Time course of recovery from nerve injury in skeletal muscle: energy state and local circulation

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PERIPHERAL NERVE INJURY affects skeletal muscle volume, contractile ability, and energy metabolism (6, 7, 11, 24) in association with the impairment of mitochondrial coupling (11), loss of oxidative delivery (4), and alteration of glycolytic enzyme activity (9). Phosphorus-31 magnetic resonance spectroscopy (31P-MRS) can demonstrate these changes in energy metabolism and intracellular pH that occur in damaged skeletal muscle. Many metabolic changes during and after nerve crush have been reported, such as an increase in the Pi/Pi + phosphocreatine (PCr) ratio (12) and a rise in the intracellular pH (6, 25), which were more pronounced with severe nerve injury than with mild nerve injury (25). With neural recovery, these parameters returned to normal (12). These findings were observed with resting muscles. During the period of nerve regeneration, it is more important to assess these parameters with contraction. Furthermore, it is more important to observe both energy metabolism and local circulation dynamics of the contracting muscles at the same time because there is a correlation between energy metabolism and oxygen supply fueled by local circulation dynamics in skeletal muscles during exercise (10). There have been few reports documenting the pattern of both metabolic changes and local circulation changes in skeletal muscle with contraction during the recovery process from nerve injury (8). Peripheral nerve injury also induces a loss of mitochondrial function. The recovery of both mitochondrial function and local circulation dynamics are essential to recovery of the muscle metabolism. We hypothesized that the changes in the local circulation dynamics may be faster than those of the energy metabolism, with contraction during the neural recovery process.

To investigate this hypothesis, this study was designed to examine the time course and the pattern of metabolic changes with 31P-MRS and those of local circulation with fluorine-19 magnetic resonance spectroscopy (19F-MRS) in contracting rat hindlimb muscle after sciatic nerve compression and subsequent nerve regeneration. 31P-MRS and 19F-MRS enable the observation of both metabolic and local circulation changes at the same time in vivo. In a previous study, we showed the time course of the metabolic changes in rat skeletal muscle before, during, and after stimulation by using 31P-MRS (22). 19F-MRS provides the opportunity to noninvasively and repeatedly assess blood volume with perfluorotributylamine (1), which is known to remain in the vascular space for several hours but does not induce any known physiological disturbance (16). In addition, it could be easily correlated with information on muscle structure, as given by 31P-MRS. As estimation of neural function, the sciatic functional index (SFI) was used (17). Because it is noninvasive and repeatable, SFI enables us to assess the neural function in addition to other observations in the same animal. In this present study, we showed the differences between metabolic and local circulation changes of skeletal muscle by observation of the muscle function and muscle weight during the neural recovery process.

MATERIALS AND METHODS

Animal model. Figure 1 outlines the details of the animals studied. A total of 50 male Wistar rats weighing 250–280 g were used. The animals were housed in a room at controlled temperature (25°C) with a 12:12-h light-dark cycle and allowed free access to food and water until used in the experiments. Nidifferencesoftheamountsoffoodandwater were observed between groups were observed. Thirty-seven animals under-went compression of the right sciatic nerve for 2 wk and were evaluated for physiological changes, including neural function, tetanic tension, Pi/(Pi + PCr) ratio, and intracellular pH as well as local circulation of the hindlimb muscles. Thirteen of these animals were evaluated at 2 wk postcompression (CN group), 11 after recovery for 4 wk postcompression (R4 group), and the remaining 13 after recovery for 6 wk postcompression (R6 group). Thirteen were evaluated at 2 wk after a sham operation as a control group (SO group) for the CN group. All measurements except tetanic tension were performed on all the rats in each group.

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Surgical procedure. The right sciatic nerve was exposed through gluteal muscle splitting incision under pentobarbital sodium anesthesia (50 mg/kg body weight) and was compressed by a 1-cm-long silicone tube (0.8-mm ID) around the sciatic nerve at the nerve trunk (15). Three 7-0 nylon sutures were then used to reconstitute the tubing. No operation was done on the left side. In the R4 and R6 groups, the tubes around the sciatic nerve were detached by using a similar surgical approach after 2 wk of compression and the animals were allowed a period of 4 and 6 wk for recovery, respectively. In the SO group, no nerve lesion was performed after the nerve was exposed.

Functional assessment. The neural functional assessment was determined from the prints of the hind feet of walking rats by using the SFI described by Medinaceli et al. (17). The SFI is assessed from four factors: distance to opposite foot, print length, total spreading, and distance between intermediary toes. These variables were measured on each side and were entered into the formula shown in Fig. 2. With use of the SFI, a value of zero represents normal function and −100 represents the complete loss of function of the sciatic nerve. Bromophenol blue paper and water were used for the footprint. It is a noninvasive, accurate, repeatable, and simple method. Each rat in all the groups was measured at 3, 7, and 10 days, 2 wk, and then every week until the number of weeks shown in Fig. 1 after the first surgical procedure.

