Exercise-induced satellite cell activation in growing and mature skeletal muscle

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DARR, KEVIN C., AND EDWARD SCHULTZ. Exercise-induced satellite cell activation in growing and mature skeletal muscle. J. Appl. Physiol. 63(5): 1816-1821, 1987.—The time course and extent of satellite cell activation were studied in the soleus (m-SOL) and extensor digitorum longus (m-EDL) muscles of untrained growing and mature rats after a single bout of prolonged eccentric treadmill running. At 24, 48, 72, and 120 h postexercise, satellite cell mitotic activity was quantitated in autoradiographs of whole-fiber segments after injection of [3H]thymidine. Fiber damage and localization of labeled cells were also examined in muscle cross sections. Labeling in growing muscles progressively increased to peak levels (~250% of control) at 72 h postexercise, whereas mature muscles exhibited an earlier peak (~250% of control) at 24 (m-SOL) and 48 (m-EDL) h, followed by a more rapid decline to control levels by 120 h postexercise. In all exercised muscles the calculated satellite cell activation was far greater than required to repair the small extent of satellite cell activation were studied in the soleus (m-SOL) and extensor digitorum longus (m-EDL) muscles of untrained growing and mature rats after a single bout of prolonged eccentric treadmill running. At 24, 48, 72, and 120 h postexercise, satellite cell mitotic activity was quantitated in autoradiographs of whole-fiber segments after injection of [3H]thymidine. Fiber damage and localization of labeled cells were also examined in muscle cross sections. Labeling in growing muscles progressively increased to peak levels (~250% of control) at 72 h postexercise, whereas mature muscles exhibited an earlier peak (~250% of control) at 24 (m-SOL) and 48 (m-EDL) h, followed by a more rapid decline to control levels by 120 h postexercise. In all exercised muscles the calculated satellite cell activation was far greater than required to repair the small number (~3.0%) of necrotic fibers identified at the light-microscopic level. These results suggest that satellite cells were activated not only on fibers exhibiting overt necrosis but also on those with lesions not discernible with light microscopy.

eccentric running; muscle degeneration; regeneration; autoradiography; connective tissue

A SINGLE BOUT OF PHYSICAL EXERCISE, such as prolonged running, can cause a variety of lesions in untrained skeletal muscle (11, 13, 18, 19, 26). The amount of damage incurred during running can be accentuated by biasing the muscle activity toward an eccentric type of contraction (4, 15). Focal areas of inflammatory and degenerative changes appear 1-3 days after eccentric exercise (4, 19) and are associated with an elevation in the number of macrophages and other mononuclear cells in the region of injured and necrotic fibers. In addition to overt fiber necrosis, fibers exhibiting disruption of intracellular components have also been reported after acute prolonged treadmill running (7, 19, 26, 27). These fibers exhibit no degenerative changes at the light microscopic level and apparently survive the exercise-induced injury (7).

Satellite cells, the myogenic stem cells of mammalian skeletal muscle (14), are assumed to participate in the repair of damaged myofibers after exercise (2, 6). However, the degree of their participation is not known and may depend on the type and/or extent of damage incurred by the muscle. For example, if satellite cells are activated in only those damaged fibers that ultimately undergo necrosis and represent <5% of the population (4), it is unlikely that exercise would produce a measurable response in proliferative activity. However, if satellite cells are activated on fibers exhibiting subcellular injury, which have been reported to comprise half of the fibers in the muscle (7), a more extensive response would be expected.

In the present study, mitotic activation of satellite cells in growing and mature rat soleus (m-SOL) and extensor digitorum longus (m-EDL) muscles was monitored at various time intervals following an acute bout of prolonged eccentric treadmill running.

MATERIALS AND METHODS

Eccentric Treadmill Running

A total of 30 male Sprague-Dawley rats (Sasco/King Labs, Oregon, WI) 1 [96 ± 11 (SD) g body wt] and 3 (401 ± 16 g) mo of age were used for this study. The animals were run on a motor-driven treadmill down an incline of 18% for a period of 105 min. Immediately before exercise the animals were familiarized with treadmill running at a slow speed. The speed was progressively increased over 15 min until the animals could run comfortably at 24 m/min. The intermittent protocol involved three 30-min bouts at speeds of 24, 20, and 16 m/min and one 15-min bout at 16 m/min. Each bout was separated by 5 min of rest. The protocol is similar to that used by Armstrong (3), which has been demonstrated to produce muscle fiber damage in mature rats. Animals unable to complete the protocol were removed from the study. Control animals (n = 3/age group) were kept in cages until they were killed.

