Activated satellite cells fail to restore myonuclear number in spinal cord transected and exercised rats

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Du Pont-Versteegden, Esther E., René J. L. Murphy, John D. Houle, Cathy M. Gurley, and Charlotte A. Peterson. Activated satellite cells fail to restore myonuclear number in spinal cord transected and exercised rats. Am. J. Physiol. 277 (Cell Physiol. 46): C589–C597, 1999.—In this study, possible mechanisms underlying soleus muscle atrophy after spinal cord transection and attenuation of atrophy with cycling exercise were studied. Adult female Sprague-Dawley rats were divided into three groups; in two groups the spinal cord was transected by a lesion at T10. One group was transected and killed 10 days later, and another group was transected and exercised for 5 days starting 5 days after transection. The third group served as an unjured control. All animals received a continuous-release 5′-bromo-2′-deoxyuridine pellet 10 days before they were killed. Transection alone and transection with exercise lead to activation of satellite cells, but only the exercise group showed a trend toward an increase in the number of proliferating satellite cells. In all cases the number of activated satellite cells was significantly higher than the number that divided. Although the number of cells undergoing proliferation increased with exercise, no increase in fusion of satellite cells into muscle fibers was apparent. Spinal cord transection resulted in a 25% decrease in myonuclear number, and exercise was not associated with a restoration of myonuclear number. The number of apoptotic nuclei was increased after transection, and exercise attenuated this increase. However, the decrease in apoptotic nuclei with exercise did not significantly affect myonuclear number. We conclude that apoptotic nuclear loss likely contributes to loss of nuclei during muscle atrophy associated with spinal cord transection and that exercise can maintain muscle mass, at least in the short term, without restoration of myonuclear number.

Muscle atrophy; apoptosis; myogenin; spinal cord injury

Adult skeletal muscle is capable of changing its size depending on the demands placed on it. Several conditions, such as disuse, denervation, malnutrition, and microgravity, lead to atrophy of muscles. Other stimuli, such as resistance exercise and overload, are associated with muscle hypertrophy. In a previous study we showed that spinal cord transection rapidly decreased fiber size in affected muscles and that this atrophy was ameliorated by cycling exercise (12). The cellular and molecular mechanisms underlying these changes are poorly understood, but regulation of protein synthesis and degradation is likely involved (37). In addition, it has been shown that hindlimb unweighting and spaceflight decrease muscle mRNA levels (4, 17, 38), and thus the rate of transcription may be changed by conditions that lead to muscle atrophy. A change in transcription could be the result of a decrease in nuclear number and/or a decrease in transcription per nucleus.

Myonuclear number has been shown to decrease after spinal cord isolation (2), spaceflight (3), hindlimb suspension (10), and denervation (40). This loss of nuclei was most prevalent in slow-twitch muscles and in slow-twitch fibers of mixed or predominantly fasttwitch muscles. The mechanism by which these nuclei disappear is not clear, although a possibility is that programmed cell death or apoptosis. Apoptosis is characterized by a series of morphological changes that accompany the death of cells in a wide variety of tissues. These changes include chromatin compaction and segregation, rapid overall cellular condensation, budding to produce membrane-enclosed apoptotic bodies, and disposal of the apoptotic bodies without an inflammatory response (18). It has been shown that heterokaryons can exhibit apoptotic changes in a subset of nuclei without affecting the survival of the other nuclei within the same cell (11). Adult skeletal muscle fibers contain numerous nuclei within one cytoplasmic unit, and these nuclei can independently undergo apoptotic changes under certain conditions. Allen et al. (1) showed that hindlimb unweighting increased apoptotic myonuclei, which appeared to be randomly distributed within muscle fibers without signs of degeneration. Apoptotic nuclei also have been observed in muscles of patients with a variety of neuromuscular diseases (34, 35), in muscles of dystrophic mice (32), and in muscles undergoing atrophy due to denervation (36). The effect of exercise on apoptosis is controversial. Dystrophic and normal mice were shown to have increased amounts of apoptotic nuclei after running exercise (29), but in a recent study it was shown that resistance exercise decreased the frequency of apoptotic myonuclei observed after hindlimb unweighting (1).

