THE N^{τ}-METHYLHISTIDINE CONTENT OF MYOSIN IN STIMULATED AND CROSS-REINNERVATED SKELETAL MUSCLES OF THE RABBIT

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1. Introduction

It has been known that the characteristics of slow- and fast-twitch muscles are not immutable and transformation from the slow to the fast type of muscle and vice versa can take place [1–7]. Recently we reported that fast muscle subjected to chronic low-frequency stimulation acquired an electrophoretic pattern of light chains and a light meromyosin paracrystal staining pattern, identical to that of slow muscle [8,9]. We also reported a similar, but less complete, change in fast and slow muscles following cross-reinnervation [10,11].

It was reported that N^{τ}-methylhistidine (N^{τ}-MeHis) is a normal component of the heavy chain of fast-twitch skeletal muscle myosin but it is absent from slow-twitch muscle myosin of adult rabbits [12–14]. It was of considerable interest to examine the N^{τ}-MeHis content of the myosin in stimulated and cross-reinnervated experiments, since increases or decreases in the amount of this amino acid would serve as an indicator of changes taking place in the heavy chain component of the myosin.

Our present results show that when fast muscle is subjected to a pattern of activity similar to that in a normal slow muscle, the content of N^{τ}-MeHis declines to undetectable levels. Recovery of normal levels of N^{τ}-MeHis following cessation of stimulation is also demonstrated. Cross-reinnervation, however, only partially affects the content of this amino acid.

2. Materials and methods

Adult rabbits weighing 2–3 kg were used. In one group of rabbits the left tibialis anterior (TA) and extensor digitorum longus (EDL) muscles were subjected to continuous indirect stimulation at 10 impulses per second by means of an implanted electronic device, essentially as described previously [3]. Stimulation was allowed to continue for periods up to 15 weeks, following which the animal was killed and the TA and EDL muscles of both hind limbs removed. The reversibility of the effects of stimulation was studied in an experiment of the following design. The left TA and EDL muscles were stimulated for 6 weeks. The animal was then reoperated under aseptic conditions; the blood supply to the TA muscle

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was ligated and the TA muscle removed as a whole muscle biopsy. Stimulation was discontinued and the animal was returned to his cage. Six weeks later the rabbit was killed and the left EDL muscle removed along with the control muscles of the right hind limb. In all cases the muscles were frozen rapidly in liquid nitrogen and stored below −80°C while awaiting biochemical analysis [9].

In another group of rabbits the motor nerves to the EDL and soleus muscles were cross-anastomosed, and the cross-reinnervated muscles removed 11 to 12 months later.

For N^{τ}-MeHis determination myosin (1%) in a medium 0.25 M KCl, 70 mM β-mercaptoethanol and 2 mM Tris HCl, pH 7.5 was treated with sodium dodecyl sulfate (SDS) (protein:SDS ratio 1:2, w/v) in boiling water for 5 min, diluted with cold 1 mM EDTA, and then treated with ethanol. The heavy chain was precipitated with 5 M NaCl, centrifuged, and the pellet homogenised in a small amount of cold H_2O. Gel electrophoresis confirmed that this procedure yielded a pellet which contained only the heavy chains. About 10 mg of heavy chain were treated with trichloroacetic acid, washed with ethanol and ether, and dried. One ml of 6M HCl was then added, the tubes were sealed under reduced pressure, and the protein hydrolyzed for 22 hr at 110°C. Amino acid analyses were carried out as described by Huszár and Elzinga [15].

3. Results and discussion

Tables 1 and 2 illustrate the changes in the content of N^{τ}-MeHis that resulted from chronic stimulation, and chronic stimulation followed by a period without stimulation. N^{τ}-MeHis could not be detected in the fast muscles after 6 weeks of stimulation; a loss which would be consistant with the replacement of the heavy chains characteristic of fast muscle by heavy chains of the slow muscle type. This process did not appear to proceed synchronously with the changes in the pattern of light chains (fig.1). While the TA muscle subjected to electrical stimulation for 6 weeks no longer shows any evidence of the DTNB-light chain (LC_{2f}), small amounts of the alkali light chains (LC_{1f} and LC_{3f}) persist; not until 12 weeks of stimulation have elapsed do the last trace of these chains disappear, leaving a pattern composed exclusively of light chains of the slow muscle type. This could be interpreted in two ways. One could assume in the stimulated fast muscle a synchronous turning off of all genes involved in the synthesis of the fast subunits—heavy and light chains—coupled with a slower breakdown of the fast type alkali light chains. Or, alternatively, the results could be explained by a later shutting-off of the genes coding for fast alkali light chains without involving a difference in the turnover rate of the various subunits.