Tissue Preparation and Autoradiography

After exercise the animals were returned to their cages and given free access to food and water. One hour before they were killed each animal received a single injection of [3H]thymidine (New England Nuclear, Boston, MA) 2 μCi/g body wt ip). Rats (n = 3/time interval) were killed at 24, 48, 72, and 120 h postexercise. One hindlimb was removed and placed in Carnoy’s fixative. The other was perfused with sodium cacodylate buffered (pH 7.4) 2.5% glutaraldehyde and 2.0% p formaldehyde through the common iliac artery, removed, and placed in fresh fixative. After 24 h in the fixative, m-SOL and m-EDL were
FIG. 1. Autoradiograph of (A) whole-fiber segment and (B) fiber segment at myotendinous junction. Labeled nuclei (arrows) are easily distinguished by presence of silver grains. Incidence of labeled nuclei at myotendinous junction appeared much higher than at other regions of fiber length (x400).

**TABLE 1. Labeling in eccentrically exercised soleus and extensor digitorum longus muscles**

<table>
<thead>
<tr>
<th>Time After Exercise, h</th>
<th>Control</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mo</td>
<td>m-SOL</td>
<td>19.7±4.0</td>
<td>27.2±8.5</td>
<td>31.6±19.2</td>
<td>46.2±19.0*</td>
</tr>
<tr>
<td></td>
<td>(138)</td>
<td>(180)</td>
<td>(235)</td>
<td>(185)</td>
<td>(185)</td>
</tr>
<tr>
<td></td>
<td>m-EDL</td>
<td>13.6±0.9</td>
<td>11.5±7.8</td>
<td>27.8±11.7</td>
<td>37.7±10.2*</td>
</tr>
<tr>
<td></td>
<td>(83)</td>
<td>(200)</td>
<td>(277)</td>
<td>(196)</td>
<td>(196)</td>
</tr>
<tr>
<td>3 mo</td>
<td>m-SOL</td>
<td>2.5±2.1</td>
<td>7.9±0.17*</td>
<td>6.5±1.60</td>
<td>3.5±0.07</td>
</tr>
<tr>
<td></td>
<td>(316)</td>
<td>(260)</td>
<td>(140)</td>
<td>(88)</td>
<td>(88)</td>
</tr>
<tr>
<td></td>
<td>m-EDL</td>
<td>0.78±0.1</td>
<td>0.75±0.25</td>
<td>1.9±1.24</td>
<td>1.05±0.50</td>
</tr>
<tr>
<td></td>
<td>(96)</td>
<td>(245)</td>
<td>(135)</td>
<td>(67)</td>
<td>(67)</td>
</tr>
</tbody>
</table>

Values are means ± SD, expressed as no. of labeled nuclei per 1,000. Values in parentheses represent percent of control; m-SOL, soleus muscle; m-EDL, extensor digitorum longus muscle. * Significantly different from control (P < 0.05).

Muscles dissected free and stored in 0.1 M sodium cacodylate buffer (pH 7.4).

Muscles fixed in Carnoy’s fixative were prepared for autoradiography as described by Kopriwa and Moss (12). Briefly the procedure involved separation of individual muscle fiber bundles with forceps and their incubation in a 2.5% solution of collagenase at 37°C (Sigma, St. Louis, MO) for ~3 h. Once separated by mechanical agitation, the fibers were washed with distilled water, mixed with Kodak NTB2 emulsion (Kodak, Rochester, NY), and spread out on glass slides. After 4 wk of exposure at 4°C, the slides were developed and lightly stained with Gill’s hematoxylin. A minimum of 1,000 nuclei (satellite cell nuclei and myonuclei) were counted per muscle. Under the conditions of this experiment only satellite cells were labeled after injection of [3H]thymine; all myonuclei were unlabeled (14). Nuclei were quantitated by focusing through the fiber along its length and tabulating labeled and unlabeled nuclei; only those fiber segments completely free of adhering connective tissue were used to count nuclei. This selection process ensured that only satellite cells and myonuclei were included in our counts (21). Labeling in the exercised muscles was expressed as the number labeled per 1,000 and as a percent of control. In the same way, labeling in fibroblasts located in clumps of connective tissue free from myofibers was counted in 3-mo-old m-SOL at 48 h postexertion and expressed as a percent of control.

Muscles fixed in glutaraldehyde were transversely cut into two equal segments and prepared for histological examination and quantitation of degenerating and/or necrotic fibers. One-half was embedded in Epon, and 1-μm, semithin sections were cut. The other half was frozen in isopentane chilled in liquid nitrogen and cut in serial transverse sections starting from midbelly of the muscle. The number of degenerating fibers per soleus cross section was counted and expressed as a percent of the total number of fibers (~3,000 fibers based on counts by Parsons et al. (16)). The frozen sections were dipped in NTB2 emulsion and exposed for 4 wk. The autoradiographs were developed and stained with Gill’s hematoxylin. These slides were used to determine the localization and distribution of labeled cells in cross section.

