Myosin Adenosine Triphosphatase Activity during Work-induced Growth of Slow and Fast Skeletal Muscle in the Normal Rat

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SUMMARY

The influence of work-induced hypertrophy on the myosin of skeletal muscle was studied. Unilateral tenotomy of the gastrocnemius muscle (single tenotomy procedure) in normal rats led to rapid compensatory growth of the synergistic plantaris and soleus muscles. Total myosin increased with hypertrophy. The myosin from these hypertrophied muscles, however, was enzymatically and structurally identical to the myosin from the contralateral limb muscles. Tenotomy of the plantaris in addition to the gastrocnemius (double tenotomy procedure) caused more rapid hypertrophy of the soleus than that observed with the single tenotomy procedure. The specific activity of the myosin ATPase from these hypertrophied soleus muscles was 11% lower (p < 0.05) than that of the myosin from the controls. However, the alkali stability of the ATPase activity and the light chain content of the myosin from the control and hypertrophied soleus muscles were identical. Thus, only the specific activity of the myosin ATPase accumulated in the soleus muscles during work-induced growth appears to depend on the rate of muscle hypertrophy. Preliminary studies on adult soleus, plantaris, and newborn rat muscle myosin showed that the decrease in specific activity of the hypertrophied soleus myosin ATPase cannot be explained by the selective accumulation of fast muscle or fetal muscle myosin.

MATERIALS AND METHODS

Compensatory hypertrophy of the soleus and plantaris muscles was induced by tenotomy of the synergist gastrocnemius muscle (single tenotomy procedure) in 250 to 300 g Sprague-Dawley rats under chloral hydrate (35 mg per kg intraperitoneal) anesthesia. In some experiments, the plantaris tendon was also severed (double tenotomy procedure) to increase the extent and rate of hypertrophy of the soleus muscle. A sham operation was performed on the contralateral limb; the tenotomy and sham operations were alternated between the right and left leg in successive hypertrophy experiments.

Adult rats were sacrificed by cervical fracture and the plantaris or soleus muscles or both rapidly excised, rinsed in 0.85% saline, chilled on ice, weighed, and minced. The hindlimb muscle tissue of 1-day-old Sprague-Dawley rats was used as a source of fetal myosin. In all cases, myosin was extracted from 1 g of minced muscle by a modified Szent-Györgyi method (12). All steps were carried out at 4°C, and all solutions were made 0.5 M with respect to KCl. The homogenate was centrifuged for 10 min at 1000 x g, and the precipitate washed three times with the same solution. Nine milliliters of the clear supernatant fraction were precipitated overnight in 207 ml of distilled water. The sediment was collected at 6000 x g for 10 min and the supernatant fraction was centrifuged at 30,000 x g for 20 min. Nine milliliters of the clear supernatant fraction were precipitated overnight in 207 ml of distilled water. The sediment was collected at 6000 x g for 10 min and the supernatant fraction was decanted; the sediment was made 0.5 M KCl equal to the wet weight of the sediment.

1 The abbreviations used are: TES, N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid; SDS, sodium dodecyl sulfate.

2 J. C. Seidel, personal communication.
dissolved by the addition of 0.5 M KCl to make the final concentration of protein equal to 3 to 4 mg per ml.

Myosin ATPase activity was assayed according to Sreter et al. (3) with a final concentration of 0.05 M Tris-HCl at pH 7.5, 5 mM ATP, 0.2 mg of myosin per ml, and either 0.05 M KCl, 10 mM CaCl₂, or 0.5 M KCl-1 mM EDTA to measure the Ca²⁺- and EDTA-activated ATPase activities, respectively. The incubation was started by addition of the ATP, and was stopped by addition of one-half the volume of cold 15% trichloroacetic acid. Inorganic phosphate (P₃) was determined by the method of Rocheh and Herrin (13). A zero time control was carried out by reversal of the order of addition of the trichloroacetic acid and ATP. Protein was measured by the Lowry method (14) with bovine serum albumin as a standard.

