The Effect of Denervation and Subsequent Reinnervation on the Morphology of Rat Soleus Muscles

HARUTOSHI SAKAKIMA, PT, MS1), YOSHIHIRO YOSHIDA, MD1), NORIO MORIMOTO, MD1), KIYOHIRO SAKAE, MD1)
1) School of Health Sciences, Faculty of Medicine, Kagoshima University:
8–35–1 Sakuragaoka, Kagoshima 890-8506, Japan. TEL +81 99-275-6801, FAX +81 99-275-6804, E-mail sakaki@health.nop.kagoshima-u.ac.jp

Abstract. To investigate the effect of denervation and subsequent reinnervation on skeletal muscle, a histochemical study was performed on the soleus muscles of rats. Partial denervation was carried out by freezing the sciatic nerve locally, and the change in the nerve and the soleus muscles was examined for 5 weeks. The muscle fiber cross-sectional area of the denervated soleus muscles progressively declined to a minimum 2 weeks after the injury (type I fibers, 1209.1 ± 248.3 µm²; type II fibers, 802.4 ± 126.8 µm²) and began to reverse the decline at 3 weeks. The type II fiber ratios to total fiber of the denervated sides were consistently higher than the control levels, and muscle fibers stained in both acid preincubation and alkaline preincubation were observed. The proportion of type II fibers in the soleus muscles showed an increase and consequently a decrease with a short delay in response to denervation and consequent reinnervation. These data suggest that denervation elicits an alteration in fiber type composition and a reduction in fiber size. The increase of type II fibers seemed to occur in hybrid fibers containing both myosin heavy chains I and II at varying ratios in the same fibers. The reinnervation took the crucial role of recovering from atrophy and composing the integrity of the soleus muscles. However, the ability to generate muscle tension needs a much longer time to recover. This suggests a need to investigate interventions to facilitate the functional recovery of partially-denervated muscle.

Key words: Partial denervation, Muscle fiber type, Soleus muscle atrophy.

(This article was submitted Oct. 9, 2001, and was accepted Nov. 12, 2001)

INTRODUCTION

Skeletal muscle atrophy occurs due to prolonged bed rest, plaster cast immobilization, tenotomy and denervation. Among these conditions, denervation produces the most serious atrophy1, 2), and results in profound alterations in the morphology of skeletal muscle. These alterations include changes in fiber type composition and fiber size3).

Clinically, peripheral nerve injury often occurs due to musculoskeletal injuries and compression by cast immobilization. The peripheral nerve system is capable of rapid and extensive reconstruction after nerve injury, as nerve fibers proximal to the injury send out new sprouts that cross the lesion and eventually reestablish their original connections. One objective of rehabilitation treatment is to prevent the muscular atrophy associated with the degeneration of nerves. It is important for the physical therapist to know the change in the partially-denervated muscles, when physiotherapy is introduced.

There are many reports on denervation of skeletal muscle. Most investigations have focused on the effects of denervation, while relatively few studies have dealt with the regenerative processes. Bodine-
Fowler et al. observed the time course of muscle atrophy and recovery after injuring the sciatic nerve of a rat with phenol, but the denervation was incomplete. It is reported that long-term denervated muscles recover poorly, perhaps due to the deposition of interstitial collagen. However, not enough attention has been paid to the time course of alterations in muscles after reinnervation. Moreover, the change in muscle fiber ratio after denervation remains controversial. Therefore, the aim of this study was basic research on the partially-denervated muscle. In this study, a frozen sciatic nerve was induced in rats as an injury and the resultant degenerative and regenerative changes in the soleus muscles were observed.

MATERIALS AND METHODS

Animals and experimental protocols

Thirty-six 8-week-old female Wistar rats weighting 170–188 g were used in this study. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The skin covering the right buttock was cut and the right sciatic nerve was isolated. The nerve was frozen and thawed several times by contact with a stainless spatula 3 mm in diameter cooled by liquid nitrogen. Care was taken not to injure other tissues. The nerve became white when frozen, and the proximal margin of the frozen portion was loosely tied with a white thread for marking. The contralateral hindlimbs were untreated and served as a control. Six normal 8-week-old rats were also used as controls, in particular to survey the ratio of the type II to total fiber areas and numbers in the normal soleus muscles. Food and water were supplied ad libitum. The degree of paralysis in the hindlimbs of the treated rats was checked every day. The animals were euthanatized via the inhalation of an overdose of diethyl ether at 1, 2, 3, 4 or 5 weeks (6 rats in each group) after the nerve freezing. The soleus muscles in both legs and the accompanying sciatic nerves of the frozen sides were removed en bloc. The distance between the proximal margin of the lesion and the nerve entrance into the soleus muscle was measured. After the nerve freezing, the rats were ambulatory and dragged the foot of the frozen side. A loss of active movement on the ankle and toe joints was observed. The paralysis was alleviated and voluntary extension of digits was noticed approximately 3 weeks after the operation.