**Statistical Analysis**

Peak labeling counts for each of the experimental muscles was compared with its appropriate control using the Kruskal-Wallis nonparametric analysis of variance (24). A 95% confidence interval was used to determine significant differences between groups.
RESULTS

Fiber Segment Labeling

After isolation by the Kopriwa and Moss technique (12), fibers were broken into segments of various lengths. Only fibers devoid of connective tissue elements were used to count nuclei. Well-stained myonuclei were easily distinguished from the surrounding, lightly stained cytoplasm. Labeled nuclei were identified by the presence of silver grains (Fig. 1A).

The pattern of labeling in m-SOL and m-EDL was similar at each age but differed markedly between 1 and 3 mo of age (Table 1). The labeling pattern in the 1-mo-old muscles was characterized by a progressive increase to peak levels at 72 h, followed by a sustained elevation above control through 120 h postexercise. In contrast, the pattern in the 3-mo-old animals exhibited an earlier labeling peak at 24 (m SOL) and 48 (m EDL) h, followed by a decline to control levels by 120 h.

Extensive labeling was also observed in free connective tissue elements of the fiber segment preparation in both the 1- and 3-mo-old m-SOL. At 48 h postexercise, fibroblast labeling in the 3-mo-old m-SOL was ~600% above control levels. In addition, labeling also appeared strong on fiber segments at the level of the myotendinous junction (Fig. 1B), although labeling was not quantitated in these regions because of their limited numbers (<25/muscle).

Histological Observations

After exercise, 1-mo-old muscles exhibited little fiber degeneration and/or necrosis; <0.5% of the fibers in the m-SOL showed degenerative changes 72 h postexercise. No fiber damage was observed in the m-EDL at any time interval postexercise. However, despite apparently minimal morphological damage in the young muscles, there was extensive labeling in the m-SOL and m-EDL around the perimeter of fibers and in the interstitial spaces (Fig. 2, A and B).

In the 3-mo-old m-SOL, multiple sites of focal degeneration and fiber necrosis were characterized by concentrations of macrophages and other mononuclear cells in the interstitial spaces and necrotic fibers (Fig. 2C). These areas were especially prominent at 24, 48, and 72 h postexercise. In the latter ~3% of the fibers exhibited degenerative changes. Labeling was within, but not exclusive to, these focal areas of degeneration (Fig. 2D). Most labeled cells were observed within and around the perimeter of degenerating and/or necrotic fibers and in the interstitial space of degenerative areas. At 120 h postexercise, small-diameter fibers, presumed to be regenerating fibers, were observed in the focal areas of degeneration throughout the muscle (Fig. 2E). EDL myofibers showed no degenerative changes (Fig. 2F). Labeling was localized primarily in the interstitial space between apparently healthy fibers (Fig. 2F).

DISCUSSION

Extent of Activation

Muscle fibers. The results of this study demonstrate that satellite cells are activated in working muscles after an acute bout of prolonged eccentric exercise. The extent of activation was two to three times greater than control levels during peak labeling in both the 1- and 3-mo-old m-SOL and m-EDL (see Table 1). It should be noted, however, that even though the labeling peaks were similar at 1 and 3-mo of age, the actual number of activated satellite cells was greater in the m-SOL at each age and in the younger animals overall because of differences in the size of the satellite cell populations in these muscles. Satellite cell nuclei represent ~6.3% of the total nuclei (satellite cell nuclei and myofiber nuclei) in m-SOL and 4.3% in m-EDL of 3-mo-old animals (21). Consequently, the observed peak labeling at 24 h would be obtained if 11% of the total satellite cell population on the m-SOL and 5.0% in the m-EDL were activated. Likewise, satellite cell nuclei comprise 9.6 and 7.0% of the total nuclei in 1-mo-old m-SOL and m-EDL, respectively (8). Peak labeling in both muscles would represent activation of ~50% of the satellite cell population. Thus exercise appears to have had a profound effect on the proliferative behavior of mitotically active satellite cells in young, growing muscle in terms of both the proportion and total number of cells that were labeled and also on the mitotically quiescent satellite cells of 3-mo-old muscle.

In all cases, the calculated activation of satellite cells in the exercised muscles appears far greater than expected if the proliferative activity was associated solely with the repair and/or regeneration of the small number of damaged fibers (<3% of the fibers in all the muscles). For example, if in the 1-mo-old m-SOL the estimated satellite cell population of 5.2 × 10^6 (8) is equally distributed among the 3,000 fibers (16), activation and labeling of satellite cells on 0.5% of the damaged fibers would result in a 0.5% increase in labeling of the satellite cell population. This value is not only far below the increase we obtained but would likely not have been detectable in our assay because the relatively small number of damaged fiber segments that contained most of the labeled cells would be randomly dispersed throughout the preparation. Consequently, these results suggest that satellite cells are activated on far more fibers than appear to be damaged in histological sections.