A modification of the method of Seidel (4) was used to inactivate myosin under alkaline conditions. All solutions were 0.5 M with respect to KCl. Myosin, 0.4 ml, (3 mg per ml) was incubated with 0.1 ml of 0.4 M glycine-NaOH buffer, pH 10.0, for 10 min at 100°C unless otherwise specified. The pH was lowered to 7.0 with 0.1 ml of 0.4 M potassium acetate-acetic acid buffer (pH 4.5) previously diluted to be equivalent to the glycine buffer, and the myosin (2 mg per ml) was placed on ice. The ATPase activity was measured immediately (at pH 7.5) and was compared to the activity of a control solution made by addition of 0.2 ml of a 1:1 mixture (pH 7.0) of the glycine and potassium acetate-acetic acid buffers to 0.4 ml of myosin (3 mg per ml).

Polyacrylamide SDS gel electrophoresis was performed by the method of Weber and Osborn (15) as modified by Adelstein et al. (16), except that samples were prepared by dialysis overnight against distilled water, followed by lyopholization, and reduction in 1% SDS in 1 mM sodium phosphate buffer (pH 7.0) with 50 mM dithiothreitol at 100°C for 5 min.³ Actin standards were kindly furnished by Dr. Adelstein. In some cases, samples were reduced and subjected to electrophoresis on polyacrylamide SDS gels and the approximate molecular weights of the light chains estimated from calibration curves as described by Neville (17).

RESULTS

Enzymic Activity and Structure of Myosin from Adult and Newborn Rat Muscles

Myosin Yields—The soleus and plantaris muscles of the normal adult rat yield 36 ± 5 mg and 32 ± 2 mg of myosin per g wet weight, respectively. These yields are similar to those obtained by Barany and Close (8) in their study of the transformation of myosin in cross-innervated soleus and plantaris muscles in the rat. They calculated that this yield represented 50% of the total myosin of the adult rat muscle. The pooled hindlimb muscle tissue of 1-day-old rats yielded only 10 ± 1 mg of myosin per g wet weight.

ATPase Activity—For each of the different muscles, the myosin ATPase activity showed the usual linear dependence on time and protein concentration in the Ca²⁺- and EDTA-KCl-activated assays. Heating of the enzyme for 5 min at 100°C caused complete loss of activity. Various ions activated and inhibited the ATPase activity in a fashion similar to that reported by Seidel (4) for purified rabbit muscle myosin (Table I). The Ca²⁺-activated ATPase activity was strongly (>95%) inhibited by a low concentration of Mg²⁺ ions. This inhibition of myosin

³ R. S. Adelstein, personal communication.

Myosin was extracted from normal adult rat plantaris and soleus muscles, and from 1-day-old rat hindlimb muscles as described under “Materials and Methods.” The ATPase activity was measured at 25°C for 5 min in 0.05 M KCl, 0.05 M Tris-HCl, pH 7.5, and 0.2 mg of myosin per ml with the additions noted below. Each value is the average of duplicate determinations. Similar results were observed in two other independent experiments.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Specific activity of myosin ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plantaris</td>
</tr>
<tr>
<td>None</td>
<td>0.01</td>
</tr>
<tr>
<td>5 mM MgCl₂</td>
<td>0.01</td>
</tr>
<tr>
<td>10 mM CaCl₂</td>
<td>0.01</td>
</tr>
<tr>
<td>10 mM CaCl₂</td>
<td>0.52</td>
</tr>
<tr>
<td>1 mM EDTA + 0.5 M KCl</td>
<td>1.22</td>
</tr>
</tbody>
</table>

ATPase activity is in contrast to the activation of actomyosin (18) and particulate (19) ATPase activities by Mg²⁺ ions, and suggests that large amounts of these contaminants are not present in our myosin preparations.

The specific activity of the myosin ATPase from the three different muscles was compared. Both the Ca²⁺- and the EDTA-KCl-activated ATPase activities of myosin from the soleus (slow) muscle were less than the corresponding activities of the myosin from the plantaris (fast) muscle (Table I). The specific activity of the rat plantaris myosin ATPase is very similar to that reported by Sreter et al. (3) for rabbit fast muscle myosin using the same extraction and assay techniques. On the other hand, the rat soleus muscle myosin ATPase has a higher specific activity than Sreter et al. (3) reported for rabbit soleus muscle myosin. This finding may reflect the fact that the adult rat soleus muscle, unlike the adult rabbit soleus muscle, is not homogeneous and contains some fast fibers (20). The newborn rat hindlimb muscle myosin ATPase specific activity was similar to that of adult rat soleus muscle myosin, but higher than has been previously reported (7). The inclusion of EDTA in our extraction technique to prevent oxidation of myosin may account for our higher values. Dow and Stracher (21) have shown that fetal myosin is more susceptible to loss of activity due to oxidation than is adult muscle myosin.

