 0.30 ± 0.02 liter, respectively.

The effects of internal and external intercostal nerve section and left cervical phrenicotomy on the SMU activity of each respective muscle are shown for one animal in Fig. 6. Following nerve section of each respective inspiratory muscle, EMG activities were eliminated, confirming the absence of cross-contamination from other muscles.

**Protocol 3.** The mean effects of chronic HF-SCS applied over a 6-h period on airway pressure, inspired volume, end-tidal PCO2, and oxygen saturation are presented in Fig. 7. Stimulus parameters and train rate (breaths/min) were set at the same values (300 Hz, 0.5 mA, 0.2 ms, 7/min) in each animal. Throughout the initial 5.5 h of chronic stimulation, airway pressure generation and inspired volume production remained unchanged. Mean airway pressure and inspired volume were 29 ± 1 cmH2O and 0.45 ± 0.07 liter during the first hour, and 26 ± 1 cmH2O and 0.46 ± 0.12 liter during the final hour, respectively (NS for each). Mean end-tidal PCO2 and oxygen saturation during chronic stimulation are provided in Table 1. As shown, there were no significant changes in these values over the period of stimulation. Importantly, progressive elevations in PCO2 did not occur in any animal. Finally, mean blood pressure and heart rate remained stable throughout the period of stimulation at 95 ± 9 mmHg and 107 ± 7 min⁻¹ during the first hour and 88 ± 5 mmHg and 105 ± 5 min⁻¹ during the final hour of stimulation (NS for each, Fig. 7). In each animal, the increases in stimulus parameters during the final 30 min of pacing resulted in a sustained fall in end-tidal PCO2 to 26 ± 1 mmHg.

During continuous pacing, there was no limb motion, and extensor carpi radialis and quadriceps EMGs were electrically silent.

**DISCUSSION**

This study represents the first demonstration of physiological activation of the inspiratory muscles utilizing electrical stimulation techniques. This is indicated by two critical observations. First, HF-SCS results in an asynchronous pattern of both diaphragm and inspiratory intercostal muscle activation, which resembles that observed during spontaneous breathing. This pattern stands in marked contrast to the synchronous activation of all axons, as reflected in the single action potential following each stimulus spike, as seen with direct phrenic nerve stimulation, ventral root stimulation of the intercostal muscles, and all other motor nerve stimulation techniques. Second, analysis of single motor units indicates that the firing frequency during HF-SCS is not significantly different from
that observed during spontaneous breathing. Moreover, in studies on normal human subjects, other investigators have reported similar values of 10.8 ± 0.3, 11.9 ± 0.4, and 12.6 ± 0.5 Hz for the parasternal and external intercostal muscles and diaphragm, respectively (50). In previous studies in cats, investigators have also found similar firing frequency for the diaphragm (39, 52).

This study also demonstrates that HF-SCS, with a single electrode positioned on the ventral surface of the upper thoracic spinal cord, at high stimulus frequencies and low stimulus currents, results in near-maximum diaphragm and intercostal muscle activation. This is evidenced by the fact that 1) the magnitude of inspired volume achieved by this method approximates the inspiratory capacity, and 2) maximum bilateral phrenic nerve stimulation and combined intercostal and phrenic nerve stimulation result in negative airway pressures of ~44 and ~68 cmH2O, respectively (23), whereas HF-SCS resulted in a mean airway pressure of 67 cmH2O. Taken together, these results indicate that HF-SCS has the potential to provide a much more natural and effective method of inspiratory muscle pacing.

Importantly, the results of this investigation also demonstrate that artificial ventilation can be maintained for prolonged periods by HF-SCS. Based on the stability of inspired volume over several hours, and the fact that ventilation could be increased at the end of the trial resulting in a reduction in end-tidal PCO2 into the 25- to 30-mmHg range, our results further suggest that chronic pacing was accomplished without significant system fatigue.

