The effect of glutamine on locomotor performance and skeletal muscle myosins following spinal cord injury in rats

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Golding, Jamie D., Sarah T. Rigley MacDonald, Bernhard H. J. Juurlink, and Benjamin W. C. Rosser. The effect of glutamine on locomotor performance and skeletal muscle myosins following spinal cord injury in rats. J Appl Physiol 101: 1045–1052, 2006. First published June 15, 2006; doi:10.1152/japplphysiol.00428.2006.—Following initial spinal cord injury (SCI), a cascade of pathological events, including oxidative stress, leads to secondary injury. Glutathione (GSH) plays a critical role in oxidant scavenging. Maintenance of GSH concentrations after SCI lessens secondary injury and improves recovery. Since glutamine promotes GSH synthesis, this nonessential amino acid was examined for therapeutic potential. Denervation alters the expression of myosin heavy chain (MHC) isoforms within skeletal muscles. The hypotheses of this study were that glutamine administration to SCI rats would lead to improved functional recovery and more preserved MHC phenotypes in representative locomotor muscles. Male Wistar rats were divided into four groups: healthy, sham with laminectomy, laminectomized SCI untreated, and laminectomized SCI treated with glutamine. Functional performance was measured weekly for 6 wk using Basso-Beattie-Bresnahan scale and angle board methods. MHC composition of rat soleus and extensor digitorum longus muscles was determined using SDS-PAGE. Glutamine-treated rats had significantly higher angle board scores (P < 0.001) and Basso-Beattie-Bresnahan scores (P < 0.01) than untreated SCI rats. Soleus of healthy rats contained 94% type 1 myosin isoform. Treated rats maintained 68%, which was significantly (P < 0.001) greater than 28% in untreated rats. The extensor digitorum longus of healthy rats contained 55% type 2b myosin. There was a significant (P < 0.001) decrease in this isoform following SCI, but no significant difference between treated and untreated groups. There were strong correlations between higher functional scores and more preserved MHC phenotypes. Our findings suggest glutamine improves functional recovery and helps preserve myosin phenotype by reducing secondary SCI, thereby maintaining more nerves.

Glutathione

Glutathione plays a critical role in oxidant scavenging. Maintenance of GSH concentrations after SCI lessens secondary injury and improves recovery. Glutamine promotes GSH synthesis, which is an ATP-dependent process, and therefore ATP production by formation of glutathione (30). The synthesis of glutathione may also be acted upon by glutamate dehydrogenase to form α-ketoglutarate, thereby promoting ATP synthesis (30). The synthesis of GSH is an ATP-dependent process, and therefore ATP production by formation of α-ketoglutarate may play a role in increasing GSH (9) and, in general, promote recovery from injury.

Our laboratory has shown that administration of glutamine following SCI results in 75% of basal spinal cord GSH concentration being maintained compared with the 50% level found in SCI rats that did not receive glutamine (46, 47). Increased GSH production after SCI in rats decreases secondary injuries and increases the likelihood of regaining locomotor function in rats (22). Preserved GSH levels were also shown to reduce the products of lipid peroxidation in the spinal cords of SCI rats (28).

Skeletal muscle phenotype is strongly influenced by neuromuscular activity, and studies have shown that change in neural input affects the myosin heavy chain (MHC) composition of a muscle (16, 50, 54, 61). Mammalian skeletal muscles contain distinct muscle fiber types that are described broadly as either slow or fast twitch, depending on the type of MHC isoforms they express (7). Neuronal disruption following SCI causes a shift in the expression of MHC isoforms in rat, cat, and human fast and slow muscles (17, 34, 61).

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This study tests the hypotheses that glutamine administration to SCI rats will lead to an improved functional recovery and a more preserved MHC phenotype in representative locomotor muscles. Testing of locomotor performance was carried out weekly, and after 6 wk, MHC composition of the soleus and extensor digitorum longus (EDL) was determined using SDS-PAGE. The results indicated that glutamine is a beneficial therapeutic intervention following SCI.

