Long-Term Effects of Spinal Cord Transection on Fast and Slow Rat Skeletal Muscle

I. Contractile Properties

RICHARD L. LIEBER, CARINA B. JOHANSSON, H. L. VAHLSING, ALAN R. HARGENS, AND EARL R. FERINGA

Division of Orthopaedics and Rehabilitation, Departments of Surgery and Neurology, Veterans Administration Medical Center and University of California, San Diego, California 92161

Received June 26, 1985; revision received October 14, 1985

Contractile properties of rat soleus and extensor digitorum longus muscles were studied 1 year after complete thoracic spinal cord transection (spinal cord level T9). Force-generating capacity and contraction speed were unchanged in the extensor digitorum longus 1 year after transection. However, the rate of contraction and relaxation increased in the soleus as reflected by a decrease in time-to-peak tension and increase in fusion frequency. Additionally, the soleus muscle cross-sectional area decreased significantly (50%) while generating the same absolute tension. Thus, a large increase in soleus specific tension (force per unit area) was observed. These data, in conjunction with the increase in contractile speeds, suggest soleus slow-to-fast fiber type conversion secondary to cordotomy. Discriminant analysis of the contractile properties yields fusion frequency as the best discriminator between muscle groups. Thus, following cordotomy, predominantly slow muscles are affected to a greater extent than fast muscles. © 1986 Academic Press, Inc.

INTRODUCTION

The plasticity of skeletal muscle has been demonstrated using a variety of models which alter muscular environment. Under conditions such as limb immobilization (7, 33, 38, 40), chronic electrical stimulation (26, 31, 34, 36), exercise training (2, 16, 37), peripheral nerve lesion (27), cross-innervation (5, 8, 9), and central nervous system lesion (4, 15, 24, 30, 32, 35), skeletal muscle adapts to changes in use, nervous system input, and electrical activity.

Abbreviations: EDL—extensor digitorum longus, SOL—soleus.

The authors thank Dr. Roland R. Roy for his excellent criticism and comments on this work. Please address reprint requests to Dr. R. L. Lieber, Division of Orthopaedics and Rehabilitation, V-151, VAMC, San Diego, CA 92161.
Previous studies of spinal cord transection in the rat (13) and cat (4, 32, 35) suggest that transection decreases muscle force-generating capacity. In addition, contraction speed generally increases in “slow” muscle (13, 35) but remains unchanged in “fast” muscle (13, 32, 35). The short duration of previous studies relative to the life span of the animal (to 10% of life span) leave unanswered questions regarding the extent of muscle adaptation to spinal cord transection. Because of the relatively short life span of the rat (2 to 3 years), it is possible to use this model to study long-term effects of spinal cord transection.

Using the rat transection model, we studied the loss of corticospinal neurons from 5 weeks after spinal cord transection to 25 weeks after transection (19, 20). To quantify further changes occurring after 25 weeks, we extended these observations to one year (21, 22), when muscle contractile properties were also evaluated. Although contractile properties of skeletal muscle are not the most sensitive indicator of muscle change, they indicate the functional significance of fiber type changes that occur secondary to cordotomy. Our purpose was thus to determine the contractile properties of the rat soleus (SOL) and extensor digitorum longus (EDL) 1 year (about 50% of the rat life span) following spinal cord transection.

METHODS

Experimental Preparation. An inbred isogeneic strain of Wistar rats was developed by brother-and-sister mating for more than 80 generations in order to decrease fiber type and size variability between rats (17). Two groups of rats were studied. Control rats (N = 14) were permitted normal growth for 1 year after entry into the study at age 6 weeks. At age 6 weeks [about 2 weeks after the muscle fiber types were differentiated (12)], experimental rats (N = 10) were anesthetized with acepromazine/ketamine (1 and 50 mg/100 g body mass, respectively). After T6/T7 laminectomy, the spinal cord and its coverings were completely transected with a scalpel. The completeness of the transection was verified by two investigators using the operating microscope while applying gentle traction to the animal's head and tail. No portion of the spinal cord was removed. Longitudinal sections from the injured area in similarly treated rats have demonstrated that this procedure results in complete anatomic transection of the cord (17, 18).