**Mechanism of inspiratory muscle activation via HF-SCS.** In contrast to the pattern of motor unit recruitment observed during DP, spontaneous breathing is characterized by an orderly recruitment of diaphragm motor units, which has been shown to follow Henneman’s size principle (3, 11, 26, 37, 38, 40, 51). According to this principle, smaller motoneurons that have lower membrane surface area and higher input resistance are more excitable. Consequently, smaller motoneurons have a lower recruitment threshold than larger ones and are recruited first. In support of this principle, studies have shown that motor units with slower axonal conduction velocities, which are...
Table 1. Ventilation parameters monitored during 6 h of continuous HF-SCS

<table>
<thead>
<tr>
<th>Time, hour</th>
<th>ETCO$_2$, mmHg</th>
<th>SaO$_2$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>38.7±1.7</td>
<td>97.5±0.5</td>
</tr>
<tr>
<td>2</td>
<td>38.3±0.9</td>
<td>96.0±1.0</td>
</tr>
<tr>
<td>4</td>
<td>38.0±0.6</td>
<td>97.5±0.5</td>
</tr>
<tr>
<td>5.5</td>
<td>37.7±0.7</td>
<td>97.5±0.5</td>
</tr>
<tr>
<td>6</td>
<td>25.7±0.7</td>
<td>100±0.0</td>
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Values are means ± SE. HF-SCS, high-frequency spinal cord stimulation; ETCO$_2$, end-tidal $\text{PCO}_2$; SaO$_2$, oxygen saturation.

known to correlate with smaller motoneuron size, are recruited first during inspiratory efforts (12, 51). Based on this thesis, motoneuron activation and therefore motor unit recruitment proceed in an orderly fashion from slow to fast units. Consequently, slow fibers, which are fatigue resistant and generate relatively low-twitch and maximum forces, are recruited first. This is followed by the progressive recruitment of faster fibers, which are more fatigueable, but capable of generating much larger forces (11, 30, 43). Implicit in this concept is the fact that control of motoneuron recruitment pattern resides at the level of the motoneuron pools and does not require input from bulbospinal premotoneurons (11, 12, 43).

With regard to HF-SCS, the observation that the applied stimulus currents result in a physiological pattern of activation indicates that the mechanism of inspiratory muscle activation entails stimulation of spinal cord tracts that synapse with the diaphragm and inspiratory intercostal motoneuron pools. In contrast to virtually all functional electrical stimulation applications (24, 25, 31, 44), this method allows for processing of the stimulus within the motoneuron pools resulting in a more physiological recruitment pattern. The mechanism of pathway activation arises from the fact that motoneurons, previously thought of as passive integrators of synaptic input, possess intrinsic membrane properties (as described above) that allow processing of afferent signals (3, 6). It is important to mention, however, that the precise pattern of motor unit recruitment was not investigated in the present study. Further investigations will be necessary, therefore, to assess this parameter.

While the specific spinal cord tracts mediating this response are not known, Decima and von Euler (7) and Decima et al. (8, 9) have described afferents from the lower thoracic intercostal and abdominal muscles (T$_9$–T$_{13}$) that reflexly facilitate phrenic motoneuron activity via spinal cord pathways (intercostal to phrenic reflex). Afferents from the inspiratory and expiratory intercostal muscles monosynaptically excite the homonymous motoneurons of the same spinal segment and also motoneurons of adjacent segments (intercostal to intercostal and intercostal to phrenic facilitatory reflexes) (27, 28). We speculate that activation of the inspiratory motoneuron pools by HF-SCS may be mediated by these same excitatory tracts.

