Rapid and reversible changes in myosin heavy chain expression in response to increased neuromuscular activity of rat fast-twitch muscle

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Chronic 10 Hz stimulation of rat fast-twitch muscle induced rapid and reversible changes in the tissue levels of fast myosin heavy chain (HC) mRNA isoforms. These changes consisted of a rapid decrease in HCIIb mRNA and a progressive increase in HCIIa mRNA. After 15 days, the HCIIb mRNA normally amounting to approximately 80%, had decreased to less than 5% of the sum of the two HC mRNA isoforms. HCIIb mRNA was again detectable one day after cessation of stimulation and progressively increased at the expense of HCIIa mRNA with ongoing recovery. These results point to a down-regulation of the HCIIb gene by the applied stimulus pattern which, conversely, enhances the expression of the HCIIa gene.

Chronic low-frequency stimulation; Myosin heavy chain mRNA isoform; Fast-twitch muscle

1. INTRODUCTION

Adult skeletal muscle fibers exist as dynamic structures and are capable of adapting to altered functional demands. This capacity is amply documented by the changes induced in fast-twitch muscle by chronic low-frequency nerve stimulation [1]. At the myosin level, stimulation causes a progressive exchange of fast with less fast and, depending on the species, slow subunit isoforms [2–7]. In rat muscle, low-frequency stimulation mainly affects the fast myosin isoforms [4–7], and changes in myosin heavy chains (HC) follow the order HCIIb ⟶ HCIId ⟶ HCIIa [5].

The present study aims at stimulation-induced alterations in myosin HC expression at the mRNA level. We followed the time course of changes in myosin HC mRNA isoforms and also addressed the question as to the reversibility of these changes.

2. MATERIALS AND METHODS

2.1. *Animals and chronic stimulation*

Adult male Wistar rats weighing 350–450 g were used. Chronic low-frequency stimulation (10 Hz, 10 h daily) of the fast-twitch tibialis anterior (TA) muscle of the left hindlimb was performed via electrodes implanted laterally to the peroneal nerve [8]. The animals were sacrificed after various periods of stimulation (2, 4, 7, 10, 11, 14, 15, 19, 28, 35, 42 and 56 days, n = 1–4). The interval between cessation of stimulation and killing of the animals was 14–15 h. For studying the reversibility of stimulation-induced changes, animals were stimulated for 15 days. Four animals were sacrificed immediately, the other animals at various time points after cessation of stimulation: 12 h (n = 8), 21 h (n = 4), 45 h (n = 4), 62 h (n = 4), 4 days (n = 2), 5 days (n = 2), and 6 days (n = 2).

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2.2. *Preparation of total RNA and S1-nuclease mapping*

Total muscle RNA was purified as previously described [7]. S1-nuclease mapping in DNA excess was carried out using a modification [8] of the technique of Berk and Sharp [8]. The restriction fragments were end-labelled with [α-^{32}P]dATP or with [γ-^{32}P]dATP, strand-separated, and the strand complementary to the mRNA was purified. Ten or 20 μg of total cellular RNA was hybridized to 3–6 × 10^4 cpm of probe in 25 μl of 80% deionized formamide/0.4 M NaCl/0.05% (w/v) sodium dodecylsulfate/1 mM EDTA/10 mM Pipes, pH 6.4 (Sigma, Deisenhofen, FRG). In all cases, the probe was in vast excess to the corresponding mRNA. The hybridization mixture was incubated at 65°C for one hour, then adjusted to 42°C, and incubated for 15 h. Digestion was performed during one hour at room temperature with 150 units of S1-nuclease (Boehringer, Mannheim, FRG) in 300 μl of 200 mM NaCl/3 mM $ZnSO_4$/30 mM sodium acetate (pH 4.5). These conditions were found to be optimal in control studies with different amounts of RNA (including zero controls), as well as with varying amounts of S1-nuclease (50, 100, 150 units). In particular, the single-stranded radioactive probe was quantitatively removed in assays with RNA preparations that did not contain the specific mRNA. The digestion was stopped by ethanol precipitation. The dried pellets were dissolved in 85% formamide and subjected to electrophoresis on a 7% polyacrylamide gel containing 8 M urea. The gel was dried and exposed for autoradiography on Hyperfilm (Amersham, Braunschweig, FRG). The cDNA probes for myosin HCIIb (pMHC62), HCIIa (pMHC40) and HC_{neo} (pFOD5) were previously described [11,12]. The probe for HCIIb mRNA was the 304-nt 3′ end *Pst*I fragment of pMHC62, specific for the HCIIb gene. This probe contains 215 nt of the 3′-terminal coding sequence and the entire 89-nt 3′ untranslated sequence of this gene. It produces a 304-nt fully protected fragment when hybridized to the homologous mRNA. The