The sensitivity to alkali of myosin ATPase activity from the three different muscles was compared. The ATPase activity of the myosin was determined at pH 7.5 before and after incubation under alkaline conditions as described in Fig. 1. The ATPase activity of the enzyme after incubation in alkali showed the same order of activation and inhibition by EDTA-KCl, Ca²⁺, and Mg²⁺ as before incubation, demonstrating that the surviving ATPase activity is in fact due to myosin (4). The activity of myosin ATPase from adult plantaris muscles was more stable than that from adult soleus muscles at all alkaline incubation conditions that were examined (Fig. 1). The greatest difference was observed when the myosin solutions were incubated at pH 10.0 for 10 min (Fig. 2). Plantaris muscle myosin retained 95% of the initial ATPase activity, whereas soleus muscle myosin retained only 34%. This result is in accord with similar measurements by Samaha et al. (22). The ATPase activity of myosin extracted from the newborn rat hindlimb muscle tissue showed a stability in alkali similar, although not identical,
muscle myosin contained three different types of light chains described under "Materials and Methods." The plantaris 27,000 and 20,000; the molecular weights were estimated as types of light chains with approximate molecular weights of 25,000, 18,000, and 16,000. Sarkar et al. (5) and Lowey and Risby (6) reported similar molecular weights for slow and fast adult rabbit muscle myosin light chains. Faint bands corresponding to the plantaris muscle light chains were seen in the soleus muscle myosin SDS gel patterns. This finding confirms the presence of some fast muscle myosin in the rat soleus as suggested by Guth (20). Interestingly, the electrophoresis pattern of myosin light chains of the newborn hindlimb muscle (muscle tissue destined to become predominantly fast muscle) was identical to that of the adult plantaris. That the myosin preparations were substantially free of actin was confirmed by inclusion of actin standards in some gels.

Subunit Structure of Rat Muscle Myosins—The subunit composition of myosin from the three different muscles was compared by SDS gel electrophoresis. No difference in the molecular weight of the heavy chains was found in 7.5% polyacrylamide gels that were prepared as described by Adelstein et al. (16). The light chain content of the different muscle myosins was determined in similarly prepared 10.0% polyacrylamide gels (Fig. 3). Soleus muscle myosin contained predominantly two types of light chains with approximate molecular weights of 27,000 and 20,000; the molecular weights were estimated as described under "Materials and Methods." The plantaris muscle myosin contained three different types of light chains with the approximate molecular weights of 25,000, 18,000, and 16,000. Sarkar et al. (5) and Lowey and Risby (6) reported similar molecular weights for slow and fast adult rabbit muscle myosin light chains. Faint bands corresponding to the plantaris muscle light chains were seen in the soleus muscle myosin SDS gel patterns. This finding confirms the presence of some fast muscle myosin in the rat soleus as suggested by Guth (20). Interestingly, the electrophoresis pattern of myosin light chains of the newborn hindlimb muscle (muscle tissue destined to become predominantly fast muscle) was identical to that of the adult plantaris. That the myosin preparations were substantially free of actin was confirmed by inclusion of actin standards in some gels.

Fig. 1. Alkali stability of myosin ATPase activity. Aliquots of myosin (3 mg per ml) from adult (250 g) rat soleus and plantaris muscles were incubated in 0.5 M KCl with 0.4 M glycine-NaOH buffer over a range of pH values for various times at 25° as described under "Materials and Methods." The pH was lowered to 7.0 with 0.4 M potassium acetate-acetic acid buffer (pH 4.5) that had been previously diluted to be equivalent to each glycine buffer, and the myosin (2 mg per ml) placed on ice. The ATPase activity before and after incubation in alkali was measured in 0.5 M KCl, 0.05 M Tris-HCl, pH 7.5, 5 mM ATP, and 1 mM EDTA with 0.2 mg of myosin per ml for 5 min at 25°. The stability of the activity to incubation in alkali was calculated from the activity before and after incubation in alkali as a percentage of the initial ATPase activity. The control sample was made by addition of a 1:1 mixture (pH 7.0) of the corresponding buffers to an aliquot of the original myosin solution. Initial activities of these control samples were 0.88 ± 1.22 μmoles of P released per mg of myosin per min for soleus and plantaris myosin, respectively. Each point is the average of duplicate determinations. Similar results were obtained in two other independent experiments. The pH of the glycine buffers used is noted on the graphs.

