Effects of chronic electrical stimulation on paralyzed expiratory muscles

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DiMarco AF, Kowalski KE. Effects of chronic electrical stimulation on paralyzed expiratory muscles. J Appl Physiol 104: 1634–1640, 2008. First published April 10, 2008; doi:10.1152/japphysiol.01321.2007.—Following spinal cord injury, the expiratory muscles develop significant disuse atrophy characterized by reductions in their weight, fiber cross-sectional area, and force-generating capacity. We determined the extent to which these physiological alterations can be prevented with electrical stimulation. Because a critical function of the expiratory muscles is cough generation, an important goal was the maintenance of maximal force production. In a cat model of spinal cord injury, short periods of high-frequency lower thoracic electrical spinal cord stimulation (SCS) at the T_{10} level (50 Hz, 15 min, twice/day, 5 days/wk) were initiated 2 wk following spinalization and continued for a 6-mo period. Airway pressure (P)-generating capacity was determined by SCS. Five acute, spinalized animals served as controls. Compared with controls, initial P fell from 43.9 ± 1.0 to 41.8 ± 0.7 cmH_{2}O (not significant) in the chronic animals. There were small reductions in the weight of the external oblique, internal oblique, and rectus abdominis muscles (not significant for each). There were no significant changes in the population of fast muscle fibers. Because prior studies (Kowalski KE, Romaniuk JR, DiMarco AF. J Appl Physiol 102: 1422–1428, 2007) have demonstrated significant atrophy following spinalization in this model, these results indicate that expiratory muscle atrophy can be prevented by the application of short periods of daily high-frequency stimulation. Because the frequency of stimulation is similar to the expected pattern of clinical use for cough generation, the daily application of electrical stimulation could potentially serve the dual purpose of maintenance of expiratory muscle function and airway clearance.

METHODS

Experiments were performed in 10 cats (weight: 4.3 ± 0.2 kg). Studies were performed with the approval of the Institutional Animal Care and Use Committee of Case Western Reserve University. All surgical procedures and measurements were the same for each animal. Comparisons were made between five chronic animals that were maintained for 6 mo with daily electrical stimulation and five animals studied acutely. Animals were anesthetized with xylazine (0.45 mg/kg) followed by ketamine (2.2 mg/kg intramuscularly) after 10 min. Following administration of atropine (0.05 mg/kg intramuscularly), animals were intubated. Halothane (0.5–1.5% provided by a vapor-

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izer) was used to maintain subsequent anesthesia. Body temperature was maintained at 38 ± 0.5°C with a homeothermic blanket (Harvard Apparatus, Cambridge, MA). A rapidly responding CO2 analyzer (OR SARAcap, PPG Biomedical System, Lenexa, KS), positioned at the tracheal opening, was used to monitor end-tidal PO2.

Under aseptic conditions, laminectomies were performed at the T6 and T10 spinal cord levels in five chronic animals. The spinal cord was transected with watchmaker forceps at the T6 level. A hook forceps was lifted across the area of transection to verify complete sectioning. The transection site was packed with Gelfoam to minimize bleeding. A disc electrode (4 mm) was then inserted onto the dorsal epidural surface at the T6 level.

Following surgery, each of the chronic animals was kept in a separate, large cage on crate mattresses covered by absorbent tissue. Buprenorphine (0.01–0.02 mg/kg every 12 h subcutaneously, for 48 h postoperatively) was administered for pain control. Ringer solution (15–25 mL·kg−1·day−1) was also provided for the first 2 days following surgery for hydration. Vetropycin was placed in each eye to prevent corneal ulcers. Prophylactic antibiotics (amoxicillin, 2.2 mg/kg twice a day) was also provided for 10 days following surgery. The bladder was expressed three times per day by manual compression. Elimination of stool was also manually supported. Each animal was weighed weekly.

