Novel transitions in MHC isoforms: separate and combined effects of thyroid hormone and mechanical unloading

VINCENT J. CAIOZZO,1,2 MICHAEL J. BAKER,2 AND KENNETH M. BALDWIN1

Departments of 1Physiology and Biophysics and 2Orthopaedics, College of Medicine, University of California, Irvine, California 92717

CAIOZZO, Vincent J., Michael J. Baker, and Kenneth M. Baldwin. Novel transitions in MHC isoforms: separate and combined effects of thyroid hormone and mechanical unloading. J. Appl. Physiol. 85(6): 2237–2248, 1998.—Single-fiber (n = 3,818 fibers) electrophoretic analyses were used to delineate the separate and combined effects of hyperthyroidism (T3) and hindlimb suspension (HS) on the myosin heavy chain (MHC) isoform composition (1-, 2-, and 4-wk time points) of the rat soleus muscle. The key findings of this study are as follows. First, T3 and HS both altered the distribution of MHC isoforms at the single-fiber level; however, the populations of fibers produced by these two interventions were clearly different from one another. Second, T3 + HS rapidly converted the soleus into a fast muscle, producing large increases in the relative contents of the fast type IIX and IIb MHC isoforms which were primarily expressed in several populations of hybrid fibers (e.g., types I/IIa/IIX, I/IIX/IIb, I/IIa/IIX/IIb). Finally, T3 + HS produced unique populations of hybrid fibers that did not adhere to the I→IIX→IIb sequential scheme of MHC plasticity. Collectively, the findings of this study demonstrate that the intervention of T3 + HS is a powerful model for manipulating and studying MHC isoform plasticity in slow skeletal muscle.

myosin heavy chain; single-fiber electrophoresis; muscle plasticity; transitions; rat; soleus muscle; hybrid fibers; polymorphic expression

IN PREVIOUS STUDIES, WE (2–5, 7–8, 11, 24) and others (1, 6, 14–17, 21, 25) reported that both hyperthyroidism (T3) and hindlimb suspension (HS) produced significant increases in the fast type IIX and IIb MHC isoforms which were exclusively expressed in several populations of hybrid fibers (e.g., types I/IIa/IIX, I/IIX/IIb, I/IIa/IIX/IIb). However, in subsequent studies with the use of immunohistochemical techniques, we observed that T3 (5) and HS (7) affected some, but not all, slow type I fibers in the soleus muscle. As a consequence, we speculated that the soleus muscle contains so-called “refractory” fibers, i.e., fibers that are incapable of altering their MHC phenotype.

We addressed the issue of refractory fibers further in a recent study (2) that examined the combined effects of T3 and HS. This combination of stimuli produced remarkable alterations in both whole muscle and single-fiber MHC isoform phenotype, such that all slow type I fibers in the soleus muscle were transformed into hybrid fibers expressing various combinations of both slow or fast MHC isoforms. Hence, on the basis of this finding, it is clear that the soleus muscle does not contain so-called refractory fibers. Importantly, the single-fiber analyses also yielded two other key findings. First, the soleus muscles appeared to express subpopulations of slow type II fibers that differed in their response to altered thyroid and mechanical loading conditions; this suggests that T3 and HS alter MHC isoform phenotype via divergent processes. Second, we observed populations of hybrid fibers (polymorphic expression) with unique combinations of MHC isoforms (I/IIX and I/IIX/IIb) that were inconsistent with the I→IIX→IIb scheme of MHC plasticity proposed by Pette and coworkers (see Refs. 13, 18, 23, 26).

Because our previous study (2) examined the effects of T3, HS, and T3 + HS at only a single time point (4 wk), the objective of this study was to perform a time course analysis of MHC isoform alterations produced by each of these interventions. This approach was utilized so that transitions in fiber populations could be described in better detail, thereby providing more in-depth analyses of 1) the separate and combined actions of T3 and HS, and 2) the specific patterns of MHC coexpression, with further emphasis on examining the I→IIa→IIX→IIb transformation scheme of MHC isoform plasticity.

