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CHANGES IN TROPOMYOSIN SUBUNIT PATTERN IN CHRONIC ELECTRICALLY STIMULATED RABBIT FAST MUSCLES

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SUMMARY: The tropomyosin subunit ratio of rabbit fast muscle (α:β = 80:20) changes to that characteristic of skeletal slow muscles (α:β = 55:45) on continuous (10 Hz) stimulation for 3 weeks. The altered myosin light chain pattern and histochemical ATPase stain also show clear changes of fast → slow transformation. However, the rate of changes in the light chain patterns of myosin are slower than those of tropomyosin subunits. These results do not support the previous finding (Amphlett et al., Nature 257, 602, 1975) that the tropomyosin subunit pattern remains unaltered during transformation of skeletal muscles and the conclusion that the genetic expression of tropomyosin is regulated under separate control from other myofibrillar proteins. Rather, our results suggest that the polymorphic patterns of all myofibrillar proteins in skeletal muscles undergo changes in a temporal manner during skeletal muscle transformation.

It is well known that distinct physiological, morphological and biochemical differences exist between vertebrate skeletal fast and slow-twitch muscles (1-4). The majority of the myofibrillar proteins present in these muscles exist in multiple forms coded by different structural genes. The presence and the relative proportion of these isozymes is related to differences in the functional properties of skeletal fast and slow-twitch muscles (5-11). Fast or slow muscle can be transformed in terms of physiological, biochemical and ultrastructural properties (13-29) into the opposite type by cross-reinnervation (12-19) or, in the case of a fast muscle, by chronic electrical stimulation of the intact nerve (20-24,29). At the biochemical level, changes in the type of myosin and troponin I, the enzymes of energy metabolism, and the Ca^{2+}-transporting activity of the sarcoplasmic reticulum accompany such transformation (14,16,18,21,24-28). These changes, as shown for myosin, are due to changes in gene expression within the

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pre-existing fibers, i.e. the same fiber switches from the synthesis of fast to slow type myosin (24,29).

In view of the manifold changes that occur upon cross-reinnervation or on stimulation induced transformation, the report that the myofibrillar protein tropomyosin (Tm)[1], whose subunit ratios are distinctly different in fast and slow muscles (8), remained unaltered following cross-reinnervation (18) was rather puzzling. We have reinvestigated this problem by using low-frequency chronic electrical stimulation of rabbit fast muscles, since this process brings about more rapid and complete transformation than cross-reinnervation (21,23). Our results indicate that the Tm subunit pattern characteristic of fast skeletal muscle is quantitatively changed to that of the slow-twitch type and that these changes take place at a relatively early stage of chronic electrical stimulation of rabbit fast muscles.

MATERIALS AND METHODS

Chronic stimulation of the rabbit left common peroneal nerve, which supplies the fast-twitch muscles (extensor digitorum longus (EDL), tibialis anterior (TA) and the peroneal muscles) was performed by the use of an implanted microstimulator as described earlier (21,23). After stimulation at 10 Hz for various lengths of time, the animals were killed and both the stimulated muscles and the contralateral unstimulated ones were quickly excised. Both EDL and TA, as well as soleus muscles, were isolated from unstimulated rabbits as control fast and slow-twitch muscles.

Myosin and Tm were isolated and purified from the muscle samples by our previously published procedures (5,30,31). Histochemical staining for myosin ATPase after acid preincubation (pH 4.3) was carried out on frozen serial thin sections (8 μM) of muscle according to the procedure described by Brooke and Kaiser (32). SDS-polyacrylamide gel electrophoresis in 10% gels, densitometric scans of the Coomassie blue-stained gels and determination of the molar stoichiometry of the myofibrillar proteins were carried out as described previously (30).

RESULTS

Tm subunit pattern of stimulated muscle: Fig. 1 shows the SDS-gel electrophoretograms of Tm isolated from stimulated and contralateral rabbit fast

[1]Abbreviations used: Tm, tropomyosin; EDL, extensor digitorum longus; TA, tibialis anterior; $LC_{1(s)}$, slow myosin light chain 1; $LC_{2(s)}$, slow myosin light chain 2; $LC_{1(f)}$, fast myosin light chain 1; $LC_{2(f)}$, fast myosin light chain 2; LC_3, fast myosin light chain 3; SDS, sodium dodecyl sulfate; SR, sarcoplasmic reticulum.