Fig. 2. Alkali stability of myosin ATPase activity. Myosin from adult rat soleus, plantaris, and 1-day-old rat hindlimb muscles was incubated for 10 min at 25° in 0.5 M KCl with 0.4 M glycine buffer at different pH values and the stability of the ATPase activity was determined exactly as in Fig. 1. The initial ATPase activity was 0.88, 1.22, and 0.88 μmoles of P released per mg of myosin per min for soleus, plantaris, and 1-day-old rat hindlimb muscle myosin, respectively. •, soleus muscle myosin; □, plantaris muscle myosin; ▲, newborn hindlimb muscle myosin.

To that of plantaris muscle myosin (Fig. 2). Therefore, incubation at pH 10.0 for 10 min was selected as the condition for determining the alkali stability of rat myosin ATPase activity. There is a 3-fold difference in the stability of adult soleus and plantaris myosin ATPase activity to this incubation, and the stability of newborn hindlimb muscle myosin ATPase activity was comparable to adult plantaris myosin ATPase activity.

Subunit Structure of Rat Muscle Myosins—The subunit composition of myosin from the three different muscles was compared by SDS gel electrophoresis. No difference in the molecular weight of the heavy chains was found in 7.5% polyacrylamide gels that were prepared as described by Adelstein et al. (16). The light chain content of the different muscle myosins was determined in similarly prepared 10.0% polyacrylamide gels (Fig. 3). Soleus muscle myosin contained predominantly two types of light chains with approximate molecular weights of 27,000 and 20,000; the molecular weights were estimated as described under "Materials and Methods." The plantaris muscle myosin contained three different types of light chains
FIG. 3. Myosin light chains. Electrophoresis of adult rat soleus, plantaris, and 1-day-old rat hindlimb myosin was done in 10.0% polyacrylamide SDS gels prepared as described by Adelstein (16). The samples had ATPase activities similar to those in Table I. Samples were prepared as described under "Materials and Methods," and electrophoresis was carried out in 0.02 M sodium phosphate buffer (pH 7.0) containing 0.1% SDS at a constant current of 6 mA per gel for 70 min. The length of the gels was 5 cm; they were stained with aniline blue black (Canalco). The amount of each type of rat muscle myosin applied is noted below each gel. Note that the mixture of rat soleus and plantaris muscle myosin and the mixture of soleus and newborn hindlimb muscle myosin showed more resolvable bands than the corresponding unmixed samples, whereas the mixture of plantaris and newborn hindlimb muscle myosin had the identical number of bands seen in the unmixed samples.

**Table II**

ATPase activity of myosin extracted from mixtures of adult and newborn rat muscles

<table>
<thead>
<tr>
<th>Proportion of muscle by wet weight</th>
<th>Specific activity of myosin ATPase</th>
<th>Alkaline stability of EDTA-KCl-activated myosin ATPase activity (as % of initial activity remaining)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cap+ activated</td>
<td>EDTA-KCl-activated</td>
</tr>
<tr>
<td>10 Adult plantaris</td>
<td>0.54</td>
<td>1.17</td>
</tr>
<tr>
<td>9 Adult soleus</td>
<td>0.53</td>
<td>1.15</td>
</tr>
<tr>
<td>8 Newborn hindlimb</td>
<td>0.51</td>
<td>1.11</td>
</tr>
<tr>
<td>5 Adult plantaris</td>
<td>0.46</td>
<td>1.07</td>
</tr>
<tr>
<td>10 Adult soleus</td>
<td>0.32</td>
<td>0.85</td>
</tr>
<tr>
<td>9 Newborn hindlimb</td>
<td>0.32</td>
<td>0.85</td>
</tr>
<tr>
<td>8 Adult soleus</td>
<td>0.32</td>
<td>0.85</td>
</tr>
<tr>
<td>10 Newborn hindlimb</td>
<td>0.32</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Tenotomy of the synergistic gastrocnemius muscle causes compensatory hypertrophy of the soleus and plantaris muscle that is complete in 5 to 6 days (11). In the present study we have also examined hypertrophy of the soleus muscle in response to simultaneous tenotomy of both the plantaris and gastrocnemius muscles; this technique increases both the rate and extent of hypertrophy of the soleus muscle (2). The yields of myosin (milligrams per g wet weight muscle) from the hypertrophied soleus muscles 3 and 6 days after either operation were about 10% less than that obtained from the control limb muscles. In spite of this decrease, however, the increase in wet weight of the hypertrophied muscle was such that the total muscle myosin content increased with hypertrophy (Fig. 4). This estimate of the amount of myosin that accumulated during work-induced growth agrees well with the previous report of a 10% increase in the total soleus muscle myofibrillar nitrogen content with a 30% increase in wet weight (2).