**MATERIALS AND METHODS**

**Animals and surgical procedures.** Thirty male Wistar rats (Charles River Laboratories, Laval, Quebec), weighing 238–351 g, were obtained at 11 wk of age for this study. For 1 wk before surgery, the animals were acclimatized to the research facility, researchers, and the functional performance tests used in this study. Rats received standard rat chow and water ad libitum and were kept in a 12:12-h light-dark cycle and 25°C. All protocols followed were approved by the University of Saskatchewan Animal Care Council and performed following the Guidelines of the Canadian Council on Animal Care.

Rats were randomly assigned to one of four groups. One group (n = 8) contained healthy animals that did not undergo treatment of any kind. The second group (n = 8) received sham operations of laminectomies only. The third group (n = 7) was given laminectomies and SCIs followed by intraperitoneal saline injections. The fourth group (n = 7) received laminectomies and SCIs followed by intraperitoneal glutamine injections at a dose of 1 mmol/kg body wt. This has been shown to be the optimal dose to increase GSH concentrations in rat spinal cord tissue (47). The first dose of glutamine was administered 1 h after surgery. This was repeated every 12 h, for 1 wk postsurgery, to maintain blood GSH at basal concentrations, as determined by a time response curve (47).

In preparation for surgery, each rat was anesthetized with 5% halothane (MTC Pharmaceuticals, Cambridge, Ontario) mixed with pure oxygen and later maintained with 2.5% halothane. The thoracic region of the dorsal surface was shaved and then sterilized with Hibitan (Chlorhexidine, Ayerst Laboratories, Montreal, Quebec) and 70% ethanol. A 0.05 mg/kg dose of the analgesic Buprenorphine (Reckett Benckiser Pharmaceuticals, Richmond, VA) was injected subcutaneously with five subsequent injections in decreasing doses in 12-h intervals.

A complete laminectomy was performed on T6, and standardized SCIs were performed with a modified aneurism clip (Kerr-Lougheed clip, Walsh Manufacturing, Oakville, Ontario), according to the methods of Schultke et al. (56), modified by changing the severity of the clip from 50 to 30 g. The injury was performed at the level of the sixth thoracic vertebrae to induce complete paraplegia without involving any injury to the forelimbs. The clip exerted a 30-g force for 5 s before it was removed (49). As animals that underwent SCI lost the ability to micturate, bladder expressions were performed manually until function returned or until the animal was killed. Following surgery, rats were weighed, and functional testing was done at weekly intervals. At 6 wk postsurgery, each animal was anesthetized with 5% halothane and killed by intracardiac perfusion with a cold, nonheparinized saline drip attached to an intravenous needle. Each rat was exsanguinated, and then both hind legs were removed.

**Testing of functional performance.** Rats were assessed using the angle board, also termed the inclined plane, method (48). This task assesses motor function by determining the maximum angle that a rat can maintain without falling off an angled board. The test was begun with the board at an angle of 25° to the horizontal and was then increased at 5° increments until the rat could no longer maintain its position or until a maximum angle of 55° was reached. Rats were also assessed using the Basso-Beattie-Bresnahan (BBB) open field locomotor rating scale (6), in which a 21-point scale is used to rate a rat’s ability to walk. Each animal received a score that reflected its ability to perform certain movements, including those of the three hindlimb joints, sweeping motions with the hindlimb, weight support, paw placement, forelimb-hindlimb coordination, toe clearance, and tail position. On the BBB scale, a score of 0 equals total paralysis or no hindlimb movement, and a score of 21 signifies normal locomotion. As BBB scores increase, rats display an increased range of movement at the hip, knee, and ankle joints until they are able to take occasional steps, which results in a score of 10. See Basso et al. (6) for a full description of the scale.