On the other hand, it has been demonstrated that hypertrophying conditions, such as functional overload (2, 21, 41) and endurance training (6), increase the number of myonuclei. Satellite cells most likely serve as the source of new myonuclei. Satellite cells are undifferentiated myogenic stem cells located between the sarcolemma and the basal lamina (20) and have been shown to be important during normal muscle growth (23), regeneration (5, 15), and hypertrophy of skeletal muscles (28, 30, 31). Satellite cells are acti-
vated on muscle damage and go through cell division, after which they may fuse with existing fibers to repair the damage or form new fibers if the damage is very extensive (for review see Refs. 5 and 15). Whether satellite cell proliferation is required for hypertrophy is unclear. Studies have shown that satellite cells are involved in the hypertrophic response (26) and that γ-irradiation of skeletal muscle, which kills dividing cells, prevents hypertrophy (28). However, others have shown that hypertrophy occurs in skeletal muscle even after γ-irradiation (19). Satellite cell activation is characterized by increased expression of MyoD and myogenin, and therefore these factors have commonly been used as indicators of satellite cell activation (14, 15). MyoD and myogenin are members of the group of myogenic regulatory factors that are involved in inducing muscle-specific gene expression during embryogenesis. However, their role in adult skeletal muscle is less clear (24). In a previous study we showed that satellite cells become activated without overt signs of muscle damage with spinal cord transection alone or in combination with exercise (12). This indicates that activation of satellite cells, as measured by MyoD and myogenin expression, occurs independently of muscle damage, and factors other than damage-induced growth factors may be involved in satellite cell activation. However, the fate and function of these activated satellite cells were not explored. A possibility is that satellite cells become activated but do not progress through the cell cycle. Also, the question remains whether satellite cells contribute to the decrease in atrophy with short-term cycling exercise observed after spinal cord transection (12).

The goal of this study was to investigate factors contributing to muscle atrophy observed with spinal cord transection and the attenuation of atrophy with cycling exercise. Specifically, the fate of satellite cells after spinal cord transection and exercise was investigated, and the involvement of apoptotic nuclear loss in atrophy was studied. We focused on early cellular responses of the soleus muscle, as we showed previously that this muscle was most severely affected by spinal cord transection and responded to exercise with an attenuation of atrophy (12).

**MATERIALS AND METHODS**

Animals and experimental protocol. All procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals. Adult female Sprague-Dawley rats (180–220 g) were randomly divided into three groups (n = 4–5); control rats did not undergo a spinal cord transection and were not exercised. Rats in the remaining two groups underwent a complete transection of the thoracic (T10) spinal cord by creation of an aspiration lesion 2–3 mm long while under anesthesia with ketamine (60 mg/kg) and xylazine (10 mg/kg). After surgery, manual expression of the urinary bladder was carried out twice daily, and rats received penicillin procaine G and a dextrose saline injection immediately after surgery. Rats in one group did not exercise and were killed 10 days after transection (tx10). Rats in the remaining group were subjected to pedaling exercise on a motor-driven bicycle as described (12, 16) for 60 min each day beginning 5 days after spinal cord transection (tx10e5). The tx10e5 rats were killed 5 days after the first exercise session. To measure nuclei that underwent cell division, rats received a 21-day continuous-release pellet containing 100 mg of 5′-bromo-2′-deoxyuridine (Brdu; Innovative Research America, Sarasota, FL), which was constructed to give a dose of 0.022 mg Brdu-g body wt·day⁻¹ (7). Pellets were implanted subcutaneously in the subscapular region in tx10 and tx10e5 rats at the time of transection and in the control rats 10 days before they were killed. Animals were killed with an overdose of pentobarbital sodium. Soleus muscles were carefully dissected, embedded in freezing medium, snap frozen at rest length in liquid nitrogen-cooled isopentane, and stored at −70°C.