The magnitude of the accumulation of myosin during compensatory hypertrophy noted above is such that a selective increase in fast or fetal myosin in the soleus muscle, or of slow myosin in the plantaris muscle, could be detected by a study of the distinctive properties of the various types of myosin, i.e. specific activity and alkaline stability of the myosin ATPase, and the light chain content of the myosin. If, on the other hand, increased external work results in the accumulation of the same type of myosin that was initially present in the muscle, the hypertrophied muscle myosin would be identical to that of the corresponding control limb muscle myosin.
Fig. 4. Relationship between the increase in total myosin to the gain in weight by hypertrophied muscles. The total myosin was estimated by the product of the myosin yield (milligrams per g wet weight muscle) and the average wet weight of the muscles. Net growth and the gain in total myosin by the hypertrophied muscle were both expressed as the percentage increase above that found in the corresponding muscle from the contralateral sham-operated limb. Each point is the average value of three separate determinations at 3 (open symbol) and 6 (closed symbol) days after the single tenotomy procedure (soleus, ○; plantaris, □), and the double tenotomy procedure (soleus, △).

Table III

Effect of hypertrophy on soleus and plantaris muscle myosin ATPase activity

Hypertrophy of the soleus and plantaris muscle was induced by unilateral tenotomy of the gastrocnemius muscle (single tenotomy procedure). The extent of growth was calculated from the average ratio of hypertrophied muscle wet weight per control muscle wet weight as in Fig. 4, and in accord with previous findings by Goldberg (1). Myosin was extracted and the EDTA-KCl-activated ATPase activity assayed before and after incubation at pH 10.0 for 10 min as described under "Materials and Methods." The percentage of the ATPase activity stable to incubation in alkali was calculated as in Fig. 1. Each value is the mean of duplicate determinations for three independent experiments. Note that there is no significant difference in the specific activity or the alkali stability of the myosin ATPase from the hypertrophied muscles compared to the corresponding control limb muscles.

<table>
<thead>
<tr>
<th>Type of muscle and days after operation</th>
<th>Extent of growth</th>
<th>EDTA-KCl-activated myosin ATPase</th>
<th>Alkali stability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Specific activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Hypertrophied</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μmoles Pi/mg myosin/min</td>
<td>% of initial activity remaining</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No operation</td>
<td></td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.89</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>0.89</td>
<td>0.86</td>
</tr>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No operation</td>
<td></td>
<td>1.22</td>
<td>1.22</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>1.21</td>
<td>1.21</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>1.27</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Characteristics of Myosin from Hypertrophied Muscles: Hypertrophy of Soleus and Plantaris Muscles Produced by Single Tenotomy Procedure—Moderate hypertrophy of the soleus and plantaris muscles was induced by severing the gastrocnemius tendon. This operation has the advantage of inducing growth in both a fast (plantaris) and slow (soleus) muscle. The specific activity of the EDTA-KCl-activated ATPase of myosin from the hypertrophied plantaris muscles was identical to that from the contralateral, sham-operated plantaris myosin (Table III). The small fall (3%) in the specific activity of the EDTA-KCl-activated ATPase of myosin from the hypertrophied soleus muscles compared to that of myosin from the control soleus muscles was not statistically significant (p > 0.5). The alkali stability of the myosin ATPase activity from the hypertrophied muscles was identical to that of the myosin from the corresponding control limb soleus and plantaris muscles, as was the Ca2+ activation and Mg2+ inhibition of the myosin ATPase activity. There was no qualitative difference between the 10% SDS gel electrophoresis pattern of the myosins from the corresponding control and hypertrophied muscles. Our results indicate that both during the course of hypertrophy (3 days after operation) and on completion of work-induced growth (6 days after operation), the myosin from the hypertrophied soleus (slow) and plantaris (fast) muscles is enzymatically and structurally indistinguishable from myosin in the corresponding control limb muscles.