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probe for HCIIa mRNA was the single-stranded 360-nt fragment of pMHC40 entirely composed of the coding sequence. Because of the sequence conservation among various HC cDNA clones, S1-nuclease mapping produces in addition to the fully protected HCIIa mRNA-DNA hybrid a partially protected 170-nt fragment with HCIIb mRNA. The use of this probe made it possible to evaluate densitometrically relative concentrations of HCIIa mRNA and HCIIb mRNA. The mRNA coding for the neonatal HC (HC_{neo}) was detected with the 420-nt *PstI-BglI* fragment of pFOD5. This fragment contains 105 nt of the 3′ end terminal coding sequence, the entire 140-nt 3′ untranslated sequence, and a portion of pBR322. The fully protected fragment is 245 nt long. In addition, this probe gives partially protected fragments for HCIIa (110 nt) and for HCIIb (95 nt). The embryonic HC (HC_{emb}) mRNA was detected by S1-nuclease mapping with the pD2 probe [13]. Probe pUC4H, specific for the slow HCI, is a 272 bp *HindIII-AvaI* fragment that corresponds to positions +1485 to +1757 of the rat HCI gene. This probe contains the last 102 nt of the second intron followed by the first 170 nt of the first coding exon (exon 3) of the HCI gene (V. Mahdavi, unpublished). Hybridization to HCI mRNA produces a 170 nt-long S1-nuclease-resistant hybrid.

3. RESULTS

Contrary to the rabbit [3], low-frequency stimulation did not induce appreciable increases in the amount of slow myosin HC mRNA in rat fast-twitch muscle. As compared to the intensity of the signal in slow-twitch soleus muscle, HCI mRNA remained at very low levels even after stimulation periods of 56 days (Fig. 1). Stimulation mainly affected the pattern of fast myosin HC mRNA isoforms. As verified with the pMHC62 probe (results not shown) and the pMHC40 probe (Fig. 2a), the amount of the predominant fast heavy chain mRNA, HCIIb mRNA, was markedly reduced after 4 days and became very low after 7 days. It reached values of less than 5% in 15-day-stimulated TA muscle (Table I). Conversly, stimulation evoked progressive increases in HCIIa mRNA content which, however, appeared to follow a slower time course than the decrease in HCIIb mRNA.

The stimulation-induced changes were reversible. Low amounts of HCIIb mRNA reappeared within one day after cessation of stimulation (Fig. 2b). Longer recovery periods further enhanced the reappearance of this isoform, whereas the HCIIa mRNA content decayed progressively. The time point of the reappearance of HCIIb mRNA was confirmed in a separate experimental series in which 15-day-stimulated rats were either killed immediately or were allowed to recover for 12 h, 21 h, 45 h, and 62 h after cessation of stimulation. Densitometric evaluation of the autoradiographs showed that HCIIb mRNA which amounted to 82% of the two investigated fast HC mRNAs in normal TA muscle, was reduced to less than 5% after a stimulation period of 15 days (Table I). As independently verified with the use of the pMHC62 probe (data not shown) and the pMHC40 probe (Fig. 3, Table I), recovery periods of 21 h were sufficient to evoke slight but significant increases in HCIIb mRNA. Longer periods led to further increases in

