Changes in expiratory muscle function following spinal cord section

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PREVIOUS STUDIES INDICATE that spinal cord injury exerts a profound influence on the physiological, morphological, and histochemical properties of the muscles innervated below the level of injury (1, 3, 11, 20, 25, 36, 42, 43, 48). The time course and magnitude of these changes are fundamental to the potential success of interventions that may prevent or reverse the atrophic process. In animal models of spinal cord transection (38, 45) and in humans following spinal cord injury (3, 25, 27, 42), for example, hindlimb muscle atrophy occurs at an extremely high rate during the first several months, following which the rate of atrophy slows and remains relatively stable. The rate of development and ultimate degree of atrophy, however, can vary considerably in different muscles (21, 22, 25, 37, 42), with some muscles exhibiting little or no atrophic changes (35, 40).

There are no studies that have systematically evaluated the effects of spinal cord injury on the physiological changes of the expiratory muscles (11). This is an important muscle group since patients with cervical or thoracic spinal cord injury have an increased risk of developing respiratory tract infections and atelectasis as a result of expiratory muscle paralysis, and consequently diminished ability to cough and clear airway secretions (2, 17, 29). We hypothesized that, following spinal cord injury, these muscles undergo significant atrophy, which adversely impacts their force-generating capacity.

The purpose of the present study, therefore, was to evaluate the consequences of spinalization at the midthoracic level on the capacity of the expiratory muscles to produce changes in airway pressure, expiratory muscles fiber-type composition, and muscle fiber cross-sectional area (CSA). Studies were performed in a cat model of spinal cord injury in which there is considerable previous experience (19, 37, 39, 40).

METHODS

Studies were performed on 10 cats (weight range: 2.9–5.5 kg, mean 4.4 ± 0.3 kg) with approval from the Institutional Animal Care and Use Committee of Case Western Reserve University. All animals were anesthetized initially with xylazine (0.45 mg/kg) followed by ketamine (2.2 mg/kg given intramuscularly) after 10 min and intubated. Atropine (0.05 mg/kg given intramuscularly) was given before intubation. Subsequent anesthesia was maintained with halothane (0.5–1.5% given via vaporizer). A homeothermic blanket (Harvard Apparatus, Cambridge, MA) was used to maintain body temperature at 38 ± 0.5°C. End-tidal Po2 was monitored with a rapidly responding CO2 analyzer (OR SARAcap; PPG Biomedical System, Lenexa, KS) at the tracheal opening.

In five chronic animals, laminectomies were performed at the T4 and T10 spinal cord levels under aseptic conditions. The spinal cord was transected at the T4 level with watchmaker forceps. Complete transection was verified by lifting the hook across the area of transection. To minimize bleeding, the transection site was packed with Gelfoam. In each animal, a disc electrode was inserted onto the dorsal epidural surface at the T10 level. To prevent corneal ulcers, Vetropolyvin was placed in each eye. Following surgery, each cat was kept in a separate, large cage on crate mattresses covered by absorbent tissue with free access to food and water. The animals were administered buprenorphine (0.01–0.02 mg/kg every 12 h subcutaneously) for pain control and Ringer solution (15–25 ml·kg−1·day−1) for the first 2 days. During the 10 days following surgery, a prophylactic antibiotic regimen of amoxicillin administered twice a day (2.2 mg/kg each dose) was instituted. The bladder was expressed three times per day by manual compression. Elimination of stool was manually assisted. Animals were weighed weekly.

Five acute animals served as controls (mean weight 4.2 ± 0.5 kg). In these animals, general anesthesia and final procedures were the same as described for the chronic animals.

Protocol

Assessment of pressure-generating capacity. Spinal cord stimulation (SCS) was employed in the present study to evaluate the pressure-

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generating capacity of the expiratory muscles, according to previously described techniques (7–10). Briefly, this technique results in direct activation of motor roots in the vicinity of the stimulating electrode and more distal roots via spinal cord pathways (7–10). Based on EMG analyses, the threshold for activation of motor roots in the vicinity of the stimulating electrode is quite small: <1 mA (9). Although a two-electrode system was necessary to achieve maximum pressure generation in a dog model (9), in preliminary studies in the smaller cat model, near maximum pressure generation could be achieved with a single electrode positioned at the T10 spinal level. During SCS at the T10 level, pressure generation was assessed under conditions of tracheal occlusion following hyperventilation-induced apnea.