Postoperative care of the cordotomized rats required special cage bedding to prevent pressure sores. Initially, there was a flaccid paraplegia with the limbs dragging behind the rats as they crawled about in the cage. They were able to move, using their forelimbs, and had no difficulty reaching food and water. During the first weeks, the bladder was emptied every 8 hours by the method of Crede. At approximately 3 to 4 weeks, the bladder became auto-
matic. During the same period, the paralyzed hind limbs of the animals changed from flaccid to spastic. After spasticity developed, the limbs were almost always held in extension. No recovery of voluntary activity was observed in the hindquarters of these animals.

Functional Evaluation. Prior to killing, each animal was evaluated clinically. In some animals, reflex movements of the hindquarters occurred in brief flurries of clonus-like activity. No spontaneous or stimulus-induced voluntary movements of the hindquarters were observed in any animal. This is in agreement with studies indicating that upper neuron regeneration in transected rats is meager to nonexistent (17, 18).

After clinical examination, intramuscular EMGs were recorded from both experimental and control animals. For comparative purposes, a group with lower motor neuron paralysis due to sciatic nerve transection was also studied. The experimental animals showed evidence of decreased motor activity but no evidence of lower motor neuron abnormalities, i.e., fibrillations, fasciculations, or positive waves. Animals with sciatic nerve transections demonstrated all these abnormalities. Thus, the EMG readings from the cordotomized rats were qualitatively similar to those found in upper motor neuron-paralyzed patients.

Measurement of Contractile Properties. The contractile properties of the SOL and EDL muscles were tested in both groups of rats at age 58 weeks using the apparatus shown in Fig. 1. Rats were anesthetized by injection of an acepromazine/ketamine cocktail (1 and 50 mg/100 g body mass, i.p., respectively). In this apparatus, the distal femur and proximal tibia were immobilized using 1.2-mm Kirschner wires. The distal tendon of the EDL (or

![Fig. 1. Experimental apparatus used to measure contractile properties in rat muscles. The distal femur and proximal tibia were immobilized with Kirschner wires. Contractile force from the distal portion of the soleus (SOL) or extensor digitorum longus (EDL) muscles was measured by the force transducer and acquired by the LSI-11/23+ data acquisition system.](image-url)
SOL) was attached to a force transducer using a clamp that minimized stress concentration at the transducer. The compliance of this testing system was about 10 μm/g and the resonant frequency 275 Hz. The intact peroneal (or tibial) nerve was stimulated under computer control to elicit muscle contraction and the muscle force was acquired by the LSI-11/23+ minicomputer system (28).

After setting muscle length to that at which twitch tension is maximum (L₀) by generating a length–twitch tension curve (3, 10), the following contractile properties were measured at room temperature (about 22°C): (i) time to peak tension (in ms), (ii) fusion frequency (in Hz), (iii) maximum tetanic tension (P₀, in N), and (iv) twitch:tetanus ratio (Tw:Tet ratio).

Specific tension (force per unit area) was calculated by dividing the maximum tetanic tension by the maximum muscle cross-sectional area measured morphometrically from the histologic sections. Contractile data were analyzed from digitized tension records. Time-to-peak tension values were determined by the computer analysis algorithm with a time resolution of 0.33 ms (data acquisition rate of 3000 Hz). To decrease the effect of signal noise, successive trace variation, and analog-to-digital converter resolution, three twitch traces were acquired from each muscle and averaged to yield one value for time to peak tension. Practical time resolution using this protocol was about 4 ms for each muscle. Fusion frequency was determined by stimulating the muscle from 5 Hz to 60 Hz in 5-Hz increments. The trace that showed minimal tension fluctuation at the stimulation frequency was defined as the fused tetanus. Typically, this value was determined with an accuracy of about 5 Hz. Maximum tetanic tension was determined by computer. To minimize error, the maximum tension values from three separate traces were averaged to obtain a single value for maximum tension.