**Myosin purification.** EDL and soleus muscles were removed from each limb. The EDL and soleus were chosen as they are, respectively, commonly studied representive fast-twitch and slow-twitch muscles (15, 19). They are also probably the most commonly studied mammalian muscles. Each muscle was weighed and then frozen in isopentane cooled by liquid nitrogen (57). The average weights for left and right EDL and left and right soleus muscles from each animal were determined and used in all subsequent calculations. The diaphragm muscle of several animals from the healthy group was also excised and frozen for use as a control muscle in SDS-PAGE gels. All parts of the diaphragm were utilized for this purpose. All tissue samples were stored at −80°C.

Rat skeletal muscle MHC isoforms were separated using a protocol from Talmadge and Roy (62) with modifications (personal communication, D. Syme, University of Calgary). Briefly, all homogenates, suspensions, and buffers were kept on ice. Tissue was ground using a mortar and pestle in dry ice. Tissue was then separated into two or more centrifuge tubes, and 0.8 ml of buffer 1 (250 mM sucrose, 100 mM KCl, 5 mM EDTA, pH 7.2) was added to each tube. The tissue was homogenized by hand using a pestle in a 1.5 ml G-tube (Bio Plas, San Rafael, CA) for 1 min additional. The sample was then spun at 3,340 g for 10 min at 4°C, and the supernatant discarded. The sample was resuspended in buffer 2 (150 mM KCl, 10 mM Tris, 0.5% Triton-X 100, pH 6.8), homogenized as in the preceding for 1 min, and then centrifuged at 835 g, 4°C, for 3 min. The sample was then resuspended in buffer 2, homogenized for 1 min, and then centrifuged at 835 g, 4°C, for 3 min a second time. The sample was then washed in 0.8 ml of buffer 3 (150 mM KCl, 10 mM Tris, pH 7.0), homogenized for 1 min, vortexed for 20 s, and then centrifuged at 835 g, 4°C, for 3 min. The sample was subsequently washed in buffer 3, homogenized for 1 min, and centrifuged at 835 g, 4°C, for 3 min three additional times, discarding the supernatant each time after the sample was centrifuged. Enough buffer 3 was then added until the pellet of purified myofibrils was just submerged. The sample was homogenized by hand for 1 min in a centrifuge tube, vortexed for 30 s, and then stored at −80°C until electrophoresed.

**Preparation of muscle samples for electrophoresis.** Muscle samples were diluted 1:1 with glycerol and then mixed with an equal volume of protein stock buffer (28% glycerol, 2.8% SDS, 1 M β-mercaptoethanol, 4× stacking buffer, 0.001% bromophenol blue). The 4× stacking buffer had a pH value of 6.8 and contained 6.1 g/100 ml Tris base, 10% SDS, and deionized water. Samples were contained in 1.5-ml centrifuge tubes and placed in boiling water for 5 min. Samples were then centrifuged at 9,280 g at room temperature for 45 s, and the supernatant was drawn off and the pellet discarded.

**Gel electrophoresis.** Gels were prepared according to the methods of Talmadge and Roy (62). The only modification made was to use a 5% instead of a 4% stacking gel. The Mini-PROTEAN three-cell gel electrophoresis apparatus (Bio-Rad, Mississauga, Ontario) was used to produce optimum visualization, and predetermined amounts of prepared myofibril samples were loaded into gel lanes with a Hamilton syringe (Hamilton, Reno, NV). A broad-range SDS-PAGE molecular weight standard (Bio-Rad) with a marker for myosin at 200,000 was also loaded into the gel. The lower and upper chambers of the apparatus were filled, respectively, with lower and upper running buffer (56). The entire apparatus was then placed into a 4°C walk-in refrigerator and attached to a 100-V transformer for ~30 h. When complete, the gel was removed from the apparatus and rinsed.
with distilled water. The gel was then stained with 0.25% Coomassie blue for 15 min and then destained using 10% methanol and 10% acetic acid until the MHC bands were visible and the background staining was minimized. Gels were then stored in 10% glycerol, subsequently wrapped in cellophane membrane (Bio-Rad), and then allowed to air dry for 2 days before being filed.