During the initial surgical procedure in the chronic animals, the effects of SCS at the T10 level (15 mA, 50 Hz, 0.2-ms pulse width) were assessed but only at functional residual capacity (FRC). A more comprehensive evaluation of pressure-generating capacity was not performed in attempt to minimize operative time and potential for postoperative complications. Six months after spinalization, each of the chronic animals underwent a final procedure during which the effects of SCS at the T10 level on airway pressure generation were determined at FRC and over a wide range of lung volumes (0.3 liter below to 1.5 liters above FRC). Although lung deflations and inflations were applied with a volume syringe, the magnitude of inflation was assessed by the corresponding change in airway pressure (precontractile airway pressure). The relationship between stimulus frequency (20–80 Hz) and airway pressure generation at FRC was also assessed by the corresponding change in airway pressure (precontractile airway pressure). The relationship between stimulus frequency and airway pressure generation at FRC during SCS in the chronic animals was 41 ± 6 cmH2O; in the acute animals, this value was 44 ± 1 cmH2O (P = not significant). Six months after spinalization in the chronic animals, pressure generation fell to 28 ± 3 cmH2O (P < 0.05).

The effects of SCS, over a wide range of lung volumes, on expiratory airway pressure generation are shown in Fig. 1. Generated airway pressures were lowest at lung volumes below FRC and increased progressively with increasing lung volume. However, generated airway pressures following spinalization were lower than control values at each lung volume (P < 0.05). These findings are illustrated for a single animal in Fig. 1A.

### RESULTS

#### Animal and Muscle Weight

Except for an initial loss of body weight (11 ± 2%), body weight of the chronic animals remained unchanged (4.6 ± 0.3 kg at baseline and 4.4 ± 0.4 kg after 6 mo) over the course of the experimental period.

Muscle mass of all muscles innervated by spinal cord segments below the lesion decreased compared with control values (Table 1). Mass of the external oblique, internal oblique, and transversus abdominis muscles decreased significantly by 29 ± 3, 27 ± 2, and 21 ± 2%, respectively (P < 0.01). The reduction in RA muscle mass did not reach statistical significance. Mass of the internal and external intercostal and muscles were also significantly reduced below control values by 35 ± 6 and 41 ± 6%, respectively (P < 0.05). Soleus muscle mass also decreased by 58 ± 4% (P < 0.01). The mass of those muscles innervated by spinal cord segments above the lesion was not significantly different.

#### Effects of Spinalization on Expiratory Muscle Pressure-Generating Capacity

During the initial surgical procedure, mean pressure generation at FRC during SCS in the chronic animals was 41 ± 1 cmH2O; in the acute animals, this value was 44 ± 1 cmH2O (P = not significant). Six months after spinalization in the chronic animals, pressure generation fell to 28 ± 3 cmH2O (P < 0.05). These findings are illustrated for a single animal in Fig. 1A.

### Table 1. Respiratory and nonrespiratory muscle weights following spinalization expressed as a percentage of control

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Total Muscle Weight Reduction, %control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscles innervated by spinal segments below the lesion</td>
<td></td>
</tr>
<tr>
<td>External oblique</td>
<td>71±3†</td>
</tr>
<tr>
<td>Internal oblique</td>
<td>73±2†</td>
</tr>
<tr>
<td>Transversus abdominis</td>
<td>79±2†</td>
</tr>
<tr>
<td>Rectus abdominis</td>
<td>91±11</td>
</tr>
<tr>
<td>Internal intercostal (10th interspace)</td>
<td>65±6*</td>
</tr>
<tr>
<td>External Intercostal (10th interspace)</td>
<td>59±6*</td>
</tr>
<tr>
<td>Soleus</td>
<td>42±4†</td>
</tr>
<tr>
<td>Muscles innervated by spinal segments above the lesion</td>
<td></td>
</tr>
<tr>
<td>Parasternal intercostal (3rd interspace)</td>
<td>99±5</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>98±6</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *Significantly greater reduction than control (P < 0.05). †Significantly greater reduction than control (P < 0.01).