FIG. 2. (See facing page.) Transverse sections of 1-mo-old exercised muscles 72 h postexercise. In both soleus (m-SOL, A) and extensor digitorum longus (m-EDL, B) muscles, labeling appears to be associated with fibers that show no morphological evidence of damage. It cannot be determined whether label is associated with cells in muscle or interstitial compartment (×200). C and D: transverse sections of 3-mo-old exercised muscles 72 h postexercise (×200). In C, fiber at top of field has been infiltrated by macrophages and is adjacent to apparently normal fibers. Autoradiograph of degenerating fiber (D) shows several labeled nuclei (arrow). E: transverse sections of 3-mo-old m-SOL 120 h postexercise exhibiting 4 focal areas of small diameter, centrally nucleated fibers (×200). F: transverse section of 3-mo-old m-EDL 72 h postexercise. At no time after exercise were m-EDL different from control either in incidence of damaged fibers or in number of labeled nuclei (×200).

EXERCISE-INDUCED SATELLITE CELL ACTIVATION 1819
Recent ultrastructural studies by Friden et al. (7) have shown that 30 min of high-intensity eccentric ergometric exercise produces extensive myofibrillar disruption in human muscle fibers. Approximately one third of the fibers in the vastus lateris exhibited some focal disturbances in the cross-banding pattern 1 h after exercise and one-half by 72 h. After 6 days of recovery only 12% showed focal disturbances, suggesting that the exercise-induced ultrastructural injury did not lead to overt fiber degeneration or death. In the present study labeling of satellite cells on fibers that exhibited no injury at the light-microscopic level could account for the disproportionate proliferation and extensive labeling in the 1- and 3-mo-old muscles.

A possible mechanism for induction of the observed increase in satellite cell proliferation activity may be the release of mitogenic factors by the exercised muscles. Satellite cell mitogenic factors have been isolated from avian (9, 10) and mammalian (5) muscles where it is postulated that they are localized in the extracellular matrix or within myofibers. Such factors, released when the muscle is damaged, may play a role in activating satellite cells into a regeneration response. In the present study, satellite cells were activated even in muscles that exhibited no fiber necrosis. Therefore, if such factors induced the activation of satellite cells in exercised muscle, they were likely derived from fibers exhibiting focal disruptions at the ultrastructural level and/or from the connective tissue compartment which was presumably damaged in all the exercised muscles.

Connective tissue. The connective tissue compartment has previously been shown to exhibit signs of damage after eccentric exercise (1, 25), especially in the distal third of the muscle (3). Increased ratios of hydroxyproline to creatinine further suggested that there is proportionately more injury to the interstitium than to myofibers (1). Our labeling studies lend further support to the suggestion that the interstitium is damaged by intense eccentric exercise. At 48 h postexercise there was a proportionally greater increase in labeling of cells in the interstitium corresponding to the time when glucose 6-phosphate dehydrogenase levels were at their peak (4).

Pattern of Activation

The primary role of satellite cells in nongrowing, mature muscle is to repair or regenerate damaged myofibers (2). Satellite cells are activated to start proliferating on muscle injury, a response that produces a rapid increase in labeling within 24 h (23) and is sustained until repair of the myofibers is complete. Labeling in the mature exercised muscles followed this pattern. For example, labeling in the m-SOL was markedly elevated by 24 h postexercise and returned to control levels by 120 h. The reduction in labeling was coincident with the appearance of small-diameter basophilic fibers. Likewise the m-EDL also exhibited a similar labeling pattern that suggests a regeneration/repair response. However, the actual number of cells involved in the response appeared small, consistent with the observation that no morphologically identifiable damaged fibers were present and suggesting that satellite cells may have been involved in the repair of fibers that exhibited lesions only at the ultrastructural level.

In contrast, neither the amount of fiber necrosis nor the pattern of labeling in younger animals appeared consistent with a simple repair/regeneration paradigm. The major distinguishing characteristic of the labeling pattern in younger animals was the slow onset to peak labeling and sustained elevation above control levels. This pattern is similar to what has been reported to occur during compensatory hypertrophy of muscle (17, 20). Satellite cells in immature muscle function to provide myonuclei to growing myofibers as well as to repair damage. It is possible that in the absence of or with minimal myofiber damage, the greater force development resulting from eccentric contractions (3) could have stimulated the already growing muscles to hypertrophy. Further studies are needed to determine the fate of these newly formed cells.

Previous studies have demonstrated that severe muscle injury significantly reduces the proliferation potential of satellite cells that participate in repair of injured muscle (22). The observation of increased proliferative activity of satellite cells in acutely exercised muscles raises the possibility that repeated bouts of acute exercise over a lifetime might eventually compromise the regeneration potential of muscle. The extent to which training will abolish satellite cell activation in acutely exercised muscle is presently being examined.

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