The key findings of the present study demonstrate that the similarities/dissimilarities of the responses to the separate interventions of T3 and HS are both MHC isoform specific and time dependent, and these changes can only be accurately portrayed by single-fiber analyses. Additionally, the unique intervention of T3 + HS clearly produced populations of hybrid fibers (as early as 1 wk) that did not conform to the I→IIX→IIb sequential scheme of MHC isoform plasticity. These findings are complemented by a companion report (11) that shows that these MHC isoform transitions are strongly regulated by pretranslational processes, with temporal components consistent with the findings reported herein.

METHODS

Animal Care and Experimental Manipulations

Before this experiment was conducted, approval was obtained from the Institutional Review Board of our university. Adult female Sprague-Dawley rats (250–300 g) were assigned to the following four groups: 1) control (Con; n = 24); 2) thyroid treatment with 3,5,3'-triiodothyronine (T3; n = 24); 3) hindlimb suspension (HS; n = 24); and 4) T3 + HS (n = 24). Animals were given food and water ad libitum and were housed individually. Animals assigned to the HS groups were suspended for either 1, 2, or 4 wk, as described in the companion paper (11). Those animals assigned to the T3 groups (T3 and T3 + HS groups) received daily injections of T3 (150 µg/day) throughout a given HS period (i.e., 1, 2, or 4 wk).
The results presented in the present study are part of a larger study (11) that also examined the separate and combined effects of T3 and HS on the pretranslational regulation of MHC isoform expression in a number of slow and fast skeletal muscles. The body weights and indexes of hyperthyroidism for the animals used in the present study are slightly different (mean difference ±SE of 1.5 ± 2.0%) from those reported in the companion paper (11). This is attributable to several factors: 1) whole muscle MHC isoform analyses in this study were performed on the left soleus muscles, whereas those in the companion paper were performed on the right soleus muscles; and 2) differences in staining procedures (silver staining versus Coomassie blue). Importantly, it should be emphasized that the trends noted in this study are identical to those observed in the companion study (11). Hence, the small differences are inconsequential relative to the interpretation of the data.

Discontinuous SDS-PAGE Determination of Single-Fiber MHC Isoform Composition

As described above, a transverse section (~5 mm) from the middle of the soleus muscle was immersed into a relaxing solution (in mM: 2 EGTA, 1 MgCl2, 4 ATP, 10 imidazole, 100 KCl, and 50% glycerol; pH 7.0). These segments were cut longitudinally into small bundles and stored in a glycerol-relaxation solution overnight (0°C). Then, 24 h later, the sample was placed into a fresh glycerol-relaxation solution and stored (~20°C) until it was processed. Approximately 40 single-fiber segments were dissected from each sample by using fine Dumont tweezers and a dissection microscope. The length of each fiber segment was standardized (~3 mm). Each single-fiber segment was transferred into 500-µl microcentrifuge tubes containing 30 µl of the following solution: 62.5 mM Tris (pH 6.8), 1.0% (wt/vol) SDS, 0.01% (wt/vol) bromophenol blue, 15% (vol/vol) glycerol, and 5.0% (vol/vol) β-mercaptoethanol. These samples were then heated at 70°C for 2 min, and 15 µl of this solution were loaded into a well of a gel. The composition of the gel and the electrophoretic conditions were identical to those described above for whole muscle analyses. The relative content of each MHC isoform found in a given fiber was determined by using the densitometric procedures described above. Analyses were performed on a total of 3,818 single-fiber segments (i.e., ~40 segments per muscle).

Immunohistochemical Analyses of MHC Composition

Frozen samples from each muscle in the 4-wk groups were sectioned in a cryostat at a thickness of 10 µm. Each sample was allowed to air dry, and then the primary monoclonal antibody (MAB) was applied to the section (2 h, 37°C). The primary MABs used in this study were BA-D5 and SC-71. These MABs have been characterized previously (21, 22) to recognize the slow type I and fast type IIa MHC isoforms, respectively. The sections were then washed twice in a Tris-buffered saline solution. The appropriate second MAB labeled with biotin was then applied to the sections (1 h, 37°C). The sections were then washed again by using the Tris-buffered saline solution. The avidin-biotin complex detection method (Vector Laboratories, Burlingame, CA) was used to label fibers containing the specific MHC isoform of interest. The percentage of fibers expressing a specific MHC isoform was determined by randomly examining ~2,000 fibers from each sample and determining the relative number of fibers that stained positive for a particular isoform. We also produced MABs from the RT-D9 (specific for types IIx and IIb) and BF-F3 (specific for type IIb) hybridoma cell lines, but we were unable to get strong labeling in control fast muscle. Hence, these MABs were not used in our analyses.