Immunocytochemistry. Cross sections of soleus muscles were cut on a cryostat (8 µm), air dried, and stored at −20°C. Myogenin was detected as described previously (12). Briefly, sections were rehydrated in PBS and reacted with 0.25% H₂O₂ to block endogenous peroxidase activity. Sections then were fixed in 2% paraformaldehyde in PBS and permeabilized using 1% Igepal CA-630 (Sigma Chemical, St. Louis, MO) to allow access of antibody to the nucleus. All subsequent washes and incubations were performed with 0.1% Igepal in PBS. Myogenin antibody was applied at a concentration of 2–5 ng/µl, and sections were incubated for 1 h. Myogenin (F5d) antibody was supplied by W. Wright (University of Texas Southwestern Medical Center, Dallas, TX) (9). An IgG1 biotin-conjugated secondary antibody (Zymed, San Francisco, CA) was applied at a dilution of 1:100. After incubation, streptavidin-horseradish peroxidase antibody (Zymed) was added, and dianinobenzidine (DAB) peroxidase substrate (Vector Labs, Burlingame, CA) was applied for color development. Sections were dehydrated and coverslipped. Positive nuclei were counted in an area occupied by 70–100 fibers. Number of positive myogenin nuclei was expressed per 100 fibers.

Brdu incorporation was detected using a Brdu antibody (Boehringer Mannheim, Indianapolis, IN) according to manufacturer’s instructions. Briefly, soleus muscle sections were rehydrated in PBS and reacted with 0.25% H₂O₂; then they were fixed in absolute methanol. Sections were incubated in 2 N HCl for 60 min at 37°C to denature the DNA, then neutralized in 0.1 M borate buffer at pH 8.5. Muscle sections then were incubated in PBS containing 1.0% Igepal (Sigma Chemical) to permeabilize the tissue, and all further washes contained 0.1% Igepal. Brdu antibody was applied at a concentration of 6–8 ng/µl and incubated for 1 h at room temperature. After the sections were washed, a secondary rat anti-mouse IgG1 biotin-conjugated antibody (Zymed) was applied at 1:100 dilution for 1 h at room temperature. Streptavidin peroxidase was applied, and then DAB peroxidase substrate (Vector Labs) was applied for color development. The number of Brdu-positive nuclei per whole muscle section was determined.

Detection of dystrophin and myogenin on the same section was performed as described previously (12). Briefly, sections were cut at 8 µm and reacted with 0.25% H₂O₂ to block endogenous peroxidase activity. Dystrophin antibody (NC-L-DYS2, Vector Labs) diluted 1:4 in PBS was applied. An alkaline phosphatase-conjugated IgG secondary antibody (Zymed) was applied; then the section was incubated with alkaline phosphatase substrate (Vector Labs) for color development. Myogenin staining was performed as described above, except blocking was eliminated.

Detection of BrdU and laminin on the same section was performed as follows. Sections were first stained for BrdU as described above, and then laminin staining was performed.
Laminin antibody (Sigma Chemical) was applied at 1:40 dilution; then a rat anti-rabbit IgG alkaline phosphatase-conjugated secondary antibody (Zymed) was added at a 1:100 dilution. Color development was performed using the alkaline phosphatase substrate kit.

To detect BrdU and dystrophin on the same section, muscle sections were rehydrated in PBS and incubated in 0.25% H$_2$O$_2$ in PBS. After the sections were washed, a dystrophin antibody (mouse anti-human dystrophin, NCL-DYS2, Vector Labs) was applied at a 1:4 dilution; then a rat anti-mouse IgG1 alkaline phosphatase-conjugated secondary antibody (Pharmigen, San Diego, CA) was applied. The alkaline phosphatase substrate kit was used to yield a red color for dystrophin staining. Sections were then fixed in methanol, and BrdU staining was performed as described above, but without the blocking step.