Statistical Analysis. Contractile properties of the SOL and EDL were analyzed separately using BMDP program P1V (14). For each parameter listed above, a one-way ANOVA was used to compare a given parameter (see above) across all four muscle groups (normal-SOL, transected-SOL, normal-EDL, and transected-EDL). After the preliminary one-way ANOVA, multiple t tests were carried out between all possible pairs of means. Thus, values presented for P include compensation for the number of tests conducted (39). Differences between groups were considered significant for P values < 0.05. After the ANOVA, discriminant analysis was carried out using BMDP program P7M with F-to-enter = 4.000 and F-to-remove = 3.996. This was to determine the contractile parameter(s) that best differentiate(s) between the four muscle groups.

RESULTS

The responses of the SOL from normal and transected rats stimulated at 5 and 10 Hz are shown in the upper and lower panels of Fig. 2, respectively.
FIG. 2. Contractile records from normal SOL (upper panel) and transected SOL (lower panel). Both muscles were stimulated at 5 and 10 Hz. Note that the transected SOL showed more complete relaxation and greater tension implying faster contraction–relaxation time. Vertical calibration bar = 0.61 N, horizontal calibration bar = 180 ms.

Note that at 10 Hz, the transected SOL developed a greater force and was less fused, implying faster contraction and/or relaxation. Unfused tetani of the EDL stimulated at 10, 20, and 30 Hz are shown in Fig. 3. Note that the differences between the normal EDL (upper panel) and transected EDL (lower panel) were much less dramatic than those observed for the SOL.

All contractile parameters measured are presented in Table 1, and selected parameters in Fig. 4. Note that no significant differences between normal and transected EDL were observed for any parameters measured. For the SOL muscle, however, dramatic changes were observed in all properties measured. Time-to-peak tension decreased by about 50% (Fig. 4A), indicating a change in the properties of the sarcoplasmic reticulum (23). In addition, fusion frequency increased 100% (Fig. 4B) indicating an increase in twitch contraction and/or relaxation speed. Absolute maximum tetanic tension (in N) did not change significantly after transection. However, as SOL cross-sectional area significantly decreased (Fig. 4C) by about 50%, specific tension (in N/cm²) of the SOL significantly increased by more than 100% 1 year after transection (Fig. 4D). In summary, following transection, the SOL muscle showed an increase in contractile speed and specific tension whereas no significant contractile changes were seen in the EDL.

A summary of the discriminant analysis of the contractile data is presented in Table 2. It is seen that fusion frequency and twitch-to-tetanus ratio were the best discriminators when distinguishing between normal EDL and normal SOL, normal SOL and transected SOL, and transected EDL and transected
Normal EDL

Transected EDL

**Fig. 3.** Contractile records from normal EDL (upper panel) and transected EDL (lower panel). Both muscles were stimulated at 10, 20, and 30 Hz. Qualitative differences between normal and transected muscles were not as dramatic as those observed with the SOL muscle. Vertical calibration bar = 0.49 N, horizontal calibration bar = 180 ms.

SOL. As expected from the preliminary ANOVA, no contractile variable discriminates between the normal and transected EDL. A plot of the canonical variables for the discriminant analysis conducted on all four groups (Fig. 5) indicated that the normal SOL was very different than any of the other groups based on the discriminating variables (fusion frequency, cross-sectional area, and Tw:Tet ratio). The transected SOL was also easy to discriminate from the EDL groups, based on the significant decrease in cross-sectional area (Table 1, Fig. 4C). However, the large overlap between normal and transected EDL groups precluded adequate statistical discrimination.