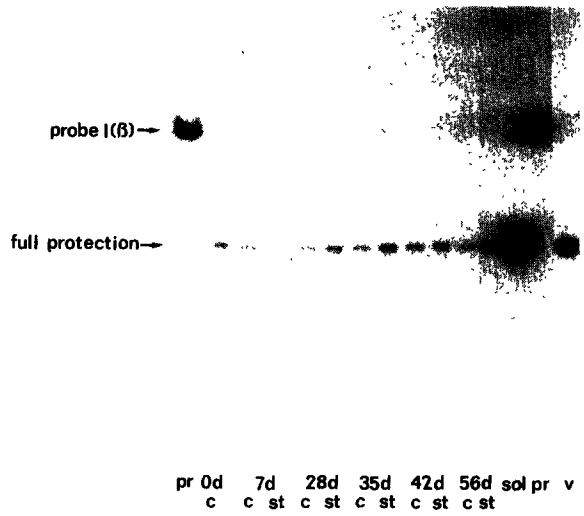

Fig. 1. Time-dependent changes of slow myosin HCI mRNA content in contralateral and stimulated rat TA muscles. Total RNA was extracted from contralateral (c) and stimulated (st) muscles and 10 μg samples were subjected to S1-nuclease mapping with the pUC4H probe for slow myosin HC mRNA. After hybridization to the homologous mRNA the 272 nt long probe yields a 170 nt long RNA-DNA, S1-nuclease resistant hybrid. For comparison, RNA from soleus (sol) and heart ventricle (v) were analysed. Note the much stronger signal in soleus than in rat heart muscle where, relatively to the α-myosin HC, the β-myosin HC (HCI) is a minor component. Figures denote time of stimulation in days (d).

HCIIb mRNA which reached a relative concentration of 43% after 62 h (Fig. 3, Table I).

Studies on HC_{emb} (data not shown) and HC_{neo} mRNA (Fig. 2, lower panel) revealed the appearance of only minute amounts of these isoforms during the first week of stimulation. Because the probe for HC_{neo} yielded partially protected fragments also with HCIIa and HCIIb mRNAs (not shown in Fig. 2, see section 2), the relative concentration of HC_{neo} mRNA could be evaluated densitometrically. Its fraction amounted to less than 1% of the fast HC mRNA isoforms in 7-day-stimulated muscles.

4. DISCUSSION

As previously shown, chronic low-frequency stimulation of rat fast-twitch muscle induces at the protein level sequential transitions in myosin HC expression in the order HCIIb $\longrightarrow$ HCIId $\longrightarrow$ HCIIa [5]. The presently observed decrease in HCIIb mRNA concomitant with an increase in HCIIa mRNA is in agreement with this sequence, although, due to the lack of a probe for HCIId, no data could be collected for this isoform at the mRNA level. However, in view of the intermediate expression of the HCIId isoform [5], the observed delay in the increase in HCIIa mRNA is conceivable.

A major finding of the present study concerns the rapid, reversible changes in myosin HC mRNA levels in