Table IV

Effect of hypertrophy on soleus muscle myosin ATPase activity

Experiments were performed exactly as those shown in Table III except that hypertrophy of the soleus muscle was induced by unilateral tenotomy of the gastrocnemius and plantaris muscles (double tenotomy procedure). Each value is the mean of duplicate determinations for three independent experiments. The gain in weight for this operation is greater than that shown in Table III for the soleus muscle, and is in accord with previous findings (2). The specific activity of the myosin ATPase from the hypertrophied soleus muscles is significantly (p < 0.05) less than that from the control soleus muscles.
accumulate in muscles during the early stages of hypertrophy. This study was undertaken to determine the type of myosin present in a muscle. In cross-innervation experiments (8, 9), it has been shown that changing the nerve that innervates a muscle can change the type of myosin in that muscle.

To better understand the underlying molecular basis of this decrease in specific activity of myosin ATPase, a preliminary study of the enzymatic and structural properties of rat muscle myosins was carried out.

Our results indicate that the specific activity of myosin ATPase cannot be used as the sole criterion for distinguishing between the various types of myosin in rat skeletal muscles. Whereas soleus and plantaris muscle ATPases are readily distinguished by this criterion, soleus and newborn-hindlimb myosins are not (see Table I). Another disadvantage of the reliance on this characteristic of myosin is that the value can vary by as much as 15% in different myosin preparations from the same muscle, a variation that is probably due to differences in the extent of inactivation of the myosin ATPase during the extraction procedure (3). Whereas, the specific activity of the ATPase of myosin from the adult soleus muscle is the same as that of myosin from newborn rat hindlimb muscle tissue, these myosins differ strikingly in the stability of the ATPase activity to incubation in alkali and in the molecular weight of their light chains. In further contrast to specific activity values, measurements of the alkali stability of the ATPase activity of different myosin preparations from the same muscles agreed within 2%. This consistency is probably due to the fact that the alkali stability of the myosin ATPase activity is a function only of the active enzyme present. Evidence in support of this explanation for the difference in the reproducibility of the two measurements (specific activity versus alkali stability of myosin ATPase) is that the addition of heat-inactivated myosin lowered the specific activity of the ATPase of a myosin solution, but did not alter the alkali stability of the ATPase activity of the same solution. A myosin preparation can also be reproducibly evaluated independently of the enzyme activity. SDS polyacrylamide gel electrophoresis allows not only the identification of the light chains that are present, but also provides information on other muscle proteins that might be altered during hypertrophy.

During skeletal muscle hypertrophy, Goldberg (23) reported that the rate of myosin synthesis is increased and that there is a simultaneous decrease (24) in the rate of its degradation. Our study extends these results with a description of the type of myosin accumulated. During hypertrophy of the rat soleus (slow) and plantaris (fast) muscles following the single tenotomy procedure, myosin that is indistinguishable from that initially present in these muscles accumulates. We found no evidence for the production of a different type of myosin in these hypertrophied skeletal muscles. This adaptation to increased work is in contrast to the role of neural regulation on the type of myosin present in a muscle. In cross-innervation experiments (8, 9), it has been shown that changing the nerve that innervates a muscle can change the type of myosin in that muscle.

In subsequent experiments, the work load on the hypertrophying soleus was increased by simultaneous tenotomy of the gastrocnemius and plantaris. Following this double tenotomy procedure, we found, in agreement with previous results (10), that there was a significant decrease of the specific activity of the myosin ATPase from hypertrophied soleus muscles. This result is compatible with the idea that during the rapid hypertrophy that is induced by the double tenotomy procedure, myosin of the same type as that originally present accumulates, but that the specific activity of its ATPase is low for one of the following reasons: (a) myosin is inactivated in vivo during the course of hypertrophy, e.g. by muscle cell injury, (b) myosin is inactivated during the extraction process, e.g. by denaturation.