Statistical Analyses

The whole muscle and immunohistochemical data were reported as means ± SE. The whole muscle data for a given MHC isoform were analyzed by using a three-way ANOVA. The three factors were time (i.e., 1, 2, and 4 wk), T3 status...
(i.e., euthyroid or T₃), and hindlimb suspension (i.e., no HS or HS). Differences between groups at any given time point were determined by using a Tukey honestly significant difference test. The electrophoretic single-fiber data are represented as populations of fibers categorized by the MHC isoforms found in a given population. Differences between population distributions (e.g., hypothesis A = distributions at the 1-wk time point are dependent on T₃ and HS status) were determined by using chi-square analysis. Only those chi-square tests that were statistically significant are reported in Results. All statistical analyses were performed by using a computer program (Systat, Evanston, IL). All statistical analyses were considered significant if \( P \leq 0.05 \).

**RESULTS**

**Whole Muscle Analyses**

Slow type I MHC isoform. The whole muscle time course for the slow type I MHC data are reported in Fig. 1. The slow type I MHC isoform content of the Con group was unchanged over the time course studied. At both the 1- and 2-wk time points, T₃ and HS produced small reductions in the slow type I MHC isoform content. After 4 wk, the effects of T₃ and HS were more substantial, with T₃ producing a greater reduction in the slow type I MHC isoform content than did HS. The combined treatment of T₃ + HS produced a more rapid and substantial decrease in the relative slow type I MHC isoform content than was produced by either intervention alone (see Fig. 1). Importantly, at the 4-wk time point, the slow type I MHC isoform represented only \( \approx 20\% \) of the total MHC pool. Interestingly, at the 1- and 4-wk time points, T₃ + HS produced a response (defined as “additive”) similar to the sum of the separate effects.

Fast type IIa MHC isoform. As shown in Fig. 2, the Con groups expressed very little fast type IIa MHC isoform. However, as early as 1 wk, both T₃ and HS produced small but statistically significant increases in the relative fast type IIa MHC isoform content. The relative fast type IIa MHC isoform content of both the T₃ and HS groups continued to increase over the 4-wk treatment period, with both treatments producing similar responses.

Fast type IIx MHC isoform. As shown in Fig. 3, the Con groups expressed very little fast type IIx MHC isoform. However, as early as 1 wk, both T₃ and HS produced small but statistically significant increases in the relative fast type IIx MHC isoform content. The relative fast type IIx MHC isoform content of both the T₃ and HS groups continued to increase over the 4-wk treatment period, with both treatments producing similar responses.
HS produced a dramatic increase in the relative fast type IIx MHC content throughout the 4 wk of treatment, with this isoform representing 40% of the total MHC pool at 4 wk. The T3+HS effect on the fast type IIx MHC at the 4-wk time point was similar to the sum of the separate effects of T3 and HS.

Fast type IIb MHC isoform. The muscles in the Con groups did not express any detectable amounts of the fast type IIb MHC isoform (see Fig. 4). As shown in Fig. 4, the separate interventions of T3 and HS produced only small increases in the relative fast type IIb MHC isoform content (<5% of the total MHC pool). However, T3+HS produced a large increase in this isoform, such that the fast type IIb MHC isoform represented 20–25% of the total MHC pool (see Fig. 4). The effect of T3+HS was much greater than the sum of the individual effects. Additionally, the effect of T3+HS on the fast type IIb MHC isoform appeared to peak at 2 wk.

Single-Fiber Analyses

Validity of single-fiber analyses of MHC isoform composition. The single-fiber approach can only be considered valid if 1) fibers are sampled randomly and 2) a large number of fibers are sampled. If both of these criteria are met, then it should be possible to predict the whole muscle MHC isoform composition from the single-fiber data. In a previous study (2), we found very high correlation coefficients between single-fiber and whole muscle analyses for each of the MHC isoforms. A similar approach was again used in the present study (see Fig. 5), and very high coefficients of determination were found for all of the MHC isoforms. Importantly, for each MHC isoform, the slope approximated 1.0 (i.e., slope ≈ 0.9).