Data analyses. Images of gels were captured using a high-performance monochrome interline transfer charge-coupled device camera with a resolution of 640 × 480 pixels (Cohu, San Diego, CA) connected to a Macintosh G4 computer (Apple Computer, Cupertino, CA). Scion Image (developed by US National Institute of Health and available on the internet by anonymous FTP from Zippy.nimh.nih.gov; Frederick, MD) was used to visualize pictures of the gels. Densities of MHC isoforms were measured using Scion Image as employed by other researchers (23, 44), and calculations were done to determine the relative percentage of each MHC isoform present in each lane.

Statistical analyses. ANOVAs were performed followed by Bonferroni post hoc tests to determine where significant differences existed when there was a significant interaction between main effects. Arcsine transformations were carried out where the data were expressed as percentages (69). Linear regressions were performed to determine the correlation between myosin isoforms and BBB scores, as well as between myosin isoforms and angle board scores. Parametric statistical tests were used to determine the significance of BBB locomotor scores, as suggested by Scheff et al. (53) and as employed by other researchers (6, 56). A P value of <0.05 was considered to be statistically significant. Values are expressed as mean with standard deviation (SD).

RESULTS

Effect of SCI and glutamine treatment on muscle weight. At 6 wk postsurgery, the soleus muscles from untreated SCI rats weighed, on average, significantly less (one-way ANOVA, 0.0575 ± 0.0069 SD, P < 0.001) than the soleus from all other groups (Fig. 1A). In rats treated with glutamine, the soleus muscle made up a significantly greater (0.0752 ± 0.0063, P < 0.001) percentage of body weight compared with untreated SCI rats. However, the soleus muscle from treated rats made up a significantly lower percentage of body weight compared with healthy (0.0935 ± 0.0084, P < 0.001) and sham (0.0883 ± 0.0086, P < 0.05) rats. Healthy and sham animals were not significantly (P > 0.05) different from one another. At 6 wk postsurgery, the average EDL weight expressed as a percentage of body weight was not significantly (one-way ANOVA, P > 0.05, Fig. 1B) different between any of the four groups.

Analysis of hindlimb strength and locomotor recovery. One-way ANOVAs with Bonferroni posttests were performed at each week for angle board and BBB scores. Healthy and sham groups of rats were able to maintain an angle of 55°, the upper limit of the measurement, throughout the 6 wk and were not significantly (P > 0.05) different from one another at any time point. Untreated SCI rats were unable to maintain their position on the angle board for the first 2 wk after surgery. Treated SCI rats performed significantly better than untreated rats at weeks 3, 4, 5 (P < 0.001), and 6 (P < 0.01; Fig. 2A). Untreated and treated groups were significantly (P < 0.001) different from healthy and sham groups at all time points.

Healthy and sham groups received consistent BBB scores of 21 throughout the study, indicating that they had normal locomotion. Treated and untreated SCI groups had BBB scores significantly (one-way ANOVAs, P < 0.001) lower than healthy and sham groups at all time points. Treated rats’ mean BBB scores were significantly higher than those of untreated rats at week 1 (P < 0.01) and weeks 2–6 (P < 0.001) postsurgery (Fig. 2B).

Effect of SCI and treatment on rat MHC isoforms. Glutamine-treated SCI rats had a more preserved MHC phenotype than untreated SCI rats (Table 1, Table 2, Fig. 3). A two-way ANOVA comparing each MHC isoform as a percentage of total myosin for the four different groups found that a significant (P < 0.0001) interaction took place between the treatment groups and MHC isoforms expressed in the soleus muscle. Bonferroni posttests were performed to determine where the significant differences occurred (Table 1). The soleus muscle from healthy and sham rats contained over 90% type 1 MHC, with type 2a and 2x accounting for the remaining MHCs. The soleus from glutamine-treated rats was more like healthy and sham rats, as it displayed significantly more type 1 MHC and significantly less type 2a and 2x than did soleus from untreated SCI rats.