Protocol

In the chronic animals, electrical stimulation was applied with a FOCUS neuromuscular stimulator (EMPI, St. Paul, MN), which delivers balanced biphasic stimulation. Stimulation was applied (15 mA, 50 Hz pulse width, 0.2 ms) with a 50% duty cycle, 5 s on/5.0 s off, for 15-min periods twice a day, 6 days/wk for 6 mo. Stimulation was initiated ~2 wk following surgery to allow for healing of wounds and resolution of edema and inflammation at the electrode site.

Assessment of pressure-generating capacity. The pressure generating capacity of the expiratory muscles was assessed by spinal cord stimulation (SCS) at the T10 level, according to previously described techniques (7, 11–13). This technique results in direct activation of motor roots in the vicinity of the stimulating electrode and more distal roots via spinal cord pathways. Pressure generation was always assessed during SCS under conditions of tracheal occlusion and following hyperventilation-induced apnea.

In the chronic animals, the effects of SCS at the T10 level (15 mA, 50 Hz, 0.2-ms pulse width) were assessed during the initial surgical procedure at functional residual capacity (FRC). A more comprehensive evaluation of pressure-generating capacity was not performed in these animals to minimize operative time and consequent potential for postoperative complications. Each of the chronic animals underwent a final procedure 6 mo after spinalization during which the airway pressure generating capacity during SCS (15 mA, 50 Hz) was assessed over a wide range of lung volumes (between 0.3 liter below and 1.5 liters above FRC). A 2-liter calibration volume syringe was used to apply lung deflations and inflations. The magnitude of inflation or deflation was assessed by the corresponding change in airway pressure and is referred to as precontractile airway pressure. The relationship between stimulus frequency over a 20- to 100-Hz range (15 mA), stimulus amplitude over a 2- to 20-mA range (50 Hz), and airway pressure generation at FRC was also assessed. A comprehensive evaluation of pressure-generating capacity was also performed in the acute animal studies.

Muscle weight. Parasternal intercostal (PA, 3rd interspace), external (EI) and internal (II) intercostals (10th interspace), external oblique (EO), internal oblique (IO), transversus abdominis (TA), rectus abdominis (RA), triceps brachii (TB, long head), and soleus (Sol) muscles were removed in their entirety and weighed postmortem, according to previously described technique (31).

Immunohistochemistry procedures. Biopsy samples (~6 × 6 mm) from the same muscles from the contralateral side of the animal were obtained for immunohistochemistry. Biopsy samples from the abdominal muscles were obtained from the midportion of the abdominal wall. Standard 3-μm-thick sections were cut, deparaffinized, and stained for slow and fast myosin heavy chain (MHC) using specific mouse monoclonal antibodies from Sigma-Aldrich (slow myosin clone NO7.5.4D and fast myosin clone MY-32; St Louis, MO). Antigen-bound primary antibodies were detected using the Ventana Medical Systems DAB detection kit (Tucson, AZ). All stains were performed using NexES IHC staging system (Ventana Medical System). Primary antibodies and detection kit were used according to manufacturers’ recommendations. Slides were counterstained with hematoxylin and coverslipped. Subtyping of type II fibers was not performed. Stained tissue sections were systematically photographed using a light microscope (Olympus BH2-RPCA, Olympus, Tokyo, Japan) connected to a computer with a video-display image-analysis system (Spot version 2.1 for Windows 95/NT). The relative frequencies of fiber types and cross-sectional areas (CSAs) were determined from an average of ~150 fibers per biopsy specimen.

Data analyses. Airway pressures resulting from SCS at specific precontractile airway pressures were obtained by interpolation of plots from individual animals. These data were used to determine mean relationships between passive precontractile and generated airway pressures during SCS. Data from the acute and chronic animal groups were compared statistically by one-way ANOVA and Student’s t-tests. Data are reported as means ± SE. Statistical significance was determined by P values that were <0.05.

RESULTS

Body weight of the acute and chronic animals at baseline were not significantly different (4.3 ± 0.3 and 4.2 ± 0.1 kg, respectively, for each group). Six months following spinalization, however, the weight of the chronic cats had increased to 4.6 ± 0.1 kg (P < 0.05).