Muscle stimulation procedure. After the appropriate period, the rats were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt) and placed in the prone position. Two oval electrodes (4-mm diameter) were then fixed on the skin of their right lower leg. The lower leg muscles were stimulated electrically through the electrode (model SEN-3301, Nihon Kohden, Tokyo, Japan) with 0.5-ms square-wave pulses of 40 V for 20 min. Stimulation frequency was 40 Hz, which induces a tetanic contraction, for a duration of 1 s every 2 s. The frequency of 40 Hz continuously produced the minimal tetanic tension and was most suitable to the magnetic resonance spectroscopy. This stimulation procedure was applied to the measurement of tetanic tension, 31P-MRS, and 19F-MRS because with frequencies >40 Hz (67 and 100 Hz) the changes in among the four groups could not be shown because of significant decrease in signals of high-energy phosphates in the preparatory experiments.

Measurement of tetanic tension. The tetanic tensions were measured with the animals’ rectal temperatures at 36 ± 1°C in a room with controlled temperature (25°C). The temperature of the animals was not controlled, but it was warm in the magnet bore because of its power supply. The tendons of the gastrocnemius, plantaris, and soleus muscle groups were exposed at the ankle and then were cut and attached to a strain gauge (model TB611, Nihon Kohden) with a noncompliant thread. The strain gauge was connected to a polygraph system (model RM-600, Nihon Kohden). Tetanic tension development was recorded with a pen recorder. In this study, we showed not the absolute values but the relative values in the tetanic tension among four groups because these tension recordings were not completely isolated and could not be considered absolutely accurate.

31P-MRS and 19F-MRS measurements. The right leg of the rat was inserted into a 25-mm-diameter solenoid coil (double tuned) to record 31P- and 19F-MRS spectra. Spectra were recorded with a BEM250/80 nuclear magnetic resonance measurement instrument (Otsuka Electronics) equipped with a horizontal 250-mm-diameter-bore magnet (1.9-T) magnetic resonance spectrometer. 31P- and 19F-MRS spectra were obtained at 32.3 and 75.1 MHz with a pulse width of 35 μs (90° pulse) and 45 μs (90° pulse), respectively. Each spectrum was obtained by 60 scans with a pulse-repetition time of 2 s. To acquire both 31P and 19F signals in the same rat, a time-shared 31P and 19F spectroscopy was performed. Thus, while excited 31P nuclei relax toward equilibrium, 19F signals can be acquired; and vice versa, during the wait for the excited 19F nuclei to relax, 31P signals can be observed.

In the 31P-MRS study, the tissue levels of PCr and P_i were estimated from the areas under individual signals. The energy states were evaluated by the ratio formula of P_i/(P_i + PCr) (2, 3). The intracellular pH was calculated from the chemical shift (d) of the P_i signal from that of PCr by using the following equation (5) (Fig. 3A).

\[
\text{intracellular } \text{pH} = 6.90 - \log [(d - 5.805)/(3.290 - d)]
\]

For the 19F-MRS study, perfluorotributylamine (FC-43; Green Cross) was injected via the tail vein (3 ml/kg body wt) as an intravascular tracer. FC-43 has four 19F signals that have been assigned (CF3, CF2, CF2, and CF2; Fig. 3B), of which the CF3 signal has been found to be more sensitive than the three CF2 signals. The area under the CF3 signal at rest was standardized to that of an external reference (ref; 5-fluorouracil), and the CF3/ref ratio was considered the blood volume (1, 19). The local circulation dynamics during and after stimulation were expressed as relative to that before the stimulation.
The compression of the sciatic nerve resulted in significant loss of muscle weight (P < 0.01). The weight of hindlimb muscles in both the R4 and R6 groups showed some recovery.

Tetanic tension. The maximum tensions as relative values to the SO values at 40 Hz of the three groups were as follows: CN, 57.6; R4, 82.0; and R6, 107.0% (SO, 100%). The compression of the sciatic nerve resulted in significantly lower tension (P < 0.01). The values of both the R4 and R6 groups were higher than that of the CN group (P < 0.05 and P < 0.01, respectively).