Data Analyses

All comparison analyses were performed between the control group (acute animals) and chronic animals. Generated 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 precontractile airway pressures and generated airway pressures. The statistical significance of the differences between group means was determined by one-way ANOVA and Student’s t-tests. Data are reported as means ± SE. Statistical significance was determined as a P value of <0.05.
The mean changes are shown in Fig. 1. Mean control airway pressures during SCS were 29 ± 2, 44 ± 1, and 76 ± 5 cmH₂O at -10, FRC, and 30 cmH₂O precontractile pressures, respectively. Mean airway pressures for spinalized animals were 16 ± 3, 28 ± 3, and 54 ± 6 cmH₂O for -10, FRC, and 30 cmH₂O, respectively (P < 0.05). The magnitude of changes in airway pressure following spinalization was ~55–70% of control values over the vital capacity range.

The effects of varying stimulus frequencies on airway pressure generation during SCS are shown in Fig. 2. Results from representative animals are shown in Fig. 2A; mean results are presented in Fig. 2B. In the control group, increases in stimulus frequency at FRC resulted in a steep rise in pressure generation between 20 and 40 Hz, followed by a much more gradual rise between 40 and 80 Hz. In the chronic animals, airway pressure generation during SCS also rose between 20 and 50 Hz and remained at those levels at higher stimulus frequencies. Pressure generation in the chronic animals, however, was significantly lower compared with control values at all stimulus frequencies [P < 0.05 (20–40 Hz) and P < 0.01 (50–80 Hz)].

As shown in Fig. 3, the weight of each of the expiratory muscles (except RA) also correlated significantly with airway pressure generation in the acute and chronic animals (r > 0.7 and P < 0.05 for each; RA: r = 0.03). Mean fiber CSA of each of the expiratory muscles (except RA) also correlated significantly with airway pressure generation (r = 0.55 and P < 0.05 for each; RA: r = 0.03) (Fig. 4).

Fiber Types

In general, fast- and slow-fiber stains were reciprocally exclusive. Fibers that stained positively with antibody-recognizing fast myosin did not stain with antibody directed against slow MHC isoform, and those that did not stain with antibodies directed to fast MHC isoform were always strongly positive for slow MHC isoform stains. Only a small percentage of fibers reacted with both fast and slow antibody types. Fiber-type results are therefore presented in terms of the relative percentages of fast fibers for each muscle (Table 2). In the chronic animals, each of the expiratory muscles innervated by spinal cord segments below the lesion (except for RA) had a significantly higher percentage of fast fibers compared with control animals. Two other muscles innervated by spinal segments below the lesion, i.e., external intercostal and soleus muscles, demonstrated higher percentages of fast muscles fibers (P < 0.05) as well. Muscle fiber compositions for muscles innervated by spinal cord segments above the lesion were not significantly different.
The frequency distributions of expiratory muscle fiber CSA for both slow and fast fibers are illustrated in Table 3. With few exceptions, there was a significant reduction of fiber size in each of the respiratory muscles innervated by spinal segments below the lesion compared with the control group ($P < 0.05$). There were no significant changes in CSA of the RA or slow fibers of the internal and external intercostal. CSAs of muscle fibers innervated by spinal cord segments above the lesion were not statistically different with exception to fast fibers of the triceps brachii ($P < 0.05$).

**DISCUSSION**

This is the first study to systematically examine the potential effects of spinalization on expiratory muscle function. Paralysis of the expiratory muscles by this method results in substantial reductions in the airway pressure-generating capacity of this muscle group. These alterations were not a function of...
resting muscle length since significant reductions in pressure generation were evident over a wide range of precontractile muscle lengths encompassing the entire vital capacity range. Moreover, reductions in pressure generation were maintained over a wide range of stimulus frequencies (20–80 Hz).

The reductions in pressure generation appear to be secondary to atrophy of the external and internal obliques, internal intercostal, and transversus abdominis muscles, as the muscle weights and average muscle fiber CSAs of each of these muscles correlated positively with pressure generation. In contrast, the RA muscle was not a significant factor in this response. From a functional standpoint, it is unlikely that the smaller degree of atrophy of the rectus muscle served to prevent even further declines in the overall pressure-generating capacity of the expiratory muscles. It has been noted previously that the rectus muscle has only a negligible expiratory role in the generation of maximum airway pressures in the range of those produced during a normal maximal cough effort (6).