The mass of each of the expiratory muscles innervated by spinal cord segments below the lesion and subjected to chronic electrical stimulation were somewhat lower than control animals, but these changes were not significant (Table 1). In contrast, Sol muscle mass decreased by 53.1% (P < 0.01). The mass of muscles innervated by spinal cord segments above the lesion (PA and TB) was unchanged compared with the control group.

Table 1. Respiratory and nonrespiratory muscle weights for control and chronically stimulated groups

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Control, g</th>
<th>Stimulated, g</th>
<th>Stimulated, % Control</th>
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<tbody>
<tr>
<td><strong>Muscles innervated by spinal segments below the lesion</strong></td>
<td></td>
<td></td>
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<tr>
<td>External oblique</td>
<td>23.64±0.39</td>
<td>22.88±0.32</td>
<td>97±2</td>
</tr>
<tr>
<td>Internal oblique</td>
<td>13.32±0.30</td>
<td>12.52±0.37</td>
<td>94±3</td>
</tr>
<tr>
<td>Transversus abdominis</td>
<td>16.78±0.61</td>
<td>16.30±0.32</td>
<td>97±2</td>
</tr>
<tr>
<td>Rectus abdominis</td>
<td>21.56±0.62</td>
<td>20.54±0.32</td>
<td>95±2</td>
</tr>
<tr>
<td>Internal intercostal</td>
<td>1.39±0.04</td>
<td>1.36±0.03</td>
<td>98±2</td>
</tr>
<tr>
<td>External intercostal</td>
<td>1.37±0.05</td>
<td>1.35±0.01</td>
<td>99±1</td>
</tr>
<tr>
<td>(10th interspace)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>4.98±0.14</td>
<td>2.65±0.05</td>
<td>53±1*</td>
</tr>
<tr>
<td><strong>Muscles innervated by spinal segments above the lesion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasternal</td>
<td>1.34±0.05</td>
<td>1.36±0.01</td>
<td>101±1</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>9.79±0.34</td>
<td>10.13±0.12</td>
<td>103±1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly smaller than control, P < 0.01.
Consistent with prior studies (31), postmortem examination of the stimulating electrode revealed negligible tissue reaction.

Effects of Chronic SCS on Expiratory Muscle Pressure-Generating Capacity

Mean maximal pressure generation in response to SCS (15 mA, 50 Hz, 0.2 ms) at FRC, in the chronic animals during the initial surgical procedure, was 43.0 ± 1.0 cmH2O compared with 44.6 ± 0.8 cmH2O in the acute animals [not significant (NS)]. Following spinalization and subsequent chronic SCS, pressure generation was 41.8 ± 0.7 cmH2O (NS).

The effects of SCS on expiratory airway pressure generation, over a wide range of lung volumes; are shown for one animal in Fig. 1A, and mean results are shown in Fig. 1B. As expected, airway pressure generation increased progressively with increasing lung volume. Following chronic SCS, however, airway pressure generation was essentially unchanged, compared with control values, at all lung volumes. The magnitude of airway pressure changes in the chronically stimulated animals was ~92–99% of control values over the vital capacity range. Mean control airway pressures were 29.4 ± 0.4, 44.6 ± 0.7, and 75.2 ± 4.5 cmH2O for −10 cmH2O, FRC, and +30 cmH2O respectively, and 27.0 ± 2.6, 41.4 ± 1.5, and 72.4 ± 4.7 cmH2O respectively, in the chronically stimulated animals (NS, for each).

The responses consequent to changes in stimulus amplitude during SCS at FRC are shown in Fig. 2. In both the control and chronically stimulated groups, there were progressive increases in airway pressure generation with increasing stimulus amplitude until a plateau was reached at 15 mA. Although airway pressure generation was somewhat smaller in the chronically stimulated animals, these differences were significantly different only at 2 and 6 mA (P < 0.05 for each). The airway pressure responses consequent to varying stimulus frequencies during SCS at FRC are shown in Fig. 3. In both the control and stimulated groups, increases in stimulus frequency resulted in steep rises in pressure generation between 20 and 50 Hz with subsequent smaller increases at higher frequencies. In the spinalized animals subjected to chronic SCS, airway pressure generation was somewhat smaller compared with the control group, ranging between 90 and 97% of control values. However, these differences were not significant.