To count myofiber nuclei, a Hoechst dye was applied after dystrophin staining. Dystrophin staining was performed as described above. Subsequently, sections were fixed in 2% paraformaldehyde, and Hoechst-33258 nuclear dye (Molecular Probes, Eugene, OR) was applied at 1.2 ng/ml for 30 min. Sections were viewed with a fluorescent microscope with use of an ultraviolet filter package and photographed. Nuclei within the dystrophin-positive sarcolemma were counted in 70–100 fibers, and the number of nuclei was expressed per 100 fibers. Numbers were not expressed per unit fiber cross-sectional area, because fiber area changes with the experimental manipulations (12).

Detection of apoptotic nuclei. Apoptotic nuclei were identified by using a TdT-mediated dUTP nick end labeling (TUNEL) assay (Boehringer Mannheim). This assay is based on the fact that apoptotic nuclei display DNA strand breaks, and TdT can be used to label these DNA strand breaks with fluorescein. Incorporated fluorescein was then detected by anti-fluorescein antibody conjugated with horseradish peroxidase. TUNEL detection was performed according to specifications supplied with the assay. Specifically, sections are fixed in 4% paraformaldehyde at room temperature, blocked in 0.3% peroxide in methanol at room temperature, and permeabilized in 0.1% Triton-X and 0.1% sodium citrate at 4°C. TUNEL mix was then added to the sections at a 1:7.5 dilution with 30 mM Tris, 140 mM sodium cacodylate, and 1 mM CoCl$_2$. Labeling mix was incubated at 37°C for 1 h. Sections were rinsed, and fluorescein antibody was applied for 30 min at 37°C. Sections were rinsed, DAB substrate (Vector Labs) was added for color development, and sections were dehydrated and coverslipped. The number of positive nuclei of a whole muscle section was counted.

Statistics. To test for statistically significant differences, ANOVA was used; in the case of significant differences, Tukey’s multiple comparison test was applied. Statistical significance was assumed at $P < 0.05$.

RESULTS

Satellite cells are activated after spinal cord transection. After spinal cord transection and transection with exercise, myogenin expression in soleus muscles was used as an indicator of satellite cell activation. Soleus muscle cross sections immunoreacted with myogenin (A–C) or dystrophin (red) and myogenin (brown) antibodies (D–F) are shown in Fig. 1; the number of myogenin-positive nuclei is quantitated in Fig. 2. Rare myogenin-positive nuclei were observed in control soleus muscles (Fig. 1A). The number of myogenin-positive nuclei was increased sevenfold in soleus muscles of tx10 rats (Figs. 1B and 2) and remained elevated in soleus muscles of tx10e5 rats (Figs. 1C and 2). Although exercise decreased the atrophy associated with transection (compare myofibers in Fig. 1, B and C) (12), no difference was observed in myogenin expression with exercise compared with transection alone (Fig. 2). In transected...
rat soleus muscle the average number of myogenin-positive nuclei was 98 nuclei in an area occupied by 100 fibers (Fig. 2). To distinguish activated satellite cell nuclei from myofiber nuclei that were also expressing myogenin, the sarcolemma was stained with dystrophin antibody. Nuclei expressing myogenin located within the dystrophin-positive sarcolemma are myonuclei, and nuclei expressing myogenin located outside the sarcolemma reside within satellite cells. Figure 1, D–F, shows the result of the double staining. No myogenin-positive nuclei in satellite cells were found in control soleus; however, some myogenin was detected in myonuclei (Fig. 1, A and D). In transected soleus muscles, myogenin-positive nuclei were found within the sarcolemma and outside the sarcolemma (Fig. 1, E and F). Approximately 10% of the myogenin-expressing nuclei reside within satellite cells in muscles from tx10 and tx10e5 rats. Therefore, spinal cord transection is associated with increased myogenin expression in myofiber nuclei and with activation of satellite cells regardless of exercise status.