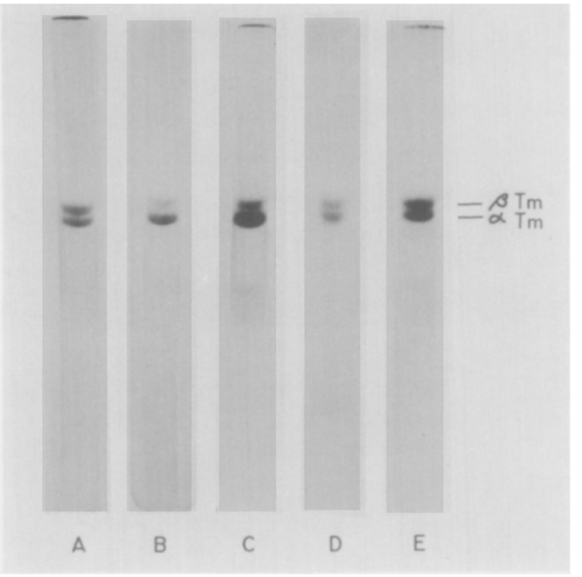

Fig. 1. SDS-polyacrylamide gel electrophoresis of Tm from control and stimulated muscles. Amount of protein used: A) Tm from slow muscle of unstimulated animal, 10 µg; B) Tm from fast muscle of unstimulated animal, 10 µg; C) Tm from contralateral fast muscle, 12 µg; D) Tm from 3-weeks stimulated fast muscles, 10 µg; E) Tm from 5-weeks stimulated fast muscles, 12 µg.

muscles as well as from unstimulated rabbit fast and slow muscles. Chronic stimulation changes the high α:β subunit ratio (80:20) of Tm of a typical fast muscle (gel B) to one characteristic of slow muscle (gel A; α:β = 55:45). The first noticeable change in the subunit picture occurred in muscle stimulated for 3 weeks (gel D). From an estimate of the subunit ratios obtained from densitometric scans of the gel runs, it was observed that at 3 weeks the Tm subunit ratio of the fast muscles changed completely and was identical to that of slow muscles (compare gel D vs. gel A). Continued stimulation beyond 3 weeks did not alter the subunit ratio any further, as has been shown with Tm isolated from 5-weeks stimulated muscles (gel E). Tm isolated from contralateral fast muscle did not show any change in the subunit ratio (gel C, α:β = 80:20).

Myosin light chain patterns and histochemical staining for myosin ATPase activities in stimulated muscles: Changes in the properties of the myosin molecule, as judged by changes in its light chain pattern and myosin ATPase activities, are correlated with the transformation of the muscle fibers as a

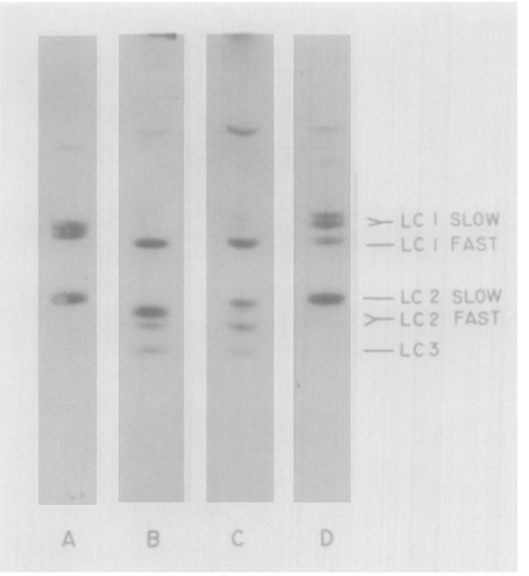

Fig. 2. SDS-polyacrylamide gel electrophoresis of myosin from control and stimulated muscles. 35 µg of myosin was applied to each gel. A) Myosin from slow muscle of unstimulated animal; B) Myosin from fast muscle of unstimulated animal; C+D) Myosin from 3 and 5 weeks stimulated fast muscles, respectively.

result of chronic stimulation (28). We have compared these changes (Figs. 2 and 3) with the changes in the Tm subunit pattern in the same stimulated muscles. Comparison of myosin light chains (Fig. 2) of 3-weeks stimulated fast muscles (gel C) with those of unstimulated fast (gel B) and slow muscles (gel A) indicates that at 3 weeks, while all three light chains of fast myosin are present, the slow myosin light chains $LC_{1(s)}$ and $LC_{2(s)}$ are also observed as minor and major components, respectively. Even after 5 weeks of stimulation (gel D), when $LC_{2(f)}$ and LC_3 were completely gone, some $LC_{1(f)}$ was still present. These results are consistent with previous reports (29) indicating that the complete conversion of myosin light chains from fast to slow type following chronic electrical stimulation takes place over a long period of time. Comparison with histochemical ATPase activity after acid (pH 4.3) incubation shows that muscles whose Tm subunit pattern changes also change from the fast to the slow type by these criteria (Fig. 3). The contralateral muscle (Panel A) showed the presence of a few slow fibers (darkly stained), but the majority of the fibers