Although preliminary studies in a cat model demonstrated that a similar degree of expiratory muscle activation could be achieved with a single electrode, it is possible that the lower most portions of the abdominal muscles were not fully activated. This should not have affected the results of the present study, however, since the single electrode should have resulted in the same degree of activation of the expiratory muscles allowing a comparison of pressures generated before and after spinalization. Moreover, biopsy specimens for muscle analysis were taken from the midregion of the abdominal wall, an area that should have realized complete activation by direct motor root stimulation.

Another concern was the potential development of fibrosis around the electrode lead, which could have interfered with transmission of electrical current leading to submaximal stimulation. Gross examination of the electrode post mortem however, demonstrated negligible tissue reaction. Moreover, the high degree of correlation between parameters of muscle atrophy and pressure generation suggests that the observed reductions in pressure generation were largely secondary to muscle atrophy.

The effects of spinalization on the triangularis sterni (also an important expiratory muscle) were not examined in the present study since this muscle is innervated by spinal cord segments above the level of spinal cord section.

Comparison to Previous Studies

Previous investigations have documented marked reductions in the strength of limb muscles below the level of injury in

Table 3. Mean muscles fiber cross-sectional areas in control and spinalized animals

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Fast</th>
<th>Slow</th>
<th>Control</th>
<th>Spinalized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscles innervated by spinal segments below the lesion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>External oblique</td>
<td>3,048</td>
<td>2,247</td>
<td>2,287</td>
<td>1,720</td>
</tr>
<tr>
<td>Internal oblique</td>
<td>3,647</td>
<td>2,667</td>
<td>2,375</td>
<td>2,084</td>
</tr>
<tr>
<td>Transversus abdominis</td>
<td>3,607</td>
<td>2,483</td>
<td>2,678</td>
<td>1,971</td>
</tr>
<tr>
<td>Rectus abdominis</td>
<td>3,718</td>
<td>2,308</td>
<td>3,086</td>
<td>2,069</td>
</tr>
<tr>
<td>Internal intercostal (10th interspace)</td>
<td>3,464</td>
<td>2,467</td>
<td>2,472</td>
<td>2,277</td>
</tr>
<tr>
<td>External intercostal (10th interspace)</td>
<td>3,183</td>
<td>1,845</td>
<td>2,315</td>
<td>1,837</td>
</tr>
<tr>
<td>Soleus</td>
<td>53</td>
<td>43</td>
<td>1,459</td>
<td>1,892</td>
</tr>
<tr>
<td><strong>Muscles innervated by spinal segments above the lesion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parasternal intercostal (3rd interspace)</td>
<td>3,247</td>
<td>1,578</td>
<td>3,111</td>
<td>1,459</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>2,941</td>
<td>1,741</td>
<td>3,229</td>
<td>1,892</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *Significantly smaller than control (P < 0.005). †Significantly smaller than control (P < 0.01). ‡Significantly smaller than control (P < 0.001).
patients with spinal cord injury (3, 46, 47) and in animal models of spinal cord injury (4, 21, 28, 40). In a similar model of spinalized cats maintained for ~6 mo, previous investigators (28, 40) found 25–50% reductions in force development, which are in the same range as described in the present study for the abdominal muscles. The findings of the present study are also consistent with the reductions in abdominal muscle strength in tetraplegic subjects previously reported by other investigators in response to magnetic and electrical stimulation (18, 23, 24). In addition, Estenne et al. (11) also found that abdominal muscle thickness assessed by ultrasound was reduced significantly in tetraplegics compared with normal subjects. Moreover, they found a positive correlation between abdominal muscle strength, as assessed by gastric pressure generation in response to magnetic stimulation, and muscle thickness. To the extent that muscle thickness represents a surrogate of muscle strength, these results are comparable to correlations between muscle weight and pressure-generating capacity of the expiratory muscles reported in the present study.