Muscle energy state and intracellular pH. The Pi/(Pi + PCr) ratio and intracellular pH of the resting muscles in each group are shown in Table 1. For all groups, there were no significant changes in both values. The time course of changes in the Pi/(Pi + PCr) ratio is shown in Fig. 5. The Pi/(Pi + PCr) ratios of the SO group and CN group showed the maxima of 0.68 ± 0.04 and 0.85 ± 0.03, respectively, 4 min after the onset of the electrical stimulation. For both groups, the Pi/(Pi + PCr) ratio decreased to about 100 within 3 days after compression of the sciatic nerve in the CN, R4, and R6 groups. The SO group did not change in the 2 wk after the sham operation.

Wet muscle weight. The hindlimb wet muscle weights of the SO, CN, R4, and R6 groups are shown in Table 1. The compression of the sciatic nerve resulted in significant loss of muscle weight (P < 0.01). The weight of hindlimb muscles in both the R4 and R6 groups showed some recovery.

Table 1. Body weights, muscle weights, SFI, Pi/(Pi + PCr) ratio, intracellular pH, and CF3/ref ratio

<table>
<thead>
<tr>
<th></th>
<th>SO (n = 7)</th>
<th>CN (n = 7)</th>
<th>R4 (n = 5)</th>
<th>R6 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>320 ± 28</td>
<td>288 ± 13</td>
<td>307 ± 7.0</td>
<td>309 ± 21</td>
</tr>
<tr>
<td>MW, g</td>
<td>1.78 ± 0.12</td>
<td>1.83 ± 0.08*</td>
<td>1.05 ± 0.11*</td>
<td>1.09 ± 0.20*</td>
</tr>
<tr>
<td>MW/BW, ×10³</td>
<td>5.57 ± 0.17</td>
<td>2.88 ± 0.33*</td>
<td>3.43 ± 0.37</td>
<td>3.31 ± 0.57*</td>
</tr>
<tr>
<td>SFI</td>
<td>0 ± 3</td>
<td>-107 ± 17*</td>
<td>-41 ± 26*</td>
<td>-8 ± 19*</td>
</tr>
<tr>
<td>Pi/(Pi + PCr)</td>
<td>0.10 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.022</td>
</tr>
<tr>
<td>Intracellular</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>CF3/ref ratio</td>
<td>0.7 ± 0.5</td>
<td>2.0 ± 0.7*</td>
<td>0.5 ± 0.2*</td>
<td>0.8 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. All data in each group were obtained from the same rats. Data of Pi/(Pi + phosphocreatine (PCr)) ratio, intracellular pH, and 19F signal of perfluorotributylamine (CF3)/external reference (ref; 5-fluorouracil) ratio were obtained from resting muscles. BW, body weight; MW, muscle weight; SFI, sciatic functional index; SO, sham operation; CN, compression nerve injury; R4, recovery for 4 wk; R6, recovery for 6 wk. Values of CN group were compared with those of SO group, and values of R4 and R6 groups were compared with those of CN group. *P < 0.01.
Pi/(Pi + PCr) ratio subsequently decreased with time and was significantly higher in the CN than SO group during stimulation (P < 0.01). During the poststimulation stage, the Pi/(Pi + PCr) ratio of both groups gradually returned to the initial value, and the Pi/(Pi + PCr) ratio of the CN group was higher than that of the SO group (P < 0.01). The effect of neural recovery was not observed in the Pi/(Pi + PCr) ratio of the R4 group but was clearly seen in the R6 group.

The changes over time in the intracellular pH are illustrated in Fig. 6. After the onset of the stimulation, the intracellular pH values of the SO group and CN group decreased to the minima of 6.6 ± 0.1 and 6.5 ± 0.2, respectively, within 4 min. The intracellular pH gradually recovered in both groups, and there was no significant difference between the SO and CN groups during stimulation. After the end of stimulation, the intracellular pH of the CN group was less than that of the SO group (P < 0.01). The time course of changes in the intracellular pH of the R4 group was similar to that of the SO group and was not statistically significant. The time course of intracellular pH in the R6 group was similar to that of the SO group, and there was a significant difference between the intracellular pH of the R6 group and the CN group after the end of the stimulation. These data were similar to those of the changes in the Pi/(Pi + PCr) ratio.

Muscle circulation dynamics. The CF3/ref ratios of the resting muscles in each group are shown in Table 1. The compression of the sciatic nerve resulted in significant increases of the CF3/ref ratio, and the effect of the neural recovery on both the R4 and R6 groups was observed in the CF3/ref ratio. The time course of the changes in the CF3/ref ratio, which was used as an index of muscle blood volume, is shown in Fig. 7. After the onset of the stimulation, the CF3/ref ratio of the CN group and SO group increased to the maxima of 120 ± 10 and 180 ± 20%, respectively. During stimulation, the CF3/ref ratio was significantly lower in the CN group than in the SO group (P < 0.01). After the end of stimulation, the CF3/ref ratio decreased to the prestimulation value of the CN group, whereas in the SO group, the value decreased but did not reach the prestimulation value (P < 0.01). In the R4 and R6 groups, the values during and poststimulation were found to improve according to the duration of the recovery period (P < 0.01).