A two-way ANOVA comparing MHC isoforms in EDL and percentage of total myosin for the four different groups found that a significant (P < 0.0001) interaction took place between the treatment groups and MHC isoforms expressed in the EDL. Bonferroni posttests were performed to determine where the significant differences occurred (Table 2). Healthy rats were not significantly (P > 0.05) different from the sham group. Treated SCI rats did not express any significant (P > 0.05) differences in the percentages of MHC isoforms expressed

Fig. 1. Soleus and extensor digitorum longus (EDL) muscles as percentage of body weight 6 wk after surgery. A: soleus muscles from glutamine-treated animals maintain a greater percentage (P < 0.001) of body weight than untreated animals. Treated rat soleus weights significantly less than sham (P < 0.05) and healthy (P < 0.001) rat soleus. B: EDL percentage of body weight is similar for all groups. For treated and untreated groups, n = 7; for healthy and sham groups, n = 8. Each bar equals mean and SD.
compared with untreated SCI rats. However, treated and untreated rats have significantly \( P < 0.05 \) less 2a and 2b MHCs and more type 2x MHC than healthy and sham animals. No significant difference existed between type 1 MHCs.

As rat diaphragm displays all four MHC isoforms, it was run as a control to visualize MHC bands in every gel. Differing amounts of MHC isoforms were seen (Fig. 3) in the various myosin samples from the diaphragm, because this muscle has a heterogeneous fiber-type distribution (60). There are more type 1 and fewer type 2b muscle fibers in the costal regions compared with the crural regions of the diaphragm (33).

**Correlation between locomotor performance and MHC isoform expression.** Rats with better locomotor performances displayed a greater amount of type 1 MHC isoform in the soleus. Normally, this one isofrom constitutes well over 90% of the MHCs in the soleus (Table 1). Our data revealed significant \( P < 0.0001 \) positive linear correlations between locomotor scores and the amount of type 1 MHC in the soleus. This was true for both the angle board (Fig. 4A, \( r^2 = 0.8686 \)) and BBB (Fig. 5A, \( r^2 = 0.8708 \)) scores.

Linear regressions were also done to determine whether better functional scores were related to a greater percentage of type 2b MHC in the EDL, as this one isoform typically makes up over 50% of the MHCs in healthy EDL (Table 2). Our data confirmed that there were significant \( P < 0.0001 \) positive correlations between functional performance and the amount of 2b MHC in the EDL. This held for both angle board (Fig. 4B, \( r^2 = 0.6084 \)) and BBB (Fig. 5B, \( r^2 = 0.6290 \)) scores. Rats with better functional scores had a greater percentage of 2b MHC in the EDL.

**Discussion**

**Changes in muscle weights.** The soleus muscle of the rat, a slow extensor of the ankle (11, 14, 15, 19), has been shown to display significant atrophy following SCI (16, 18, 61). This is expected as SCI rats are paraplegic and have little, if any, use of their hindlimbs. We found that untreated SCI rats lost, on average, 40% of soleus mass during the 6 wk after SCI. However, treatment with glutamine made a significant difference in SCI rats such that the soleus atrophy was lessened compared with that in untreated rats. Treated SCI rats lost, on average, only 20% of soleus mass by 6 wk after injury.

There may be several mechanisms by which glutamine may act to conserve the soleus during SCI. Glutamine-treated rats may be better able to maintain muscle weight compared with untreated rats following SCI, because skeletal muscle is the most important glutamine producer in the body. During states of stress and trauma, protein breakdown in skeletal muscle increases the supply of glutamine to the body (35, 58, 67). Studies of glutamine-enriched nutrition have shown increased rates of skeletal muscle protein synthesis and decreased rates of protein degradation (2). Glutamine treatment following SCI would provide an external source of glutamine to the body, thereby decreasing the need for protein degradation in skeletal muscle.