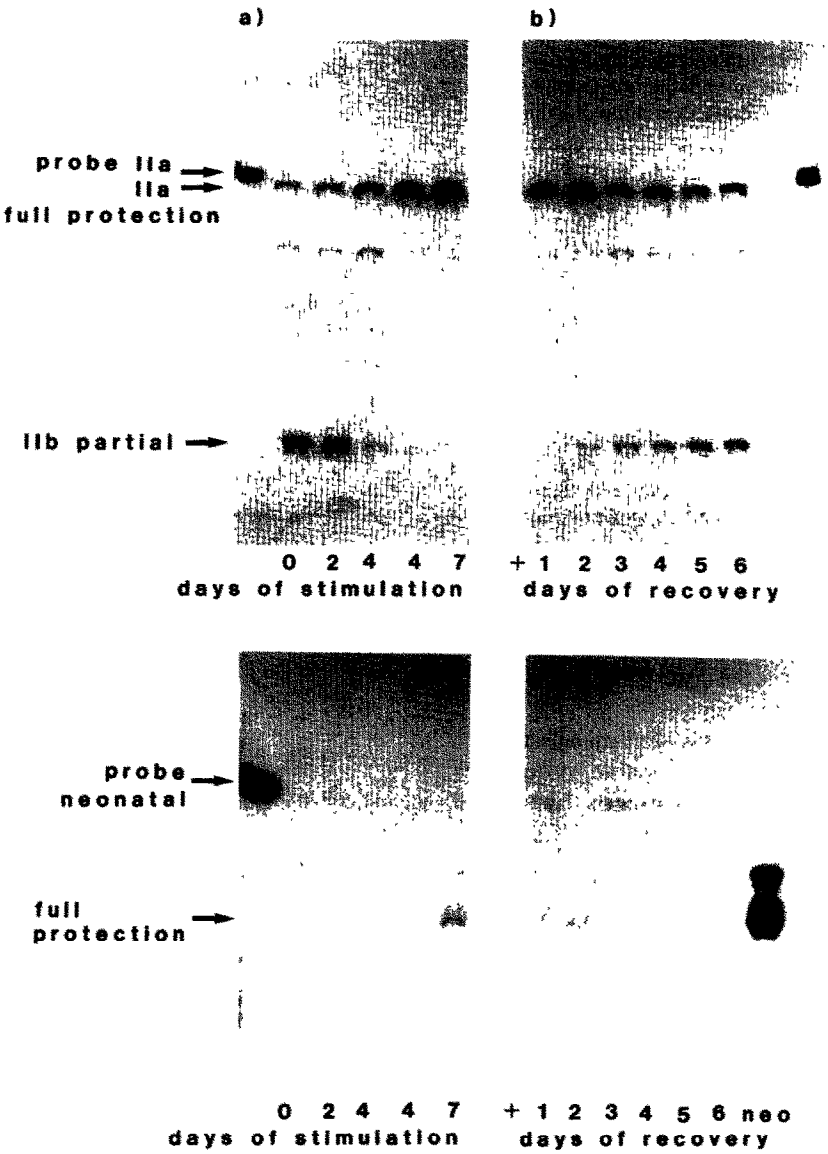

Fig. 2. Changes in HCIIa, HCIIb and HC_{neo} mRNA levels in rat TA muscles with chronic low-frequency stimulation and recovery after cessation of stimulation. Total RNA was extracted (a) from muscles stimulated during different time periods or (b) from muscles which were allowed to recover for different time periods after stimulation during 14 days. Ten μg samples were subjected to S1-nuclease mapping with the fast myosin HCIIa probe pMHC40 (upper panel) and the HC_{neo} probe pFOD5 (lower panel). The pMHC40 probe (upper panel) yields, in addition to the fully protected fragment (360 nt), a partially protected fragment (170 nt) characteristic of HCIIb mRNA. The band above the full protection signal (245 nt) of the 420 nt HC_{neo} probe (lower panel), most probably results from accidental nondigestion of the probe due to secondary structure of the DNA. *Abbreviations:* IIa, myosin HCIIa mRNA; IIb, myosin HCIIb mRNA; neo, RNA from neonatal muscle.

low-frequency stimulated and recovering fast-twitch muscle. The inverse changes of the two fast HC mRNA isoforms under study suggest their expression to be regulated in a coordinated fashion. Moreover, these rapid changes in gene expression illustrate a high degree of responsiveness to altered neuromuscular activity. The observed switch between HCIIb and HCIIa mRNA isoforms suggests that a tonic stimulus pattern, similar to that normally delivered to a slow-twitch muscle, suppresses the expression of the HCIIb gene.

The time course of the decrease in the amount of HCIIb mRNA suggests that the HCIIb gene is switched off after approximately 1–2 days of stimulation. A similar time course for the re-induction of this isoform follows from its reappearance in the recovery experiment. Although HCIIa mRNA decays after cessation of stimulation, it is not completely suppressed. This is not unexpected, because normal TA muscle expresses, in addition to the bulk of HCIIb, small amounts of HCIIa.