DISCUSSION

The major findings of this experiment were that the local circulation dynamics of denervated skeletal muscle as evaluated by 19F-MRS recover with neural recovery and that the local circulation dynamics started to return to normal faster than did the energy state (Fig. 8). These facts suggest that the recovery of the...
circulation of denervated muscle may facilitate that of the energy metabolism with time.

Peripheral nerve injury induces extensive changes in biochemical and physiological characteristics of skeletal muscle, including a loss of muscle weight, alterations in metabolic levels, and a decreased tetanic tension, which results from interruption of the nerve-muscle interaction (7, 11). As the injured nerve regenerates, these changes may reverse themselves. Previous 31P-MRS studies have shown that the energy state of denervated skeletal muscles returned to normal with neural recovery (12). It is debatable that similar patterns of changes occurred between energy state and neural function of denervated skeletal muscle during recovery.

The model of nerve compression in the present study is suitable to observe the characteristics of recovery of these properties, including local circulation. The 2 wk of compression of this model allowed us to observe the significant changes in neural function through the neural recovery, which was not possible with other amounts of compression time, such as 1, 4, and 6 wk (data not shown). Our preliminary study showed that a nerve compression by banding with a silicone tube for 2 wk induced histological changes, such as few nerve fibers, ruptured axons, and thinned myelinated fibers, indicative a mild nerve injury model compared with a nerve crush. Nerve-crush injury caused extensive damage to nerves histologically and electrophysiologically compared with our model (8). In addition, the present model has the advantage of allowing the assessment of neurological recovery after decompression of the nerve by removal of the silicone tube.

In resting muscle, there are no effects of nerve injury on energy state and intracellular pH of denervated skeletal muscles. In contrast, previous studies have shown an increase in the Pi/PCr ratio and a rise in intracellular pH of skeletal muscle during rest after a nerve-crush injury (6, 12). These findings cannot be compared with the present results because of the differences in types of nerve injury. On the other hand, it was shown that the muscle blood volume was increased threefold by 2 wk of nerve compression and that the return of resting blood volume to the control values occurred after 4–6 wk of neural recovery (Table 1). Previous studies have suggested that an increase of the blood volume of denervated muscles could be attributable to reduced sympathetic vasoconstrictive tone starting at about the 2nd day after nerve injury and muscle fibrillatory activity on the 7th day after nerve injury (8, 18, 20).

With tetanic contraction induced by 40-Hz stimulation, the muscle energy state was significantly lower in the CN group than in the SO group during contraction (Fig. 5A). After the end of contraction, intracellular pH in the CN group returned to a resting value later than in the SO group (Fig. 6A). With regard to the local circulation dynamics, the increase of muscle blood volume in the CN group remained lower than in the SO group during and after contraction (Fig. 7A). The amount of blood volume in the capillary bed increases during contraction, which results from markedly reduced resistance in the vascular bed. The resistance of the vascular bed may be reduced by myogenic, neurohumoral, and local metabolic controls (13, 21, 23). In this study, the reduced function of these controls might be caused by both reduced activity and contractile ability of muscle initiated by nerve injury. Furthermore, the reduced function of local circulation dynamics and inactivity might cause a reduced supply of oxygen and a decrease in ATP production from oxidative phosphorylation during exercise (2, 3, 10, 22). Furthermore, insuffi-
cient washing out of lactate after the end of muscle contraction may cause the low intracellular pH found in denervated skeletal muscle (14).

Parallelsism was not found between the energy state and the blood volume in recovery groups (Figs. 5B, 6B, and 7B). The energy state and the blood volume returned to the SO values after 6 wk recovery. On the other hand, the energy state showed no recovery after 4 wk recovery; however, the blood volume showed significant changes from CN values but not from the SO values (Fig. 8). Thus the recovery of muscle circulation preceded that of muscle energy state during neural recovery process. This process may be caused in the reverse order of that of nerve injury. The functional recovery of the local circulation dynamics occurred with neural recovery, and it might cause an increased supply of oxygen and an increase in ATP production from oxidative phosphorylation due to recovery of mitochondrial function. This process might cause the gap of recovery with time between the energy state and local circulation dynamics.

In conclusion, local circulation dynamics is suggested to play an important role in energy metabolism of denervated skeletal muscles. The local circulation dynamics evaluated by 19F-MRS may be a predictor of the recovery of energy metabolism and neural function of denervated skeletal muscles.

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