**Table 1.** *Soleus* MHC isofrom percentage of total myosin at 6 wk postsurgery

<table>
<thead>
<tr>
<th>Soleus Group</th>
<th>Percentage of Total Myosin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Untreated</td>
<td>7</td>
</tr>
<tr>
<td>Treated</td>
<td>7</td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
</tr>
<tr>
<td>Healthy</td>
<td>8</td>
</tr>
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</table>

**Significant Differences**

<table>
<thead>
<tr>
<th>MHC</th>
<th>Untreated</th>
<th>Treated</th>
<th>Sham</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>2a</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>2x</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>2b</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
<td>( P &lt; 0.01 )</td>
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<tr>
<td>2a</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
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<tr>
<td>2x</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>2b</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

For percentage of total myosin, values shown are means and SD; \( n \), no. of animals. Significant differences are also given for myosin heavy chain (MHC) isoforms in the soleus of treated, untreated, sham, and healthy groups. As there is a significant interaction (two-way ANOVA, \( P < 0.0001 \)) between treatment group and MHC isoform expression, Bonferroni posttests were performed. Glutamine-treated rats display a more preserved myosin phenotype in the soleus muscle than did untreated rats. NS, not significant.
Glutamine treatment may also maintain muscle weight by helping save innervation to the muscle. This was the case with our experimental model, as SCI rats receiving glutamine treatment have greater white matter sparing than untreated SCI rats (46). It is likely that the greater white matter sparing is due to better maintenance of the antioxidant defense systems, as exemplified by better maintenance of spinal cord GSH (47). Oxidative stress drives many of the secondary mechanisms of damage following SCI (19). Maintenance of GSH has been identified as a factor that is involved in the survival of spinal nerves in vitro (27), and in vivo it decreased lipid peroxidation (28), increased white matter sparing (22, 46), and increased functional recovery (22, 46).

A greater number of functioning neurons should be correlated with a greater degree of locomotion. Glutamine-treated rats had significantly greater locomotor abilities and hindlimb strength than untreated SCI rats. Increased use of the hindlimbs and hindlimb muscles, particularly postural muscles, would have contributed to decreased atrophy of the soleus in treated SCI rats. Inactive muscles undergo atrophy. This is particularly true for postural muscles that are frequently used under normal circumstances (see Ref. 3).

The EDL, a fast-twitch ankle flexor (11, 14, 15, 19), did not display significant differences in weight between any of the groups studied. Other studies of denervation and SCI have shown that the EDL atrophies less than the soleus (17–19, 59). The soleus shows comparatively greater changes than the EDL, not only in muscle atrophy, but also in contractile properties, fiber-type composition, and myosin content in response to decreased neuromuscular activity and unloading (42). When muscles are unloaded, during chronic bed rest or spaceflight, the primary targets for protein degradation are muscle fibers containing type 1 MHC. The EDL contains very little type 1 MHC (3). Additionally, as it is not a postural muscle, the EDL should atrophy less than the soleus with unloading.

Another explanation for insignificant EDL atrophy following SCI may be related to the position of this muscle following SCI. After SCI, rats pull their hindlimbs behind themselves, with the dorsal part of their foot and toes dragging on the floor. This passively stretches the EDL, but not the soleus muscle. Continuous muscle stretch has been shown to prevent muscle atrophy following disuse (25, 26, 50, 52). Passive stretch leads to muscle growth by increasing protein synthesis (67). In the present study, rats scoring below 8 on the BBB scale did not exhibit plantar placement of the foot, and rats with a BBB score below 16 did not display any toe clearance during the stepping process (6). All SCI-untreated rats had BBB scores below 7, and of the SCI-treated rats, only one attained a score above 11. Therefore, it is likely that all SCI rats were experiencing some stretching of the EDL muscle. This is a probable explanation for the insignificant difference in EDL weight between the various groups of rats.