According to Low and Goldberg [16] light chains of myosin turn over more rapidly than the heavy chains, while Morkin et al. [17] reported that the DTNB-chain synthesized at about the same rate as the heavy chain while one species of alkali light chain (LC_1) is made more slowly. Additional work is needed to clarify the mechanism of the differential changes of the subunits during stimulation and cross-innervation.

Table 1

	N^{τ}-methylhistidine moles 2 × 10^5 g
Right m. tibialis anterior, unstimulated	0.68
Right m. soleus, unstimulated	0.06
12 weeks stimulated left m. tibialis anterior	not detectable
15 weeks stimulated left m. tibialis anterior	not detectable
M. extensor digitorum longus, control	0.84
M. soleus, control	0.06
Self-reinnervated m. extensor digitorum longus	0.90
Self-reinnervated m. soleus	0.08
Cross-reinnervated m. extensor digitorum longus	0.36
Cross-reinnervated m. soleus	0.39

Amounts of N^{τ}-methylhistidine in myosin from stimulated and from cross-reinnervated rabbit muscles.

Table 2

	Ca^{2+} uptake by FSR				Myosin ATPase			N^{τ} -MeHis moles/2 × 10^5 g
	Initial rate (1)	(2)	Total uptake (3)	(4)	Ca^{2+}	Ca^{2+} after alkali preincub.*	K^+-EDTA	
Right TA, unstimulated	1.26	3.69	4.31	12.7	0.82	0.42	1.73	0.5
Right EDL, unstimulated	1.40	2.80	4.68	13.7	0.84	0.50	1.60	
Right soleus, unstimulated	0.06	0.11	0.23	0.41	0.24	0.02	0.80	not detectable
Left TA, 6 weeks stimulation	0.22	0.34	0.49	0.75	0.30	0.06	0.66	not detectable
Left EDL, 6 weeks stimulation followed by 6 weeks recovery	0.73	2.19	1.94	5.82	0.74	0.59	1.13	0.8

Reversibility of the effects of chronic stimulation
(1) μmole Ca^{2+}/min/mg of protein
(2) μmole Ca^{2+}/min/g wet muscle
(3) μmole Ca^{2+} taken up in 15 minutes/mg of protein
(4) μmole Ca^{2+} taken up in 15 minutes/g of wet muscle
* Myosin was preincubated at pH 9.2, in a solution containing 0.2 mg protein per ml, 25 mM KCl, 10 mM Tris, 10 mM $CaCl_2$ for 10 min, 25°C. At the end of this preincubation period the pH was readjusted to 7.6 by adding Tris to a final concentration of 50 mM and HCl as required. The ATPase assay was then started by adding ATP.