Immunohistochemical analyses were also used to establish the validity of the single-fiber electrophoretic approach, and as shown in Fig. 6, there was very good agreement between the two approaches. Interestingly, the single-fiber electrophoretic approach typically yielded a slightly greater percentage of fibers expressing a given MHC isoform. We believe that this is due to the fact that the electrophoretic approach is more sensitive than the immunohistochemical approach.

Control condition. The Con groups at each time point contained a large population of fibers that expressed only the slow type I MHC isoform (see Figs. 7–9). This population of fibers typically represented ~70–80% of the entire population of fibers. The rest of the fibers in each group were represented by fibers that either contained the fast type IIa MHC only (~5–10% of the entire population) or hybrid fibers coexpressing both type I and IIa MHC isoforms (~10–20% of the fibers).

Effects of T3 treatment. The single-fiber MHC isoform composition after 1 wk of T3 treatment was similar to that of the Con group, except the T3 group contained a larger population of fibers that expressed only the fast type IIb MHC isoform (see Fig. 10). This population of fibers typically represented ~20–25% of the total MHC pool. The rest of the fibers in each group were represented by fibers that either contained the fast type IIa MHC only (~5–10% of the entire population) or hybrid fibers coexpressing both type I and IIa MHC isoforms (~10–20% of the fibers).
I/IIa/IIx hybrid fibers (~10% of the entire population of fibers).

After 2 wk of T₃ treatment, a more dramatic effect was produced on the single-fiber MHC isoform composition (see Fig. 8). There was a substantial reduction in the population of fibers that only expressed the slow type I MHC isoform. Consistent with the trend noted at 1 wk, there was a progressive increase in the population of I/IIa/IIx hybrid fibers that represented, 25–30% of the entire population of fibers at this time point.

After 4 wk of T₃ treatment, the population of fibers that only expressed the slow type I MHC isoform was further reduced (see Fig. 9). At 4 wk, this population of fibers accounted for only ~10% of the entire population of fibers. At each of the earlier time points (see Figs. 7 and 8), the reduction in this population of fibers was accompanied by an increase in the I/IIa/IIx hybrid fibers that represented ~25–30% of the entire population of fibers at this time point.

After 4 wk of T₃ treatment, the population of fibers that only expressed the slow type I MHC isoform was further reduced (see Fig. 9). At 4 wk, this population of fibers accounted for only ~10% of the entire population of fibers. At each of the earlier time points (see Figs. 7 and 8), the reduction in this population of fibers was accompanied by an increase in the I/IIa/IIx hybrid fibers.

Treatment for 4 wk with T₃, however, did not increase the proportion of these fibers above that seen at the 2-wk time point. Interestingly, however, there was a large increase in the population of I/IIa hybrid fibers that was not observed at the earlier time points.

Effects of HS. The single-fiber MHC isoform composition of the HS group at 1 wk was very similar to that of the T₃ group, with the increase in the I/IIa/IIx hybrid fibers being the primary adaptive response (see Fig. 7). Beyond this time point, however, the responses of the HS and T₃ groups became increasingly more divergent (see Figs. 8 and 9). At the 2-wk time point, the HS group had a much larger population (~40–45%) of fibers that coexpressed all four MHC isoforms, and smaller populations of I/IIa and I/IIa/IIx hybrid fibers. Compared with the 4-wk T₃ group, the two most remarkable differences were that the 4-wk HS group had a much larger population of fibers that only expressed the slow type I MHC isoform and a much smaller population of I/IIa hybrid fibers.

The combined effects of T₃ + HS. Whereas 1 wk of T₃ + HS had a relatively small effect on the single-fiber MHC isoform composition, there were some interesting populations of hybrid fibers at this time point (see Fig. 7). The T₃ + HS group contained I/IIb and I/IIa/IIB hybrid fibers that were not observed in any of the other 1-wk groups. Additionally, the T₃ + HS group contained ~10% I/IIa/IIx/IIB hybrid fibers at the 1-wk time point, demonstrating that this unique population develops rapidly under the combined treatment.