The transient appearance of minute amounts of the HC_{emb} and HC_{neo} mRNA isoforms most likely relates to degenerative-regenerative processes resulting from focal lesions in some fibers. Obviously, fiber degenera-

Table I

Relative concentration of myosin HCIIb mRNA in unstimulated and 15-day-stimulated rat tibialis anterior muscles without and with recovery periods of increasing duration after cessation of stimulation

Condition of muscle	Myosin HCIIb mRNA (%)
Normal	82.5 ± 1.7 (6)
15-day-stimulated	3.8 ± 1.6 (4)
15-day-stimulated	
12 h recovery	3.4 ± 1.8 (8)
21 h recovery	11.7 ± 3.9 (4)*
45 h recovery	37.3 ± 9.0 (4)**
62 h recovery	43.4 ± 6.1 (4)**

The data (means ± SE (n)) were obtained by S1-nuclease mapping with the pMHC40 probe which yields, in addition to the fully protected HCIIa mRNA-DNA hybrid, a partially protected HCIIb mRNA-DNA hybrid. The densitometrically evaluated intensities of the two bands together were set equal to 100% and the percentage of HCIIb mRNA was calculated. Significance of changes with regard to the 15-day-stimulated TA muscle is indicated: * $P < 0.05$; ** $P < 0.001$

tion is less pronounced in the rat than previously observed in the rabbit [14,15].

Taken together, these results show that muscle fibers exist in a dynamic state [16] and are capable of quickly responding to altered functional demands by rapid changes in myosin HC gene expression. Our findings also suggest that re-expression of 'developmental' myosin HC isoforms is not a prerequisite for myosin HC isoform transitions in adult muscle fibers.

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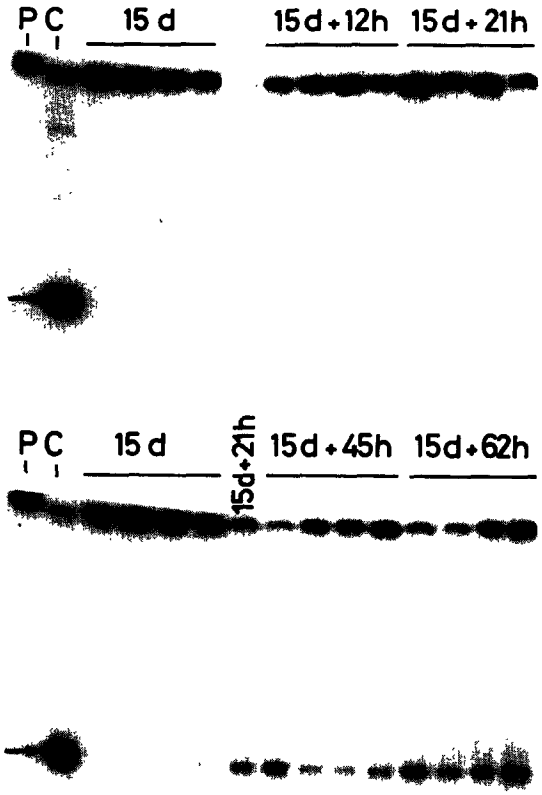

Fig. 3. Changes in myosin HCIIb and HCIIa mRNA levels in rat TA muscles after chronic low-frequency stimulation during 15 days followed by different time periods of recovery (0, 12, 21, 45 and 62 h). Ten μg RNA samples were subjected to S1-nuclease mapping with the pMHC40 probe. Four animals are shown for each time point in order to illustrate interanimal variations. The sample from the 21-h-recovered muscle which produced the strongest signal (upper panel), was used as reference on the gel shown in the lower panel. Arrow marks the partially protected 170 nt fragment for HCIIb mRNA. *Abbreviations:* C, RNA from control (unstimulated) TA muscle; P, probe.

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