Comparison to current methods of DP. There are a number of factors that may contribute to reduced inspired volume production and consequent inability to maintain satisfactory full-time ventilatory support with current methods of DP. First, with inspiratory muscle pacing and virtually all other functional electrical stimulation methods to restore muscular function, electrodes are positioned near motor roots (24, 25), on peripheral nerves (31), or at functional motor points (44). This methodology results in synchronous activation of motor axons within the field of stimulation (36) and a reversed order of motor unit recruitment. In general, large-diameter motor axons, which innervate large, more fatigueable motor units, are more easily excited by imposed electric fields. Smaller, fatigue-resistant motor units require higher stimulus levels and may not be fully activated, resulting in incomplete axonal stimulation and therefore incomplete muscle activation (42). Assuming that HF-SCS results in an orderly pattern of motor unit recruitment, the early engagement of the weakest and least fatigueable motor units with progression to stronger and more fatigueable units during inspiration (rather than synchronous activation of all motor units) serves to maximize the range of force modulation and thereby inspired volume generation.

Second, while maximum activation is usually achieved with 50-Hz stimulation during DP, stimulus frequencies of 20 Hz or less are generally applied, as chronic stimulation at higher frequencies may result in myopathic changes and muscle fatigue (5, 31–33). While low-frequency stimulation increases the endurance characteristics of electrically stimulated muscles by conversion from a mixed-fiber population to a uniform population of type I fibers, it also significantly reduces fiber diameter and maximum force generation and, in turn, maximum inspired volume generation (48). Since HF-SCS results in the activation of the inspiratory muscles at physiological firing frequencies, fiber type conversion may not occur, resulting in preserved histological characteristics and mechanical function.

Finally, there is lack of coincident inspiratory intercostal/ accessory activation during DP, a muscle group that is responsible for the generation of 35–40% of the inspiratory capacity (2, 10). The lack of coincident intercostal activation also prevents the synergistic interaction of combined intercostal and diaphragm activation to generate inspired volume and airway pressure (23). While the relative degree of diaphragm and intercostal activation resulting from HF-SCS is unknown, the EMG data of the present study suggest that both muscle groups are activated in synchrony.

Study limitations. We have previously demonstrated that upper thoracic SCS, on the ventral surface of the spinal cord at the T2 level, with conventional stimulus frequencies results in activation of the inspiratory intercostal muscles and the production of large inspired volumes (15–17). While intercostal pacing alone is not sufficient (24), combined intercostal and unilateral diaphragm pacing is successful in maintaining full-time ventilatory support (25). Intercostal pacing, however, results in nonspecific stimulation of the upper thoracic ventral roots and therefore some undesirable upper extremity movement (24, 25). Based on the results of this study, chronic HF-SCS does not result in significant upper extremity muscle activation. This is not surprising since HF-SCS does not depend on ventral root stimulation and results in maximum inspired volumes with lower stimulus currents. It is possible, therefore, that the potential clinical application of HF-SCS will not be associated with movement side effects.

The degree of inspiratory muscle activation resulting from HF-SCS is unknown. It is possible that this technique also results in activation of expiratory motoneuron pools, as well. While this is possible, it would appear that this is a negligible effect since HF-SCS results in inspired volumes that approxi-
mate the inspiratory capacity. In addition, the expiratory intercostal muscles of the upper rib cage are very thin and generate negligible opposing positive pressure (20).

In summary, HF-SCS is a unique method of inspiratory muscle activation that results in physiological activation of the inspiratory muscles. It is possible that this technique may provide ventilator-dependent tetraplegic persons a more natural and effective method of inspiratory muscle pacing. Intact phrenic nerve function is necessary to activate the diaphragm with HF-SCS. However, since HF-SCS also results in activation of the inspiratory intercostal muscles, it is possible that this technique may also be successful in maintaining ventilatory support in tetraplegic persons with suboptimal phrenic nerve function.

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GRANTS

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DISCLOSURES

A. F. DiMarco is a founder of and has a significant financial interest in Synapse BioMedical, a manufacturer of diaphragm pacing systems and holds patents for spinal cord stimulation to restore cough and respiration. A patent covering high-frequency spinal cord stimulation is pending.

REFERENCES


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