**Fig. 5.** Electrophoresis of control and hypertrophied soleus myosin in 11.0% polyacrylamide SDS gels prepared at pH 9.5 as described by Neville (17). Gels were 7-cm long and stained with Coomassie brilliant blue. The predominant low molecular weight proteins are the light chains. Although one can see some intensity differences, the mixture of the hypertrophied and control soleus muscle myosin had the same number of resolvable bands as the unmixed samples. The molecular weight of the light chains was estimated from calibration curves prepared by Neville (17) and are only approximate, as standards were not run in the same gels. The amount of control and hypertrophied soleus muscle myosin applied is noted below each gel, and the ATPase activities of the samples are the same as those shown in Table IV.

<table>
<thead>
<tr>
<th>Light Chain Molecular weight</th>
<th>Control Soleus (µg)</th>
<th>30</th>
<th>15</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophied Soleus (µg)</td>
<td>27,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>1</td>
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</tbody>
</table>

**Table IV.** Enzymatic properties of control and hypertrophied soleus myosin. ATPase activity was measured at 20°C using Mg2f as substrate. Activity of the same solution was measured at 37°C using heat-inactivated myosin as substrate. Mg2f inhibited more than 95% of the Ca2f-activated ATPase activity of both the hypertrophied and control soleus muscle myosin. The alkali stability of myosin ATPase from the hypertrophied soleus muscle myosin was identical to that of the control soleus muscle and no qualitative difference was seen in 11.0% SDS gel electrophoresis patterns of the myosins studied with the very sensitive Coomassie brilliant blue dye (Fig. 5). Identical results were obtained 3 and 6 days after the operation.

**DISCUSSION**

It has been demonstrated that tenotomy causes hypertrophy and an increase in myosin content in the synergistic muscle(s) (2). This study was undertaken to determine the type of myosin that accumulates during this work-induced growth of skeletal muscle. The possibility that fast, slow, or fetal myosin might be selectively synthesized during muscle hypertrophy was considered, especially in light of the recent studies by Margreth and Salviati (10). These authors found a significant decrease of the specific activity of myosin ATPase of hypertrophied rat soleus muscles following the double tenotomy procedure and suggested that a fetal-type of myosin might accumulate in muscles during the early stages of hypertrophy.

To better understand the underlying molecular basis of this
or oxidation, or (c) incomplete, inactive myosin is accumulated due to disproportionate rates of synthesis of the subunits. Alternatively, a new type of myosin is selectively accumulated with the following properties: the specific activity of the ATPase of this new myosin is lower than that of soleus muscle myosin, but the alkali stability of the ATPase activity and the light chain content are the same as the corresponding properties of soleus muscle myosin.

We are presently unable to distinguish between these alternatives. However, we feel that the decrease in the specific activity of the myosin ATPase from the hypertrophied soleus muscle following the double tenotomy procedure represents an in vivo change. That the decrease is not an artifact of the extraction technique, is supported by the finding that it did not characterize myosin from the hypertrophied soleus muscle following the single tenotomy procedure even at a time when a comparable extent of growth (30%) and myosin accumulation (9%) had occurred. The possibility that a new type of myosin is synthesized can be explored in more detail as more enzymatic and structural properties of myosin are elucidated. For example, Kuehl and Adelstein (25) recently reported a difference in the methylated-amino acid content of adult and fetal rabbit myosins. It would be of interest to examine myosin accumulated during skeletal muscle hypertrophy with respect to this property.

In our previous study of the contractile properties of the hypertrophied soleus muscle (26), the double tenotomy procedure was used to induce hypertrophy. Although we did not measure the ATPase activity of the hypertrophied muscles used in that study, from our present results it is reasonable to conclude that those muscles were characterized by a significant decrease in the specific activity of the myosin ATPase. Although we found that the total tension of the hypertrophied muscle was the same as, or greater than, that of the control, the maximal velocity of shortening and tetanic tension (normalized for cross-sectional area) was significantly reduced. These findings are consistent with muscle failure (27). The contractile properties of the hypertrophied soleus muscle following the single tenotomy procedure remain to be determined. If the decrease in the specific activity of myosin ATPase correlates with the presence of muscle failure in hypertrophied skeletal muscle, in the same way as it does in the heart following cardiac hypertrophy (28), then the combination of the double and single tenotomy procedures in one animal would provide an internally controlled system for the study of skeletal muscle hypertrophy with and without muscle failure.

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