### Table 2. EDL MHC isoform percentage of total myosin at 6 wk postsurgery

<table>
<thead>
<tr>
<th>EDL Group</th>
<th>n</th>
<th>1</th>
<th>2a</th>
<th>2x</th>
<th>2b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>7</td>
<td>0.17±0.53</td>
<td>0.36±0.96</td>
<td>73.50±7.37</td>
<td>25.97±7.34</td>
</tr>
<tr>
<td>Treated</td>
<td>7</td>
<td>0.33±0.87</td>
<td>2.21±4.01</td>
<td>65.51±20.52</td>
<td>31.96±17.72</td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
<td>3.52±6.62</td>
<td>13.57±8.13</td>
<td>31.69±5.51</td>
<td>51.22±9.14</td>
</tr>
<tr>
<td>Healthy</td>
<td>8</td>
<td>2.36±2.71</td>
<td>11.20±3.11</td>
<td>31.46±7.72</td>
<td>54.99±9.40</td>
</tr>
</tbody>
</table>

For percentage of total myosin, values shown are means and SD; n, no. of animals. Significant differences are also given in MHC isoforms in the extensor digitorum longus (EDL) of treated, untreated, sham, and healthy groups. There is a significant (two-way ANOVA, P < 0.0001) interaction between treatment group and MHC expression. Bonferroni posttests were performed to determine where significant differences exist. Treated and untreated rats are not different from each other, nor are healthy and sham rats. However, both treated and untreated rats show significant differences in MHC expression compared with healthy and sham rats.

![Fig. 3. SDS-PAGE gels showing rat myosin heavy chains (MHCs) from soleus and EDL muscles of various treatment groups. Each group of pictures was run in the same gel. Molecular weight markers (MW) and diaphragm samples (D) were run in every gel. The molecular weight marker contained two different isoforms of myosin from rabbit skeletal muscle. Note that myosin samples from the diaphragm contain all four MHCs. A: myosin from rat diaphragm aligns with the molecular weight marker at 200,000. B: myosin from the soleus of healthy (H) animals displays predominantly type 1 MHC, with some type 2a MHC. Conversely, myosin from untreated (UT) rats shows much less type 1 MHC and higher levels of 2a and 2x MHCs. C: myosin from the soleus of rats treated (T) with glutamine displays a MHC profile similar to that of untreated rats (B), but has relatively more type 1 MHC and less type 2a and 2x MHCs. D: the EDL of healthy rats displays the greatest expression of type 2b MHC, with some type 2a, and less 2a MHC. Untreated rats show a decreased expression of 2b MHC and an increased expression of 2x MHC. E: the EDL of rats treated with glutamine have increased expression of 2b MHC and decreased expression of 2x MHC compared with untreated rats (D), although this was not statistically significant.](http://jap.physiology.org/DownloadedFrom)
Functional performance. Using the angle board method, treated SCI rats acquired significantly higher scores than untreated SCI rats, but they never achieved scores as high as those of healthy or sham rats. This indicates that glutamine administration helps maintain hindlimb strength, but at a reduced level. On the BBB scale, which is an indicator of locomotor function, treated SCI rats also had significantly better scores than untreated SCI rats. At 6 wk, untreated rats achieved a mean score of 2.86, whereas treated rats had a mean BBB score of 9.57. Comparing locomotor function at 6 wk highlights differences between the two groups. Glutamine-treated SCI rats are able to walk, but untreated SCI rats are not.

A score of 3 on the BBB scale indicates that a rat has extensive movement of two of the three hindlimb joints, but is unable to support its weight or take steps. A score of 10 indicates that the rats do not display any forelimb-hindlimb coordination (6).

All rats in the treated SCI group obtained the same BBB scores in week 5 that they did in week 6. This may indicate that the treated rats reached a plateau in their locomotor recovery at 5 wk. In a study of moderate spinal cord contusion using the Ohio State University impact device (36), animals initially experienced hindlimb paralysis but regained limited locomotor capabilities with weight bearing within several weeks (17). The BBB scores of their injured rats reached a plateau from 3 to 10 wk postinjury.