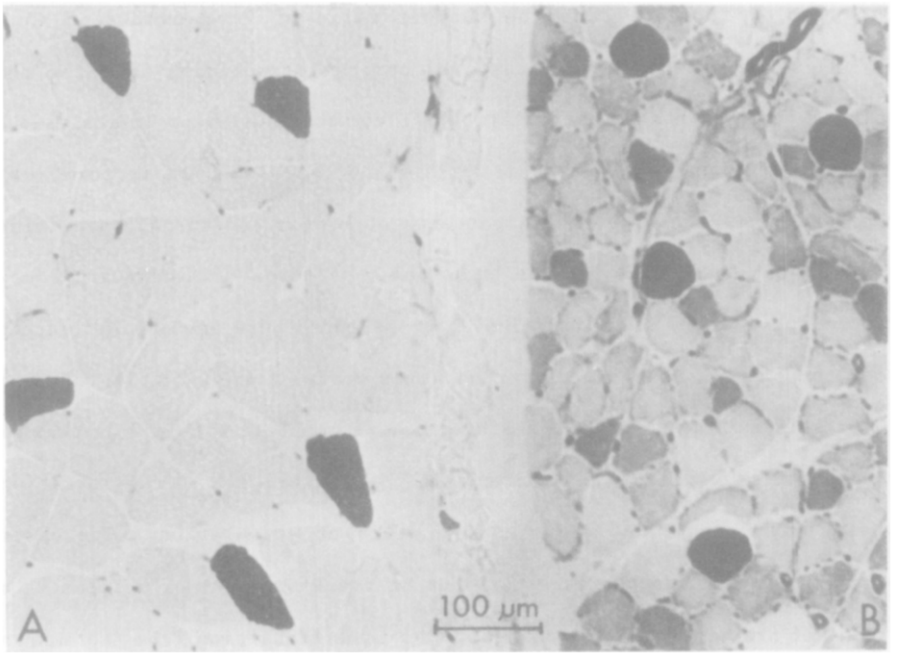

Fig. 3. Serial sections of peroneus muscles stained for ATPase activity after acid preincubation at pH 4.3. For details see Materials and Methods. A) Contralateral muscle; B) 3-weeks stimulated muscle.

were fast type and did not stain at all. However, the stimulated muscles at 3 weeks (Panel B), in addition to showing the few darkly stained fibers, contained fibers which stained with various degrees of intensity, indicating that the fibers were in the process of gradual transformation from fast (type II) to slow (type I) type. By 5 weeks this progressive transformation of fast fibers became more apparent as judged by the increase in the number of darkly stained slow fibers (results not shown).

DISCUSSION

In trying to answer the question whether or not transformation of muscles causes coordinated changes in myosin and Tm isozymes, we have chosen low frequency stimulation of intact fast muscles instead of cross-reinnervation for the following reasons. The changes induced by this method are more complete, take place more rapidly, and avoid the complications due to initial denervation atrophy of the fibers which takes place as a result of cross-reinnervation.

Our results indicate that the subunit ratio of Tm does change in fast muscles under the influence of chronic electrical stimulation, and the change takes place within 3 weeks of stimulation. The muscles used for Tm analysis show clear changes of transformation from the fast to the slow type in terms of two criteria used, viz. the light chain patterns of myosin and the ATPase stains at pH 4.3. However, the changes in the light chain patterns of myosin are somewhat slower than those of Tm subunits. The data presented here seem to indicate that different components change at different rates during transformation of muscles by chronic stimulation, e.g. SR transformation is complete within 2-3 weeks as is the transformation of the metabolite enzyme pattern (25,28). Transformation of the myosin light complement requires 6-8 weeks of stimulation. The observed early changes in the Tm subunit pattern during transformation of muscles is also reminiscent of its developmental changes, where the transition from the embryonic to adult types is complete earlier compared to myosin light chain complements in avian skeletal muscle (31).

In summary, our results do not support the previous report that the Tm subunit pattern remains unchanged during transformation of skeletal muscles, thereby excluding the possibility that the genetic expression of its subunits is under separate control from other myofibrillar proteins, such as myosin light chains and troponin I (18). Rather, they suggest that all myofibrillar proteins showing characteristic isozymes in skeletal fast and slow muscles undergo changes to the opposite type during fiber transformation. However, there are differences in the rate of transformation among different myofibrillar proteins, as has been shown here for Tm subunits and myosin light chains.

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