Histological and histochemical analysis

The soleus muscles were mounted vertically on a cork plate in tragacanth gum jelly of appropriate softness to obtain cross-sections. The mounted muscle was then frozen by immersion in isopentane solution cooled in liquid nitrogen. Transverse sections (10 µm) were cut in a cryostat cooled to −20°C, and stained with hematoxylin and eosin for general observation. Cryosections were also stained for the myosin adenosine triphosphatase (ATPase, pH 10.5, 4.3) reaction according to Guth and Samaha, and then the muscle fibers were classified into type I or II fibers. The whole cross section of each soleus muscle stained by ATPase was photographed at a magnification of 20 for fiber-type composition. The central region of the soleus muscles was photographed at a magnification of 50 and all the muscle fibers delineated by entire fiber boundaries were measured for the cross-sectional areas. A random sample of 100–110 fibers from each soleus muscle was analyzed. The proportion of the type II fiber area was expressed as a percent of the total fiber. The sciatic nerves were stained with 0.5% toluidine blue in 0.5% borate and observed by conventional light microscope. A Power Macintosh 8,500 computer was employed with NIH Image version 1.61 software (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/).

Data analysis

Statistical analysis were performed with a Macintosh computer using the StatView version 4.5 software (StatView). One-way analysis of variance (ANOVA) was used and when a significant F ratio was found, post-hoc Fisher’s Protected Least Significant Differences (PLSD) test was performed on each variable. Significance was set at P<0.05.

RESULTS

After the nerve freezing, the rats were ambulatory and dragged the foot of the frozen side. A loss of active movement on the ankle and toe joints was observed. The paralysis was alleviated and voluntary extension of digits was noticed approximately 3 weeks after the operation. The distance between the proximal margin of the
lesion and the entrance of the sciatic nerve into the soleus muscle was 20.1 ± 1.7 mm (mean ± SD).

Changes in the sciatic nerve

The distal portion of the nerve to the frozen lesion was uniformly damaged and swollen. One week after the injury, most myelin had clearly degenerated and the centers of myelin where axons had been located were densely stained. These findings were found up to 2 weeks after the injury (Fig. 1). A considerable number of macrophages, probably containing myelin debris, were observed. Central light areas indicated few axons. In contrast, 3 weeks after the injury, numerous thin formations of myelin emerged, surrounding the small light centers inside (Fig. 1).

Changes in the cross-sectional areas of each fiber type in the soleus muscles

The changes in each fiber cross-sectional area are shown in Table 1. The cross-sectional areas of muscle fiber had significantly decreased 2 weeks after the injury compared with before freezing, and began to increase at 3 weeks. Two weeks after the injury, the relative cross-sectional area in the denervated soleus muscles compared with the contralateral counterparts was 50.3 ± 9.2% in type I fibers and 39.0 ± 8.5% in type II fibers.

Changes in muscle fiber type ratio

The ratios of type II to total fiber numbers in the denervated soleus muscles were significantly higher than those in the normal rats and the contralateral uninjured side in each period (Table 2). Type II

<table>
<thead>
<tr>
<th>Groups (Number of muscles)</th>
<th>Before freezing</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
<th>5 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental side (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>1442.4 ± 123.6*†</td>
<td>1209.1 ± 248.3*†</td>
<td>1789.9 ± 348.7*</td>
<td>1953.4 ± 235.2*</td>
<td>2122.3 ± 348.7*</td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>1190.5 ± 245.5†</td>
<td>802.4 ± 126.8*†</td>
<td>1224.6 ± 400.3*†</td>
<td>1276.8 ± 287.1*</td>
<td>1327.4 ± 358.7*</td>
<td></td>
</tr>
<tr>
<td>Contralateral side (6)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Type I</td>
<td>2361.8 ± 516.1</td>
<td>2453.6 ± 441.2</td>
<td>2782.3 ± 434.8</td>
<td>2873.0 ± 406.2†</td>
<td>2943.3 ± 383.7†</td>
<td>3102.0 ± 368.8†</td>
</tr>
<tr>
<td>Type II</td>
<td>1711.2 ± 393.6</td>
<td>1609.7 ± 371.9</td>
<td>1814.1 ± 412.9</td>
<td>2031.8 ± 238.6</td>
<td>2551.6 ± 502.9†</td>
<td>2655.7 ± 478.7†</td>
</tr>
</tbody>
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Values are mean ± SD (µm²). *P<0.05 (compared with that of contralateral side), †P<0.05 (compared with that before freezing).
fibers increased and reached a maximum at 3 weeks after denervation and gradually decreased thereafter, but were still higher at 5 weeks (Fig. 2).