MHC isoform expression. The velocity of contraction of vertebrate muscle and muscle fibers is directly correlated with MHC isoform content (4, 29, 55). In mammalian muscle fibers, the velocity of contraction of fibers containing just one type of MHC isoform is $1 < 2a < 2x < 2b$ (8). Hybrid fibers, those fibers expressing more than one MHC, contract at velocities intermediate to those of fibers expressing just one MHC isoform type (41, 64). Changes in MHC content are correlated with altered contractile properties (39, 41, 43).

The soleus muscles of SCI rats displayed a shift in MHC expression from slow type 1 toward the faster isofoms. In SCI rats, compared with animals from the healthy and sham groups, there was an increased expression of 2a and 2x MHC isofoms with a concomitant decrease of the type 1 isoform. Previous studies using various models of decreased neuromuscular activity had comparable results (16, 19, 63). In the present study, however, there were significant differences in MHC isoform expression between glutamine-treated and untreated SCI rats. SCI rats treated with glutamine had significantly less 2a and 2x MHC isoform expression. The soleus muscles of SCI rats displayed a shift in MHC expression from slow type 1 toward the faster isofoms. In SCI rats, compared with animals from the healthy and sham groups, there was an increased expression of 2a and 2x MHC isofoms with a concomitant decrease of the type 1 isoform. Previous studies using various models of decreased neuromuscular activity had comparable results (16, 19, 63). In the present study, however, there were significant differences in MHC isoform expression between glutamine-treated and untreated SCI rats. SCI rats treated with glutamine had significantly less 2a and 2x MHC isoform expression.
MHC isoforms and significantly more type 1 MHC isoform expression than did untreated rats. Intact innervation is required for the maintenance of normal muscle fiber structure, function, and phenotype (14, 38–40, 54). Since glutamine administration following SCI appears to help maintain innervation (46), a more preserved MHC phenotype would be expected in the soleus of treated SCI rats.

Myosin expression in the EDL was not significantly different between treated and untreated SCI groups. The EDL of SCI rats was, however, significantly different compared with that of healthy and sham rats. One explanation is that studies showing that passive stretch contributes to increased muscle mass have also shown that passive stretch can influence MHC gene expression (27, 51). The EDL from all SCI rats had significantly less type 2a and 2b MHC isoforms, but more 2x MHC than the EDL of healthy or sham rats. Type 1 MHCs accounted for less than 5% of total MHCs and was unaffected between groups. Thus, after SCI, type 2b and some 2a MHCs were replaced by type 2x MHCs. This concurs with earlier work showing an increase in 2x MHCs (17) and a decrease in type 2b MHCs in the EDL after SCI (17, 19).

Angle board scores and BBB scores both have a strong correlation to the amount of type 1 MHC present in the soleus muscle. The correlation between functional scores and the amount of type 2b MHC present in the EDL was also strong. The strong correlations between higher functional scores and more preserved MHC profiles indicate that improved functional outcome is related to the maintenance of a muscle’s myosin profile.

How might glutamine preserve muscle function and myosin phenotype? We hypothesized that intraperitoneal glutamine administration following SCI would improve functional performance in rats and that this improvement would be accompanied by a preserved MHC profile in representative locomotor muscles. These hypotheses were confirmed, although MHC profile was preserved to a greater degree in the slow-twitch soleus than the fast-twitch EDL. Glutamine promotes GSH synthesis, and GSH plays an important role in antioxidant defense systems (20). Our laboratory has previously shown that glutamine administration to SCI rats helps preserve basal blood GSH levels as well as spinal cord GSH levels (46, 47). Our laboratory also carried out histological analyses of the spinal cords from the same group of rats used in the present study (46) and observed much sparing of the white matter in glutamine-treated rats. This was probably achieved through enhanced oxidant scavenging because of better maintained GSH levels, resulting in less secondary damage following SCI. Since nerve input regulates muscle phenotype, less damage to the spinal cord should, in turn, yield a more normal MHC phenotype and preserved locomotor function. Additionally, glutamine administration following SCI may maintain body and skeletal muscle weight by providing conditionally essential glutamine to the rats, thereby decreasing skeletal muscle protein degradation.

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