The 20–35% reductions in muscle weight and muscle fiber CSA of the expiratory muscles (except for the rectus muscle) observed in the present study are also in the same range as those observed for limb muscles in previous studies of spinalized animals (12, 20, 39) and patients with spinal cord injury (27, 36). The degree of muscle atrophy, however, varies considerably among different muscles. In both human and animal studies, evidence of marked atrophy has been documented for certain limb muscles, whereas others are barely affected (22, 35, 37, 40). The relatively minor degree of atrophy found in one of the expiratory muscles (rectus muscle), therefore, is not entirely surprising. It has been suggested that the variability in the degree of atrophy may relate to the normal level of neuromuscular activity, i.e., a decrease in the level of neuromuscular activity will have a greater impact on those muscles that are normally more active (40). Based on this hypothesis, it is possible that the smaller atrophic response of the rectus muscle may be related to a lower level of baseline activity. In fact, a much small degree of phasic expiratory activity of the rectus muscle, compared with the other expiratory muscles, has been observed in previous studies (5). There is a paucity of data concerning the fiber-type composition of the expiratory muscles. Previous investigations have found similar fiber composition in the cat internal intercostal muscles (74 vs. 68% fast fibers in the present study) (13) and RA muscle (65 vs. 60% in the present study) (16). These values are substantially different than those described in other species: 38–41% and 54% fast fibers for the internal intercostal muscles in humans and dogs, respectively (15, 30, 34), and 45% fast fibers for the rectus muscle in humans (14). However, values similar to those of the present study for the rectus muscle were reported in goats (68%) (16). These differences suggest substantial species differences in fiber-type composition of the abdominal muscles. It is not surprising, therefore, that the range observed for the remaining abdominal muscles (fast-fiber population between 68 and 70%) is much different than that previously described in humans (fast-fiber population between 42 and 45%) (14).

Consistent with most previous studies both in humans with spinal cord injury and animal models of limb muscles, we also observed a significant shift in fiber-type composition to a higher percentage of fast fibers in the expiratory muscles following spinalization (1, 12, 20, 22, 37, 42). Changes were most marked for the internal intercostal muscles. The smaller changes in fiber composition of the rectus muscle did not achieve statistical significance. CSA of fast fibers of the triceps brachii muscle, which were innervated by spinal segments above the level of spinal cord section, was significantly greater in spinalized animals compared with the control group. This may have occurred as a consequence of the spinalized animals making greater use of their forelimbs during ambulation.

The mechanisms of the skeletal muscle alterations observed following spinal cord injury are not completely understood. Disuse is thought to be the main mechanism responsible for the development of atrophy. However, other cofactors, including spasticity and microvascular damage, contribute to the morphological and enzyme histochemical changes observed in paralyzed muscles (42). Loss of muscle central control from upper centers and spasticity may be responsible for the induction of fiber-type transformation (42).

Clinical Implications

Compared with slow fibers, fast-fiber populations have a higher maximum shortening velocity, faster time to peak tension, and generally develop higher tensions (31, 41). Given the fact that cough is a ballistic maneuver involving the rapid development of high pressures for short periods (<1 s) (26), the high fast-fiber population of the expiratory muscles observed in the present study under control conditions appears well suited for this task. Although fast fibers are less fatigue resistant, this parameter is of little consequence since coughing maneuvers are generally required only intermittently.

Electrical stimulation techniques have been applied to reverse the reductions in muscle strength and endurance, which occur consequent to spinal cord injury (27, 44). In general, these studies have demonstrated improvement in muscle function consequent to specific stimulus paradigms. Relative to the expiratory muscles, several methods have been proposed to stimulate these muscles in patients with spinal cord injury in an attempt to provide a more functional cough mechanism (6, 18, 23, 24). The results of this study indicate that the expiratory muscles, like many other skeletal muscles, undergo significant atrophy within a relatively short time frame following inactivity. This would explain the relatively small positive airway pressures observed with electrical and magnetic stimulation in patients with spinal cord injury (11, 18, 23). In this patient population, therefore, it is likely that significant muscle conditioning may be necessary to restore expiratory muscle function to provide optimal pressure generation for cough production. In fact, studies performed by ourselves (6) have demonstrated improvements in expiratory muscle force generation, indicating that the reductions in muscle function are reversible. Stimulus paradigms, therefore, resulting in muscles capable of generating high forces with much less regard for fatigue resistance would appear optimal.

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EFECTS OF SPINALIZATION ON EXPIRATORY MUSCLE FUNCTION

GRANTS
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DISCLOSURES
A. F. DiMarco has 8–10% stock ownership in Synapse Biomedical, a company that manufactures diaphragm pacing devices.

REFERENCES