**DISCUSSION**

Freezing was chosen as the method of denervation since this method uniformly and definitively damages nerve fibers with reinnervation more likely than in other procedures such as nerve crushing. The fiber cross-sectional areas were regenerated 2 to 3 weeks after freezing. It was roughly estimated that it took approximately 3 weeks for the sciatic nerve to contact the soleus muscles over approximately 20 mm, which was compatible with data previously reported.

The cross-sectional area of type I and II fibers showed a progressive decrease with time after denervation. The denervation produced serious atrophy compared to immobilization due to muscle disuse caused by paralysis and tenotomy in the deprivation of trophic substances. Type II muscle fibers are generally reported to undergo preferential atrophy after total denervation and selective motor nerve cutting. In contrast, Bodine-Fowler et al. stated that the slow fibers (type I) of rat soleus muscles showed more atrophy than the fast fibers (type II). Tomanek and Lund and Herbison et al. found that type I fiber atrophy was equal to type II fiber atrophy. The morphological responses of the muscle to the denervation were fiber type specific, although both type I and II fibers of the denervated soleus muscles elicited marked atrophy. The atrophy was

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<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
<th>5 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental side (6)</td>
<td>11.7 ± 6.8</td>
<td>14.6 ± 3.9</td>
<td>11.7 ± 3.2</td>
<td>14.9 ± 3.2</td>
<td>10.6 ± 5.3</td>
<td>10.7 ± 3.3</td>
</tr>
<tr>
<td>Contralateral side (6)</td>
<td>26.7 ± 8.4*†</td>
<td>36.4 ± 8.8*†</td>
<td>46.2 ± 8.7*†</td>
<td>33.4 ± 4.3*†</td>
<td>19.7 ± 4.5</td>
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</tr>
</tbody>
</table>

Values are mean ± SD. *P<0.05 (compared with Contralateral side), †P<0.05 (compared with before freezing).
significantly greater in type II fibers. Following reinnervation, muscle cross-sectional areas steadily increased. The reinnervation took the crucial role of recovering from the atrophy and composing the integrity of the soleus muscles. However, the ability to generate muscle tension needs a much longer time to recover17).

More than 75% of the normal soleus muscles of rats is composed of type I fibers18, 19). This value increases with age and changes under varying procedures20, 21). A decrease in muscle activity generally facilitates the transformation of fiber type, from the slow to fast muscle fiber composition20). An increase in the number of type II fibers is observed not only in denervation but also in cast immobilization, unloading, and spinal cord injury22, 23). After denervation, however, the change in fiber type ratio is extremely controversial. It has been reported that the number of type II fibers in the soleus muscles after denervation decreased in mice7), hardly changed in rats9 and rabbits10, but increased in rats11 and guinea pigs16). In this study, the type II fiber ratio markedly increased and began to reverse after reinnervation after some delay (1 week or more). This finding agrees with that of Karpati et al.6), Tomanek and Lund16) and Chien and Chu24), although the extent of increase considerably differs. The discrepancy in the extent of increase is perhaps, at least in part, due to the increase of hybrid fibers containing both myosin heavy chains I and II at varying ratios within the same fiber24). Such hybrid fibers are sometimes difficult to clearly classify by the ATPase reaction alone into type I or type II fibers. The delay in fiber type change may be attributed to the time needed to produce enough new myosin to predominate in the myosin pool within each muscle fiber. The mechanism of the change of muscle fiber type remains to be elucidated and the following factors should be taken into consideration: species of the animal, the muscle examined, site of the nerve injury, duration after denervation, and whether or not the muscle is reinnervated.

CONCLUSION

Reinnervation took a crucial role in recovery from atrophy and probably was also involved in reestablishing the functional integrity of the skeletal muscle. The denervation resulted in a reduction of the fiber size, and changes in the fiber type composition. The changes evaluated in this study are to a great extent reversible after short-term denervation, although some alterations such as fiber size, fiber type ratio and contractile function may need more time to approach normal values. This suggests a need to investigate interventions to facilitate the functional recovery of partially-denervated muscle.

ACKNOWLEDGMENTS

We would like to thank Dr. S. Kawamata for his help in these investigations.

REFERENCES