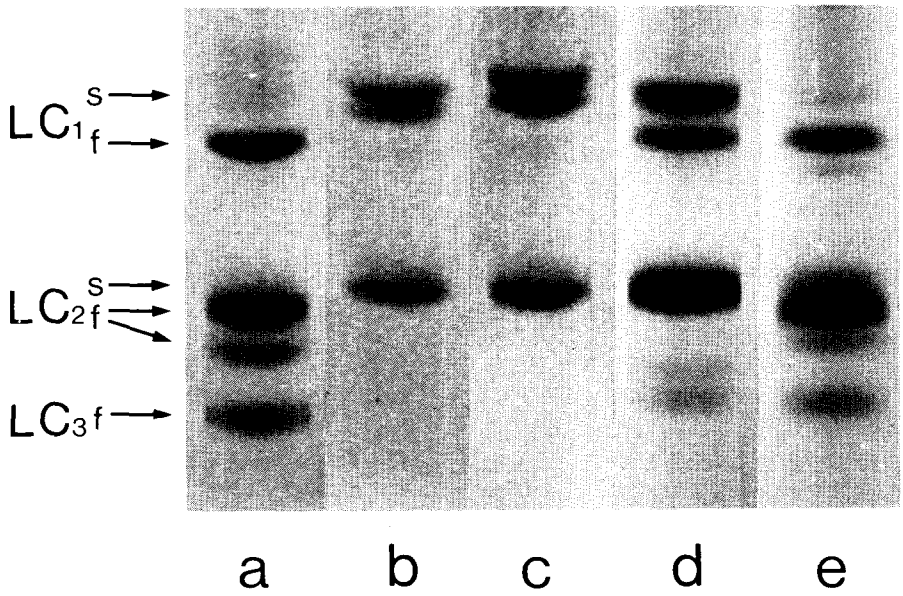

Fig.1. Pattern resulting from polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate of light chains of myosin from normal and chronically stimulated muscles. The amount of protein applied to each gel was 30 μg. (a) Control TA, (b) control soleus, (c) TA after 12 weeks of stimulation, (d) TA after 6 weeks of stimulation, (e) EDL after 6 weeks of stimulation followed by 6 weeks of recovery. Migration is from top to bottom. In (a) and (e) two bands are labelled LC_{2f}; the faster-migrating of the two is an oxidised form of the slower.

Evidence is also provided of the reversibility of the effects of chronic stimulation. In table 2 the right TA and EDL muscles and the right soleus muscle, neither of which received stimulation, serve respectively as the control fast and slow muscles. After 6 weeks of stimulation the TA muscle has acquired much of the character of a slow muscle, as reflected in the figures for Ca^{2+} uptake by fragmented sarcoplasmic reticulum (FSR) and myosin ATPase activity. After a further 6 weeks in the absence of stimulation, the same parameters, measured this time in EDL, show evidence of a return towards control levels. The table shows that the content of N^{τ}-MeHis changes correspondingly.

A complicating factor in this experiment is the compensatory hypertrophy which the EDL muscle undergoes following surgical removal of its synergist. In a more extensive study of the recovery phenomena this factor has been controlled by taking simultaneous biopsies from the control as well as the experimental hind limb in some animals, and by discontinuing stimulation without intermediate biopsy in others. Preliminary results of this study [18,19] confirm that the biopsy procedure produces in the remaining muscle an increase in the mean fibre diameter but indicate that it does not in other respects impede the recovery by that muscle of normal fast muscle characteristics. The results also support the inference which may be drawn from table 2 that a period of normal use rather longer than 6 weeks is required for complete reversal of all the effects of a 6-week period of stimulation.

It was found previously [10,11] that cross-reinnervation brought about only a partial transformation in the light chain patterns of fast and slow muscles. In the present study changes in the myosin heavy chains of cross-reinnervated muscles were likewise incomplete (table 1). The N^{τ}-MeHis content of myosin from cross-reinnervated soleus muscle rose from approximately zero to about one half of a residue per chain, while the N^{τ}-MeHis content of myosin from cross-reinnervated EDL muscle fell by about 50% to attain a similar level. These changes in N^{τ}-MeHis content, along with the changes previously observed in a number of other biochemical parameters, provide another piece of evidence for the similarity between the responses of a fast muscle to cross-reinnervation on the one hand and to chronic stimulation on the other. This similarity is most readily explained on the assumption that cross-reinnervation transposes to the fast muscle the characteristic pattern of nervous activity normally received by the slow muscle. Chronic stimulation reproduces the basic features of this pattern with an unrelieved uniformity which possibly accounts for its more profound effect.

The data presented here reinforce the conclusions reached in our previous work that under conditions of continuous low-frequency activation a fast skeletal muscle acquires all the properties of a slow skeletal muscle. During this process the myosin of such a muscle shows a reduction in both the level and the alkali stability of its ATPase activity; at the same time the light chains characteristic of fast muscle disappear, and are replaced by the light chains characteristic of slow muscle. That the heavy chains are also replaced is indicated by changes in the staining pattern of light meromyosin paracrystals [9], and by the disappearance of the N^{τ}-MeHis amino acid residue characteristic of the fast muscle heavy chain. We interpret these changes as indicating that the synthesis of certain proteins in skeletal muscle cells is regulated by factors associated with the functional activity of those cells, and that these proteins include both the light and the heavy chains of myosin.

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