After 2 wk of T₃ + HS (see Fig. 8), dramatic effects were produced, not only relative to the other groups at this time point, but also with respect to the effects of T₃ + HS at 1 wk. There were three key observations at this time point: 1) the population of fibers expressing only the slow type I MHC isoform was almost completely eliminated; 2) there were two substantial populations of hybrid fibers (I/IIb and I/IIa/IIB) that did not conform to the I–IIa–IIx–IIb scheme of MHC plasticity; and 3) all four isoforms were coexpressed on 50% of the fibers.
The major observations after 4 wk of T3 + HS were
1) none of the fibers expressed only the slow type I MHC isoform, 2) type I/IIa/IIx hybrid fibers persisted, and 3) the majority (~65–70%) of fibers coexpressed all four MHC isoforms (see Fig. 9).

DISCUSSION

Five unique findings in this study significantly add to our present understanding of MHC isoform plasticity. First, the similarities and/or dissimilarities of the responses to the separate interventions of T3 and HS were both MHC isoform specific and time dependent. The single-fiber analyses provided an important approach to discrimination between the effects of T3 vs. HS. Second, the effects of T3 + HS on whole muscle MHC isoform composition could not be consistently predicted from the separate effects of T3 and HS. Additionally, the effects of T3 + HS at the single-fiber level were clearly different from those expected based on the different populations of fibers produced by the separate treatments. To a large extent, this was due to the dramatic effect T3 + HS had on the fast type I1b MHC isoform content. Third, T3 + HS rapidly (within 2 wk) converted the slow soleus muscle into a fast muscle that expressed relatively large amounts of the fast type IIx and I1b MHC isoforms. The single-fiber analyses demonstrated that the large increases in these two isoforms were not constrained to any single population of fibers but were expressed in several populations of hybrid fibers. Fourth, the single-fiber analyses strongly suggest that there are different populations of slow type I fibers that, at a minimum, appear to differ in sensitivity to stimuli such as T3 and HS. Finally, the time course data demonstrated that the unique treatment of T3 + HS produced substantial populations of hybrid fibers that did not conform to the I--IIa--IIx--I1b sequential scheme of MHC isoform plasticity popularized in previous reports (1, 15, 16).

Do T3 and HS Produce Similar Patterns of MHC Isoform Transitions in the Rat Soleus Muscle?

Previously, we (2–5, 11, 24) and others (14–16) found that T3 downregulated the slow type I MHC isoform content of the soleus muscle while simultaneously increasing the fast type IIa/IIx MHC isoform content. Similar MHC transitions also occurred as a result of HS (1, 2, 6–8, 11, 17, 20, 25). Additionally, by using immunohistochemical techniques, we reported, in separate studies, that both T3 (5) and HS (8) seemed to act on some but not all of the slow type I fibers in the soleus muscle. Collectively, these similarities raised the possibility that T3 and HS acted on the same population of fibers and produced MHC transitions through similar pathways. As a first approach toward addressing this issue, we decided to examine the time-dependent effects of these interventions at the level of both the whole muscle and the single fiber. Single-fiber analyses were included in this study, in part, because it was possible that T3 and HS might produce similar patterns of MHC transitions at the whole muscle level while being derived from different patterns of MHC isoform expression at the single-fiber level.

As shown in Figs. 1–4, the similarities/dissimilarities between T3 and HS, at the whole muscle level, were both MHC isoform specific and time dependent. Specifically, both T3 and HS produced similar alterations in the fast type IIx and I1b MHC isoform composition but divergent responses with respect to the slow type I and fast type IIa MHC isoforms.