**DISCUSSION**

Spinal cord transection alters muscle use by altering motor neuron activity, resulting in decreased electromyographic activity (1) and decreased muscle load bearing (35). It is expected, therefore, that skeletal muscle properties would change following transection due to this altered muscular state. The contractile properties of the SOL and EDL tended to become more similar after transection. This is seen in the discriminant analysis plot (Fig. 5) in which the transected SOL moves closer to the EDL groups following transection. No significant differences in contractile properties were observed in
MUSCLE CONTRACTION AFTER CORDOTOMY

TABLE 1

Summary of Contractile Data from Four Groups of Rat Muscles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal EDL (SEM, N)</th>
<th>Normal SOL (SEM, N)</th>
<th>Xsected EDL (SEM, N)</th>
<th>Xsected SOL (SEM, N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-to-peak tension (ms)</td>
<td>35.2 (2.61, 9)</td>
<td>107 (13.4, 6)</td>
<td>40.7 (4.68, 6)</td>
<td>52.5 (2.21, 8)</td>
</tr>
<tr>
<td>Maximum force (N)</td>
<td>1.27 (0.051, 9)</td>
<td>1.15 (0.016, 6)</td>
<td>1.19 (0.0051, 7)</td>
<td>1.19 (0.025, 8)</td>
</tr>
<tr>
<td>Cross-sectional area (cm²)</td>
<td>0.0456 (0.0025, 6)</td>
<td>0.0641 (0.001, 4)</td>
<td>0.0447 (0.001, 6)</td>
<td>0.0312 (0.002, 8)</td>
</tr>
<tr>
<td>Twitch:tetanus ratio</td>
<td>0.596 (0.038, 9)</td>
<td>0.160 (0.028, 6)</td>
<td>0.719 (0.064, 6)</td>
<td>0.380 (0.039, 8)</td>
</tr>
<tr>
<td>Fusion frequency (Hz)</td>
<td>38.9 (1.11, 9)</td>
<td>18.3 (1.67, 6)</td>
<td>38.6 (1.43, 7)</td>
<td>38.8 (0.818, 8)</td>
</tr>
<tr>
<td>Specific tension (N/cm²)</td>
<td>26.3 (1.35, 6)</td>
<td>17.5 (0.314, 4)</td>
<td>26.8 (0.643, 6)</td>
<td>38.6 (2.52, 7)</td>
</tr>
</tbody>
</table>

* Mean values are connected by asterisks if the means differed significantly (P < 0.05) or by open circles if the means were not significantly different.

the EDL after transection. This can be appreciated graphically by the large overlap in normal EDL and transected EDL properties (Fig. 5). The SOL, however, demonstrated dramatic contractile changes. A large decrease in time to peak tension and increase in fusion frequency suggest a large degree of slow-to-fast fiber type transformation. This hypothesis is supported by the observation that, although the muscle cross-sectional area decreased more than 50%, no change in P₀ was observed. Thus, the large increase in specific tension (from 18 to 39 N/cm², Fig. 4D) probably reflects the larger specific tension of fast versus slow muscle fibers in rat (11).
Soleus contractile speed increases following spinal cord transection in the cat (35) and rat (13). The magnitude of the decrease in time to peak tension agrees with the values reported in this study if corrections are made for the different temperatures of the studies [22°C vs. 37°C using $Q_{10} = 2.2$, see (6)]. Additionally, significant increases in contractile speed have been reported (32) for the single motor units of the cat medial gastrocnemius, a mixed fiber type ankle plantar flexor. No such change in whole-muscle medial gastrocnemius has been reported (35). Although comparison across species and functional muscle groups is difficult, most studies agree that slow muscle contraction speed increases whereas mixed, predominantly fast muscle contraction speed remains unchanged after spinal cord transection.