Muscle Fiber Types

Fast- and slow-muscle fiber stains were, in general, reciprocally exclusive. Muscle fibers staining positively with antibody-recognizing fast myosin did not stain with antibody directed against slow MHC isoform. Similarly, fibers that did not stain with antibodies directed to fast MHC isoform were always strongly positive for slow MHC isoform stains. There was only a small percentage of fibers that reacted with both fast and slow antibody types. Therefore, fiber-type results are presented in terms of the relative percentage of fast fibers for each muscle (Table 2). Compared with control values, fiber-type characteristics were essentially unchanged for each of the expiratory muscles in the chronically stimulated group. However, the small decrease in percentage of fast fibers in the EIs (10th interspace) was significant (P < 0.05). The Sol had a significantly higher percentage of fast fibers (P < 0.01). The compositions of muscle fibers in muscles innervated by spinal cord segments above the lesion were not significantly different.

CSA

Muscle fiber CSA for the control and chronically stimulated animals, for both fast and slow fibers, are presented in Table 3. While there was a tendency toward a reduction in CSA of the fast fiber populations of each of the expiratory muscles (94–96% of control values), these differences were not significant. There were more variable changes in the slow fiber populations of the expiratory muscles, but these differences were small and also not significantly different compared with controls. There was a significant reduction in the CSA of the soleus muscle. There were no significant differences in CSA of muscle fibers innervated by spinal cord segments above the lesion.

DISCUSSION

In a previous study, in a chronic cat model of spinal cord injury (31), our laboratory demonstrated that lower thoracic spinal cord section resulted in significant reductions in the global force-generating capacity of the expiratory muscles over a 6-mo period. Force generation fell by ~36% of control values, which is similar to the reductions in force described for limb muscles in spinalized cat preparations (46, 62) and also to the reductions in abdominal muscle strength in tetraplegic subjects (12, 17, 25, 40). The reductions in force generation
were attributable to expiratory muscle atrophy, as evidenced by significant reductions in expiratory muscle mass and muscle fiber CSA. Moreover, the reductions in pressure generation correlated significantly with observed reductions in weight and CSA of the EI, IO, II, and TA muscles (31). Also consistent with previous studies of limb muscles, spinalization resulted in a shift in fiber-type composition of each of these muscles to a higher percentage of fast fibers. Of interest, there were no significant changes in weight, CSA, or fiber-type composition of the RA muscle following spinalization in our laboratory’s prior study (31). This muscle, however, is thought to have only a negligible expiratory function (8, 51) and makes only a minimal contribution to pressure generation during lower thoracic spinal cord stimulation (11). Therefore, it is not likely that preservation of rectus function impacted the observed reductions in pressure-generating capacity.

This present study represents the first systematic assessment of the potential for electrical stimulation to maintain expiratory muscle structure and function in an animal model of spinal cord injury. Our results indicate that the application of inter-

mittent high-frequency electrical stimulation via lower thoracic spinal cord stimulation is sufficient to maintain the global pressure-generating capacity of this muscle group near control values. Of importance, pressure generation is preserved over the entire vital capacity range. This is an important feature because these muscles are often engaged at high lung volumes to optimize force production, as with cough generation. Consistent with the maintenance of pressure generation, electrical stimulation served to prevent the development of muscle atrophy as evidenced by the lack of any significant decrement in expiratory muscle weight or fiber CSA. Moreover, based on the phenotypic expression techniques employed in this study, fiber type characteristics were also maintained.

**Critique of Method**

The limitations of lower thoracic spinal cord stimulation to activate the expiratory muscles in the cat have been reviewed previously (31). Briefly, most validation studies of this technique have been performed in a dog model (10–12) in which near maximal activation and positive pressure generation could be achieved with a two-electrode system. Previous studies in a cat model demonstrated that a similar degree of expiratory muscle activation could be achieved with a single electrode with recognition of the possibility that the lower most portions of the abdominal muscles may not be fully activated (31). Nonetheless, electrode placement was virtually identical in both the control and chronically stimulated animals. Therefore, the degree of activation in both groups of animals should have been the same, allowing a valid comparison. In addition, biopsy specimens for fiber typing and evaluation of CSA were taken from the midregion of the abdominal wall where muscle activation should have been supramaximal (9).