Activated satellite cells do not contribute to attenuation of atrophy in response to exercise. To investigate what happens to activated satellite cells after transection and exercise, continuous-release BrdU pellets were implanted. BrdU incorporation identifies cells that have undergone cell division after implantation of the pellets. Representative cross sections of soleus muscles immunoreacted with BrdU antibody are shown in Fig. 3, and quantitation of the labeled nuclei is depicted in Fig. 4. Compared with control (Fig. 3A), the number of BrdU-positive nuclei in a whole muscle section increased over twofold with transection and exercise combined (Figs. 3C and 4A), but not with transection alone (Figs. 3B and 4A). Soleus cross sections were immunoreacted sequentially with laminin and BrdU antibodies to estimate the total number of muscle nuclei (satellite cell nuclei + myofiber nuclei) that had undergone cell division. Laminin is a protein present in the basal lamina surrounding muscle fibers, and nuclei within the basal lamina are satellite cell nuclei or myofiber nuclei. Examples of this staining are shown in Fig. 3, D–F, and quantitation is shown in Fig. 4B. Most of the BrdU-stained nuclei were located outside the basal lamina and are therefore nonmuscle nuclei. A small percentage of BrdU-positive nuclei were muscle nuclei, and Fig. 4B shows that the number of BrdU-positive muscle nuclei tended to increase with transection and exercise combined. However, this increase failed to reach significance (P = 0.15). The average number of muscle nuclei, i.e., satellite cells and myofiber nuclei combined, per whole soleus muscle section of tx10 animals was 8 and that of tx10e5 animals was 12. These numbers are significantly lower than the number of activated satellite cells as measured by myogenin positivity. This indicates that only a small subset of activated satellite cells subsequently divide. To determine whether any activated satellite cells that had divided also fused into myofibers and whether the frequency differed between experimental groups, soleus cross sections were double stained with dystrophin and BrdU antibodies. Because myofiber nuclei are thought to be postmitotic, BrdU-positive nuclei within the sarcolemma most likely derive from satellite cells that recently fused into the fiber. BrdU-positive nuclei present in soleus muscle are shown in Fig. 3, G–I, and are quantitated in Fig. 4C. The number of BrdU-positive myofiber nuclei was not different between the three groups (Fig. 4C), indicating that there was no change in fusion of satellite cells with myofibers after transection alone or with transection and exercise combined.

Myofiber nuclei are lost after spinal cord transection and are not restored after exercise. The results described above suggest that even though satellite cells appear to be activated, they do not seem to be directly involved in restoration of muscle fiber size in response to short-term cycling exercise. This led us to investigate whether myonuclear number changed in response to transection alone or transection and exercise. Myofiber nuclei of soleus muscles from control rats (Fig. 5), tx10 rats (data not shown), and tx10e5 rats (data not shown) were counted after a dystrophin stain combined with Hoechst dye. Nuclei inside the dystrophin-positive sarcolemma were considered myofiber nuclei. With spinal cord transection, there was a decrease of 25% in the number of myofiber nuclei (see Fig. 7A). In control soleus muscle, nuclei were found in almost every myofiber on a given cross section, whereas in transected soleus muscles many small fibers did not exhibit nuclei on a cross section. The myofiber nuclear number did not increase with exercise compared with transection alone (see Fig. 7A). To investigate a possible mechanism of myofiber nuclear loss with transection, soleus muscle cross sections were assayed for TUNEL reactivity, an indicator of apoptotic nuclei (Fig. 6, A–C). Control soleus muscles showed little TUNEL positivity (Fig. 6A), but the number of TUNEL-positive nuclei was increased >35-fold with spinal cord transection (Fig. 6B, quantitated in Fig. 7B). Most of the TUNEL-positive nuclei are not muscle nuclei. The number of

Fig. 2. Increased myogenin expression in soleus muscles from tx10 and tx10e5 rats. Number of myogenin-positive nuclei per area of 100 fibers in soleus muscle cross sections from control, tx10, and tx10e5 rats is shown. Bars represent means ± SE. *Significantly different from control (P < 0.05).
TUNEL-positive nuclei decreased ~60% with exercise compared with transection alone (Figs. 6C and 7B) but remained significantly higher than control.