It is interesting that fusion frequency was the best discriminator between the various muscles (except N-EDL vs. X-EDL). Thus, although time-to-peak tension also differed significantly between muscles ($F = 32(3, 18)$ in a one-way ANOVA), fusion frequency was the better discriminator ($F = 195(3,$
TABLE 2
Summary of Contractile Discriminant Analysis

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Best discriminators (in order)</th>
<th>Percentage correct decisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-EDL vs. N-SOL</td>
<td>Fusion frequency, twitch:tetanus ratio</td>
<td>100%</td>
</tr>
<tr>
<td>N-EDL vs. X-EDL</td>
<td>No variables entered</td>
<td>NA</td>
</tr>
<tr>
<td>N-SOL vs. X-SOL</td>
<td>Fusion frequency, twitch:tetanus ratio</td>
<td>100%</td>
</tr>
<tr>
<td>X-EDL vs. X-SOL</td>
<td>Fusion frequency, twitch:tetanus ratio</td>
<td>100%</td>
</tr>
<tr>
<td>N-EDL, N-SOL vs. X-EDL, X-SOL</td>
<td>Fusion frequency, cross-sectional area, twitch:tetanus ratio</td>
<td>50-100%</td>
</tr>
</tbody>
</table>

*F-to-enter = 4.000, F-to-remove = 3.996. The scatter plot of the data indicated most difficulty in discriminating between normal (N)-EDL and transected (X)-EDL. When comparing all four groups, incorrect classifications were: 2 N-EDL classified as X-EDL, 3 X-EDL classified as N-EDL. Correct classifications were 50% of N-EDL (3/6), 100% of N-SOL (4/4), 60% of X-EDL (3/5), and 100% of X-SOL (7/7). Note that the number of data classified may not be equal to the number listed in Table 1 as only complete cases were considered for discriminant analysis.

The analysis of covariance (ANCOVA) in the second step of the discriminant analysis eliminates time-to-peak tension due to its large covariance with fusion frequency. Similar enhanced discriminating ability of the fusion

---

**FIG. 5.** Graphic representation of the discriminant analysis presented in Table 2 for all four groups. The best discriminators between all four groups were (in order): fusion frequency, muscle cross-sectional area, and tetanus-to-twitch ratio. □, Normal SOL; ○, transected SOL; △, normal EDL; and ●, transected FDI. Note the normal SOL was very different than either of the other groups. Following transection, the SOL became more like the EDL groups which were statistically indistinguishable.
frequency parameter was shown by Roy et al. (35) in which a clear shift is seen in the tension-frequency plots with no significant difference in contraction time.

Relative Tw:Tet ratios of EDL and SOL muscle (Table 1) were reversed relative to those reported in the literature for skeletal muscle at 22°C. The reason for this discrepancy is not clear. Possibly, the relatively large compliance of our measuring system (10 μm/g) masked the small twitch forces of the SOL (25) resulting in unusually small values for the SOL Tw:Tet ratio. The physiologic significance of the Tw:Tet ratio is not completely understood (12). An increase in speed of contraction and relaxation would be expected to increase twitch tension relative to tetanic tension thus increasing the Tw:Tet ratio. Additionally, slow-to-fast fiber type transformation would be expected to increase both the tetanic and twitch tension due to the larger specific tension of fast vs. slow fibers. Tetanic tension would increase to a greater extent than the twitch due to the steeper length–tetanic-tension relationship vs. the length–twitch-tension relationship (3). The combined effect of the increase in speed and fiber type transformation would thus increase the Tw:Tet ratio which was observed for the SOL, and, to a lesser extent, the EDL (Table 1). Therefore, although the absolute Tw:Tet ratios do not agree with the literature values, the change in ratio following transection is reasonable.

The maintenance of force-generating capacity reported here for both the EDL and SOL is contrary to most studies. A 20 to 60% decrease in P0 after cordotomy was reported in the cat (32, 35). (It may be difficult to compare the cat studies with the rat data presented here as the specific tension of fast and slow muscle fibers from the cat differ from those in the rat and the durations of the studies are not comparable.) The fact that the SOL maintained absolute force-generating capacity accompanied by a 50% decrease in SOL cross-sectional area implies that the muscle fibers of the SOL significantly increased their specific tension after transection. These data are explained in conjunction with the morphometric data presented in the following report (29).

REFERENCES