The single-fiber data, as mentioned above, provided a more definitive approach toward examining the similarity/dissimilarity between the effects of T3 and HS. There are three key points to be made in contrasting the response of individual fibers to these two interventions. First, at 1 wk, both interventions produced small but similar changes, increasing the IIa/IIx population of hybrid fibers. Second, at 2 wk (see Fig. 8), T3 and HS produced similar reductions in the population of slow type I MHC fibers (i.e., fibers only expressing the slow type I MHC isoform). However, the T3 group had larger...
populations of I/IIa and I/IIa/IIx hybrid fibers, whereas the HS group had a much larger proportion of I/IIa/IIx/IIb fibers. This difference in the fast type IIx MHC isoform distribution (I/IIa/IIx vs. I/IIa/IIx/IIb) at the single-fiber level is important, given that the whole muscle analyses suggested that the fast type IIx MHC isoform responded similarly to both T3 and HS. Finally, at the 4-wk time point (see Fig. 9), the T3 group had a much smaller population of slow type I fibers and a larger population of I/IIa hybrid fibers than the HS group had. Collectively, the single-fiber analyses strongly suggest that 1) there are different populations of slow type I fibers that vary in sensitivity to T3 and HS; 2) the effects of T3 and HS on slow skeletal muscle
are mediated, at least, through partially different mechanisms; and 3) the divergent response to T₃ and HS is time dependent.

Are There Different Populations of Slow Type I Fibers?

Izumo et al. (12) reported that the effect of the thyroid hormone on MHC mRNA isoform expression was muscle specific. In previous studies, using both immunohistochemical (20 wk of T₃) (4, 5) and single-fiber electrophoretic techniques (4 wk of T₃) (2), we observed that the effect of the thyroid hormone was more complex than that proposed by Izumo et al. (12). Specifically, we observed that T₃ seemed to affect some but not all of the slow type I fibers in the soleus muscle. Although the results of the
present study are consistent with the concept of sub-populations of slow type I fibers, this perspective (i.e., there are different populations of slow type I fibers) needs to be modified to include the rapidity of response. Based on population differences between weeks 1–2 and 2–4, it appears that some slow type I fibers respond very rapidly to T₃, whereas others respond slowly or not at all. The HS data also provide support for the concept that there are different populations of slow type I fibers. HS for 2 wk reduced the population of slow type I fibers from ~80 (Con) to ~40% of the total population. HS for 4 wk had no further effect on this population of fibers. Collectively, the findings of the present study provide strong support for the hypothesis that there are differ-

Fig. 9. Combinations of MHC isoforms observed at single-fiber level for 4-wk Con, T₃, HS, and T₃ + HS groups; n = no of fibers in group. No. of fibers that expressed each combination are represented as % total fiber population examined for each group and time point. Relative amount of a given MHC isoform for any pool of fibers (i.e., I/Ia/Ix/Ib) is quantitatively represented by amount of given bar occupied by that MHC isoform. Solid bar portion, slow type I MHC isoform; dark gray portion, fast type Ia MHC isoform; light gray portion, fast type Ix MHC isoform; open portion, fast type Ib MHC isoform.
ent populations of slow type I fibers and further suggest that these different populations vary in their responsiveness to different types of perturbations.

It might be possible that there are multiple slow MHC isoforms in the rat soleus muscle and that the population of fibers unresponsive to T3 and HS selectively expresses a slow MHC isoform, the regulation of which is independent of T3 and HS. Consistent with this perspective, previous investigators have noted the presence of two slow native (SM1, SM2) (27) and two slow MHC isoforms (Iβ, Iα) (9, 10) in rabbit and rat slow skeletal muscle. However, Galler et al. (10) reported that both the Iβ and Iα MHC isoforms were coexpressed in the rabbit slow type I fibers. It remains to be determined whether such a similar pattern exists in rat skeletal muscle.

Is the Effect of T3 + HS Purely an Additive Response of the Separate Effects of T3 and HS?

If T3 and HS act through similar pathways (e.g., similar transcriptional/translation factors), then the combined intervention of T3 + HS might produce an effect that is equivalent to that of either T3 or HS alone. In contrast, if T3 and HS produce MHC isoform transitions through separate mechanisms, then the combined treatment should produce an additive response. Although this “additive” model applied to a few of the time points (e.g., slow type I MHC at 4 wk), there was a lack of consistency that suggests such a model is not applicable. Consistent with this perspective, T3 + HS had an effect on the fast type IIb MHC isoform that was much greater than that predicted from the sum of the separate effects. At the single-fiber level, T3 + HS produced changes in the fiber populations that clearly were not additive. For instance, on the basis of the individual effects at 2 wk (Fig. 8), the T3 + HS group should have had substantial populations of I/IIa and I/IIa/IIx hybrid fibers if single-fiber transitions occurred in a purely additive fashion. However, the marked upregulation of the fast type IIb MHC isoform by T3 + HS altered the MHC isoform of single fibers, such that the 2-wk T3 + HS group did not contain either of these two hybrid populations but contained sizeable numbers of I/IIb and I/IIx/IIb hybrid fibers.