**DISCUSSION**

In this study we investigated myonuclear number and found that there was a 25% decrease in myonuclei accompanying atrophy after spinal cord transection. Atrophy in soleus muscle has been shown to be associated with a loss of myonuclei, independent of the manner in which atrophy was induced (2, 3), thereby maintaining the myonuclear domain (DNA-to-cytoplasmic ratio). The mechanism by which these nuclei are lost is unclear, but it may be through apoptosis. Apoptotic nuclear loss has been shown to increase with a number of muscle diseases (32, 34, 35) and with denervation (36). Furthermore, a recent study showed that apoptotic nuclear loss was increased with atrophy associated with hindlimb suspension (1). In the present study we found that the number of apoptotic nuclei dramatically increased with spinal cord transection and accompanying atrophy. With exercise, there was a decrease in the number of apoptotic nuclei compared with transection alone. Because apoptosis is a rapid process, the number of TUNEL-positive myonuclei at any given time is likely an underestimate of the actual number of nuclei that have undergone apoptosis over the 10 days after transection. Thus these results suggest that apoptotic nuclear loss is one potential mechanism by which myonuclear number decreases during muscle atrophy in response to spinal cord transection.

As the number of myonuclei decreased with atrophy due to spinal cord transection, we determined whether exercise-related reduction of atrophy was associated with a restoration in myonuclear number and whether satellite cells were involved in this response. We showed previously that muscle fiber cross-sectional area of soleus muscles decreased to ~40% of its original size 10 days after spinal cord transection. Moreover, we found that cycling exercise prevented this decrease in muscle fiber size (12, 16), and we hypothesized that satellite cells are involved in the attenuation of atrophy after exercise. Surprisingly, satellite cell activation, as measured by myogenin expression, was observed with...
spinal cord transection alone and appeared unaffected by exercise. The signals for satellite cell activation with transection alone are unclear, because no overt muscle damage occurs (12). It is known that myogenin expression increases in myonuclei in response to a decrease in electrical activity caused by denervation (13) or application of a neurotoxin (42). We also showed previously that MyoD and myogenin mRNA of whole soleus muscle increased transiently after spinal cord transection (12), likely because of the decrease in electrical activity in muscle after transection. It is possible that satellite cells also respond to a decrease in electrical activity in muscle. At issue was whether the activated satellite cells subsequently divide and fuse into fibers. We hypothesized that exercise might promote this process, allowing satellite cells to replenish myonuclei lost with atrophy. Double-labeling techniques with the BrdU antibody in combination with antibodies against components of the basal lamina and sarcolemma demonstrated that in the exercised soleus there seemed to be a preferential increase in replicating muscle cells, although most labeled nuclei were from nonmuscle cells. It has been shown that denervation increases the number of dividing satellite cells and connective tissue cells (22), but denervation is associated with nerve damage extending into the muscle, which is not the case with spinal cord transection. Therefore, spinal cord transection alone induces expression of myogenic regulatory factors in satellite cells, and thus activation, but does not appear to generate a strong enough signal for satellite cell proliferation. By contrast, exercise appears to provide signals necessary for satellite cells to enter the cell cycle.