The evaluation of the effects of electrical stimulation was limited to a single-stimulus paradigm in this study. While electrical stimulation was sufficient to maintain near normal expiratory muscle strength, it is possible that the application of more frequent stimulation may have resulted in increases in abdominal force production and associated increases in airway pressure generation. It is also possible, however, that increased levels of stimulation may have reduced force-generating ca-

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### Table 2. Muscle fiber types in cat respiratory and nonrespiratory muscles in control and chronically stimulated animals

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Percentage of Fast Fiber Types</th>
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<tbody>
<tr>
<td><strong>Muscles innervated by spinal segments below the lesion</strong></td>
<td></td>
</tr>
<tr>
<td>External oblique</td>
<td>69±5</td>
</tr>
<tr>
<td>Internal oblique</td>
<td>68±7</td>
</tr>
<tr>
<td>Transversus abdominis</td>
<td>67±13</td>
</tr>
<tr>
<td>Rectus abdominis</td>
<td>65±13</td>
</tr>
<tr>
<td>Internal intercostal (10th interspace)</td>
<td>70±6</td>
</tr>
<tr>
<td>External intercostal (10th interspace)</td>
<td>69±2</td>
</tr>
<tr>
<td>Soleus</td>
<td>0</td>
</tr>
<tr>
<td><strong>Muscles innervated by spinal segments above the lesion</strong></td>
<td></td>
</tr>
<tr>
<td>Parasternal intercostal (3rd interspace)</td>
<td>60±11</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>73±3</td>
</tr>
</tbody>
</table>

Values are mean ± SE. †Significantly greater reduction than control, P < 0.05. ‡Significantly greater reduction than control, P < 0.01.

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**Fig. 2.** Effects of lower thoracic spinal cord stimulation on airway pressure generation over a wide range of stimulus amplitudes in the acute animals (control group; ●) and in the chronically stimulated group (×). Values are group means ± SE. In both groups, there were progressive increases in airway pressure generation with increasing stimulus amplitude until a plateau was reached at ~15 mA, following which there were no further increases in pressure development. The magnitude of airway pressure generation was somewhat smaller in the chronically stimulated group compared with control animals, but these differences were significant only at stimulus amplitudes of 2 and 6 mA (*P < 0.05 for each).

**Fig. 3.** Relationship between stimulus frequency (20–100 Hz) and airway pressure generation in the acute animals (control group; ●) and in the chronically stimulated group (×). Values are group means ± SE. In the chronically stimulated group, the magnitude of airway pressure generation during spinal cord stimulation was similar to the control group at all stimulus frequencies.
pacity through either alterations in contractile characteristics or muscle damage (36). The lower limit of stimulation necessary to maintain expiratory muscle function also is unknown. Further studies may be necessary to address these issues.

The present study is also limited by the lack of a control group in which animals were spinalized but not stimulated. However, comparisons should be valid because the experimental conditions were the same as in our laboratory’s previous study (31), which assessed the physiological changes associated with spinalization in the same model.

**Previous Studies**

The maintenance of expiratory muscle weight, CSA, and global pressure-generating capacity observed in the present study in response to short-term high-frequency stimulation are generally consistent with prior animal studies in limb muscles (18, 28, 32, 33, 38, 41, 48, 55).