Evidence Against the ! −! IIa−! IIx−! IIb Sequential Scheme of MHC Isoform Plasticity

Over the past three decades, there has been a large amount of interest (see Refs. 1, 18, and 20 for reviews) in understanding muscle fiber plasticity. Recently, muscle fiber plasticity has been examined by focusing on contractile (i.e., MHC and myosin light chains) and regulatory protein (e.g., tropinin C) isoforms via electrophoresis. Moreover, Pette and coworkers (see Refs. 13, 18, 23, 26) pioneered single-fiber electrophoretic analyses of MHC isoform transitions in rabbit skeletal muscle. Using altered thyroid states and chronic low-frequency stimulation to produce MHC isoform transitions, these investigators suggested that transitions in MHC isoforms were obligated to follow a sequential scheme summarized as I→I/IIa→I/IIx→IIb. In examining the MHC transitions produced by the three different interventions employed in the present study, there are three key points to be made relative to this scheme of MHC isoform plasticity. First, neither T3 nor HS alone produced transitions in MHC isoforms that were inconsistent with the sequential scheme of MHC isoform plasticity. Second, 2 wk of T3 + HS produced two populations of hybrid fibers (I/IIb and I/IIa/I/IIb) that were inconsistent with a stringent application of this scheme, at least as it applies to rat skeletal muscle. Third, both the whole muscle MHC protein and mRNA isoform data reveal that T3 + HS upregulated the expression of the fast type IIx and IIb MHC isoforms before the upregulation of the fast type IIa MHC isoform. These findings are consistent with those observed at the single-fiber level.

With respect to the I/IIb hybrid pool of fibers, a small population of these fibers was observed as early as by 1 wk of combined treatment, representing ~10% of the total pool of fibers by the 2 wk time point. In contrast to the 1- and 2-wk time points, I/IIb hybrid fibers were not observed at the 4-wk time point. Thus the appearance and disappearance of this population of hybrid fibers clearly contradict the I−!IIa−!IIx−!IIb sequential scheme of MHC isoform plasticity in two important ways. First, this scheme postulates the existence of I/IIb hybrid fibers only if the sequential expression of the fast Ia and IIx MHC isoforms occurs before that of the fast type IIb MHC isoform. Given that the I/IIb hybrid population was first observed at 1 wk and that the half-life of a MHC molecule is ~4–7 days, it seems improbable that the IIa and IIx MHC isoforms were upregulated and completely downregulated within this time period. Additionally, as stated above (and shown in Table 2 of Ref. 11), the upregulation of the fast type IIa MHC mRNA isoform occurred after the upregulation of the fast type IIb MHC mRNA isoform. Second, the interval between the 2- and 4-wk time points was sufficient to completely repress the I/IIb hybrid pool. The 4-wk T3 + HS muscles contained only four populations of hybrid fibers (i.e., I/IIa/I/IIx, I/IIx/I/IIb, I/IIx/I/I/IIb, and I/I/IIx/I/I/I/IIb). Hence, the expression program of the I/IIb hybrid fibers observed at 2 wk must have been altered to express one or more of these other patterns, none of which is consistent with the I−!IIa−!IIx−!IIb sequential scheme of MHC isoform plasticity.