Even though the number of BrdU-labeled nuclei was increased with exercise, no increase in BrdU-labeled nuclei inside the muscle fibers themselves was observed with exercise, suggesting that satellite cells that had divided had not fused with the muscle fiber. This finding correlated with the observation that the number of myonuclei did not increase with exercise compared with transection alone. Thus, at this early time point after the onset of exercise, satellite cells are not involved in exercise-induced maintenance of muscle fiber size but may participate at later time points. The most likely explanation for the decrease in atrophy is that exercise changes protein synthesis and degradation in such a way that protein is accumulated in the muscle. Indeed, it has been shown that protein synthesis is increased to a greater extent than protein degra-
Satellite cells and myonuclear number

Fig. 6. Increased frequency of apoptotic nuclei with spinal cord transection. Cross sections of soleus muscles from control (A), tx10 (B), and tx10e5 (C) rats were assayed for TdT-mediated dUTP nick end labeling (TUNEL) reactivity (brown). Arrows indicate examples of TUNEL-positive nuclei. Bar for A–C in C, 25 µm.

Fig. 7. Quantitation of myonuclear number and apoptotic nuclei after spinal cord transection and exercise. A: myofiber nuclei per 100 fibers for soleus muscles of control, tx10, and tx10e5 rats. B: number of TUNEL-positive nuclei on a whole cross section of soleus muscles from control, tx10, and tx10e5 rats. Bars represent means ± SE. *Significantly different from control; # significantly different from tx10 (P < 0.05).

Myonuclear loss was associated with the muscle atrophy after spinal cord transection, and an increase in apoptotic nuclei is a potential mechanism to account for this loss. The attenuation of atrophy shortly after resistance exercise, resulting in a net increase in muscle protein balance (27). However, if the myonuclear domain is to remain constant, nuclei would have to be added. It is possible that satellite cells will eventually fuse with fibers to restore myonuclear number. Another possibility is that addition of nuclei to fibers with exercise is not necessary because of the fiber type switching that occurs with spinal cord transection. It has been shown that spinal cord transection is associated with a loss of slow-twitch fibers and an increase in fast-twitch fibers (16). As fast-twitch fibers have a larger myonuclear domain (2, 39), the loss of nuclei may reflect the fiber type transformation, which occurs after spinal cord transection. Thus, whereas exercise results in a reduction of atrophy, but not in restoration of slow-twitch fiber types (12, 16), satellite cells may not be required, and the larger myonuclear domains characteristic of fast-twitch fibers may be stable under these circumstances. To investigate whether satellite cells contribute to myonuclei, studies are under way to look at later time points after initiation of exercise in this model.

An important finding of this study is that the number of activated satellite cells as measured by myogenin expression is significantly higher than the number of BrdU-positive muscle nuclei in soleus muscles from tx10 and tx10e5 rats. This is deduced from the fact that we found ~98 myogenin-positive nuclei per 100 muscle fibers, with 10% of these within satellite cells. As a soleus muscle in adult female Sprague-Dawley rats contains 2,500–3,000 muscle fibers (25), it follows that there is expression of myogenin in ~250 satellite cell nuclei per whole muscle section in tx10 and tx10e5 rats. In BrdU-positive muscle, there are only 8–12 nuclei per whole muscle section. This suggests that not all activated satellite cells subsequently divide. Indeed, Tatsumi et al. (33) suggested that activation is an event separate from proliferation, since quiescent and activated satellite cells do not respond in the same way to growth signals. Therefore, different signals may be necessary for activation vs. proliferation of satellite cells. Interestingly, Cornelison and Wold (8) showed that, in some satellite cells associated with isolated muscle fibers, myogenin is expressed in the absence of proliferation, supporting the idea that activation and proliferation may be separable events. There is also the possibility that satellite cells fuse into muscle fibers on activation without dividing first; however, there is no experimental evidence to support this possibility.

In summary, myonuclear loss was associated with the muscle atrophy after spinal cord transection, and an increase in apoptotic nuclei is a potential mechanism to account for this loss. The attenuation of atrophy...
phy by short-term exercise in soleus muscles of spinal cord transected rats occurs without satellite cell fusion or an increase in myonuclear number, even though activation of satellite cells is prominent. Studies are needed to investigate whether satellite cells will eventually contribute to the increase in muscle fiber size observed with cycling exercise after spinal cord transection and to elucidate the signals responsible for the satellite cell activation vs. proliferation.

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