The specific alterations resulting from electrical stimulation have been shown to be highly dependent on the specific stimulus paradigm applied. Eerbeek et al. (16) demonstrated in a hemispinalized cat model, for example, that long-term tonic stimulation (>50% total time), regardless of stimulus frequency, resulted in slowing of muscle contractile properties with greater fatigue resistance but the muscles were weaker than normal. Rate-related effects become evident, however, with shorter periods of stimulation (<5% total time). Electrical stimulation of fast muscles with low frequencies (<20 Hz) generally results in slowing of muscle contraction and reductions in muscle mass, fiber CSA, and also tetanic tension development. In contrast, the generation of large forces (achieved by the application of high stimulus frequencies) for short periods usually results in maintenance or increases in tetanic force development, muscle weight, and fiber size. Interestingly, Kernell (28) demonstrated that superimposing even a brief period of high-frequency stimulation (100 Hz) for 0.5% of the day on a continuous low-frequency stimulation pattern (10 Hz for 5% of the day) largely prevented force loss. Although not a universal finding (18, 48), others studies have also demonstrated greater preservation of muscle mass and force development with high compared with low-stimulus paradigms (18, 48).

More recent animal studies (30) have shown that only brief periods of high-frequency stimulation are effective in reducing inactivity-induced muscle adaptations. Kim et al. (30) found that high-frequency stimulation (100 Hz) for short periods (1 s on every 30 s, 30 min/day, for 30 days) in a spinal isolation rat model significantly reduced the decrement in force development and phenotypic adaptations in the medical gastrocnemius muscle. Unlike the previously described studies in which some degree of background level of muscle activity persisted, background activity is virtually eliminated by spinal isolation. With this model, the impact of a defined pattern of neuromuscular activity on muscle properties can be determined under more controlled conditions. Because our intention was to mimic patients with spinal cord injury, however, the spinal cord transection model employed in this present study may be more relevant.

Concerning studies in humans with spinal cord injury, electrical stimulation has generally resulted in significant improvements in muscle endurance while effects on muscle strength have been inconsistent (1, 37, 59, 65). In these studies, however, study conditions varied widely in terms of the applied stimulus paradigms (1, 2, 19, 65), degree of muscle loading (1, 5, 45, 59, 65), and time following injury at which electrical stimulation was applied (1, 5, 6, 59, 64, 65). While intermittent high-frequency stimulation is usually an important feature of studies in which muscle force development increased, stimulation against an applied load (1) and initiation of stimulation in the acute phase of spinal cord injury are also thought to be important factors (1, 15, 16). It should be noted that lower thoracic spinal cord stimulation applied to a tetraplegic patient (who suffered his injury 7 yr earlier) to restore cough resulted in restoration of near-normal maximum airway pressure generation following a reconditioning program (8). While early initiation of electrical stimulation may be beneficial therefore, it may not be a critical factor in the ultimate restoration of expiratory muscle function.

**Clinical Implications**

Despite intensive respiratory management, respiratory tract infections and pneumonia are a major cause of morbidity and mortality in patients with spinal cord injury (3, 4, 24, 47, 67). For this reason, it has been suggested that the single most
important impairment of the respiratory system that occurs in patients with spinal cord injury is an ineffective cough secondary to paralysis of a major portion of the expiratory muscles (3). Maintenance of an effective cough would allow these patients to clear secretions more readily (8) and hopefully reduce the incidence of respiratory complications.

Because cough is a ballistic maneuver, which involves the rapid development of very high airway pressures for short time periods (42), it is likely that an effective cough mechanism depends on optimal function of the expiratory muscles. Muscles with high force-generating capacity with less regard for fatigue resistance would appear ideal. In fact, the expiratory muscles have a high percentage of fast fibers which are known to have a high maximum shortening velocity, faster time to peak tension, high levels of force generation, but less fatigue resistance (23, 49).

The precise amount of electrical stimulation necessary to maintain optimal expiratory muscle function is unknown. However, the daily application of short periods of high-intensity electrical stimulation, as employed in the present study, is consistent with the expected pattern of clinical use because most patients require some mechanical method of secretion removal on a daily basis. Therefore, the daily use of electrical stimulation could serve the dual purpose of maintenance of expiratory muscle function and airway clearance, and it could ultimately reduce the incidence of respiratory complications and death in this patient population.

GRANTS
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DISCLOSURES
A. F. DiMarco holds 8–10% stock ownership in Synapse BioMedical, LLC, a company that manufactures diaphragm pacing systems.

REFERENCES
ELECTRICAL STIMULATION OF PARALYZED EXPIRATORY MUSCLES