The large I/IIx/I/IIb population of hybrid fibers (~32% of the population of fibers) observed in the T3 + HS group at 2 wk provides an even stronger argument against the I−!IIa−!IIx−!IIb sequential scheme of MHC isoform plasticity and favors a discordant scheme of MHC isoform expression. Examination of the 1-wk data suggests that the 2-wk I/IIx/I/IIb hybrid fibers were derived from 1) slow type I fibers, or 2) I/A hybrid fibers, or 3) both populations of fibers. Importantly, however, the smaller population of I/I/IIa hybrid fibers could not account entirely for the larger population of I/I/IIx/I/IIb hybrid fibers observed at 2 wk. Hence, some of the I/I/IIx/I/IIb fibers observed at 2 wk must have been derived from the large pool of slow type I fibers present at 1 wk.
If the 2-wk and 4-wk data are compared, the I/IIX/IIB pool of hybrid fibers observed at 2 wk were converted to at least one of three populations (I/IIX/IIX, I/IIX/IIB, or I/IIX/IIX/IIB) observed at 4 wk. None of these transition schemes is consistent with the I→IIX→IIB sequential scheme of MHC isoform plasticity.

In addition to our observations, it should also be noted that Talmadge et al. (25) recently reported that both HS and microgravity produced small populations of hybrid fibers (i.e., I/IIX) in the soleus muscle of rats that did not adhere to the sequential scheme of MHC isoform plasticity. Collectively, our findings and those of Talmadge et al. (25) strongly suggest that there is not a clear cut scheme of MHC isoform transitions in rats. The differences between the findings of this study and those of Pette and coworkers (see Refs. 13, 18, 23, and 26) might be related to species (i.e., rat vs. rabbit) or the types of stimuli (i.e., T3/HS vs. T3/chronic stimulation).

Rationale for Single-Fiber Electrophoretic Analyses of MHC Isoform Composition

Most studies have employed histochemical and immunochemical techniques to study transitions in muscle fiber type. The single-fiber electrophoretic approach, however, offers several advantages not provided by these other techniques. First, as validated in this and a previous study (2), this approach provides a reasonable quantitative estimate of MHC isoform composition within single fibers. Immunohistochemical techniques, as presently used, only provide information about the presence/absence of a given MHC isoform in a single fiber and not its relative content. Second, the ability to estimate the relative MHC isoform composition within a single fiber is fundamental to the analysis of hybridism and MHC isoform transitions. It is becoming clear that hybrid fibers can exist under steady-state conditions and are not just a result of "fibers in transition" (2, 19). Finally, the electrophoretic approach offers a more direct and simpler approach than do present immunohistochemical techniques. Presently, a collection of MAbs that recognizes each adult skeletal MHC isoform is not commercially available. Although hybridoma cell lines that secrete MAbs specific for some of the MHC isoforms are available from the American Type Culture Collection and Deutsche Sammlung von Mikroorganismen und Zellkulturen, neither of these repositories has a cell line presently available that secretes a MAb that recognizes only the fast type IIX MHC isoform. Unfortunately, the RT-D9 (21, 22) hybridoma cell line, available through Deutsche Sammlung von Mikroorganismen und Zellkulturen, that recognizes both the fast type IIX and IIB MHC isoforms is no longer viable. Hence, to our knowledge, the approach used in the present study is the only option available for accurately analyzing transitions in the type IIX MHC isoform at the single-fiber level.

Conclusion

In summary, this study was unique in several respects. First, the time-dependent effects of T3 and HS were contrasted at both the whole muscle and single-fiber level. The whole muscle data demonstrated that the separate treatments of T3 and HS cause different effects on the MHC isoform composition of the soleus muscle. This was also confirmed at the single-fiber level. Importantly, however, the single-fiber data demonstrated that similar effects at the whole muscle level were manifested by MHC isoform transitions at the single-fiber level that differed from one another. Second, the combined intervention provided evidence to support the concept that T3 and HS act via completely or partially dependent pathways. Finally, T3 + HS produced unique transitions in MHC isoform composition at the single-fiber level that are not consistent with the I→IIX→IIB sequential scheme of MHC isoform plasticity. These unique transitions induced by T3 + HS demonstrate that this intervention is a powerful model for studying MHC isoform plasticity. On the basis of the findings reported in a companion study (11), it is apparent that the complex expression of multiple hybrid fibers is mediated chiefly by factors that regulate the MHC expression at the pretranslational level, because there is a strong concordance between mRNA and protein MHC isoform expression, especially as a new steady-state is approached.

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Address for reprint requests: V. J. Caiozzo, Medical Sciences I B-152, Dept. of Orthopaedics, College of Medicine, Univ. of California, Irvine, CA 92717 (E-mail vjcaiozz@uci.edu).

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