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Electrostimulation-induced fast-to-slow transitions of myosin light and heavy chains in rabbit fast-twitch muscle at the mRNA level

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Chronic low-frequency stimulation of rabbit fast-twitch muscle induces progressive increases in slow myosin light chain mRNAs followed by an increase in the slow myosin heavy chain HCI mRNA. Therefore, the effects of chronic stimulation are more pronounced in rabbit than in rat fast-twitch muscle. The latter responds mainly with a rearrangement of its fast isomyosin pattern.

Fast-twitch muscle; Myosin light chain isoform; Myosin heavy chain isoform; mRNA; Chronic electrostimulation

1. INTRODUCTION

Chronic low-frequency stimulation of fast-twitch muscles of the rabbit has been shown to result in a progressive exchange of fast myosin light and heavy chain isoforms with their slow counterparts [1–3]. Previous studies indicated similar fast-to-slow transitions also at the mRNA level, i.e. increases in the amounts of slow and decreases in the amounts of fast myosin light chain mRNAs [4,5]. We were interested in studying the time course of these changes for both myosin light and heavy chain mRNAs. This seemed to be of considerable importance with regard to a coordinate expression of myosin light and heavy chain isoforms. An additional aim of this study was to compare the stimulation-induced changes in rabbit with those previously observed in the rat [6,7].

2. MATERIALS AND METHODS

2.1. *Animals, chronic stimulation, muscles*

Adult male White New Zealand rabbits were used for chronic stimulation (10 Hz frequency, 12 h daily) of the left lateral peroneal nerve via implanted electrodes as previously described [8]. The animals were killed after various periods of stimulation, and stimulated and contralateral extensor digitorum longus and tibialis anterior muscles were excised, frozen in liquid N_2 and stored at −70°C.

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2.2. *Total RNA extraction, in vitro translation, S_1 nuclease mapping*

Total muscle RNA was purified by a combination of the methods of Chirgwin et al. [9] and Kirby [10] as previously described [7]. The amounts of myosin light chain (LC) mRNAs were assessed by in vitro translation in a rabbit reticulocyte system [11]. The translated, [^{35}S]methionine-labeled products were separated by two-dimensional electrophoresis. The incorporated radioactivity was determined after extraction from the excised, identified gel pieces, taking into account different methionine contents of the myosin light chains [7].

S_1 nuclease mapping in DNA excess was carried out using a modification [12] of the technique of Berk and Sharp [13]. The mRNA coding for the slow myosin heavy chain HCI was assessed with the use of the pUC4H cDNA probe. This probe is a 272 bp *Hin*dIII-*Ava*I fragment that corresponds to positions +1485 to +1757 of the rat heavy chain HCI gene. It contains the last 102 nucleotides of the second intron followed by the first 170 nucleotides of the third exon (= first coding exon) of the HCI gene (Mahdavi, V., unpublished). Hybridization of the single-stranded probe to HCI mRNA produces a 170 nucleotide long RNA-DNA, S_1 nuclease-resistant hybrid. The cDNA probe used for S_1 nuclease mapping of the mRNA coding for HCIIa was previously described in detail [14].

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3. RESULTS AND DISCUSSION

3.1. *Stimulation-induced changes in myosin light chain mRNAs*

Chronic stimulation induced changes in the amounts of in vitro translatable myosin light chain mRNAs. These changes, detected after 7 days, generally increased with prolonged stimulation and were essentially the same in extensor digitorum longus (fig.1) and tibialis anterior (not shown) muscles. However, as previously noted [3,7], muscles stimulated for identical periods of time showed variable responses, and occasionally changes were less pronounced with longer than shorter stimulation periods.

The slow LC1s$_b$ and LC2s mRNAs increased

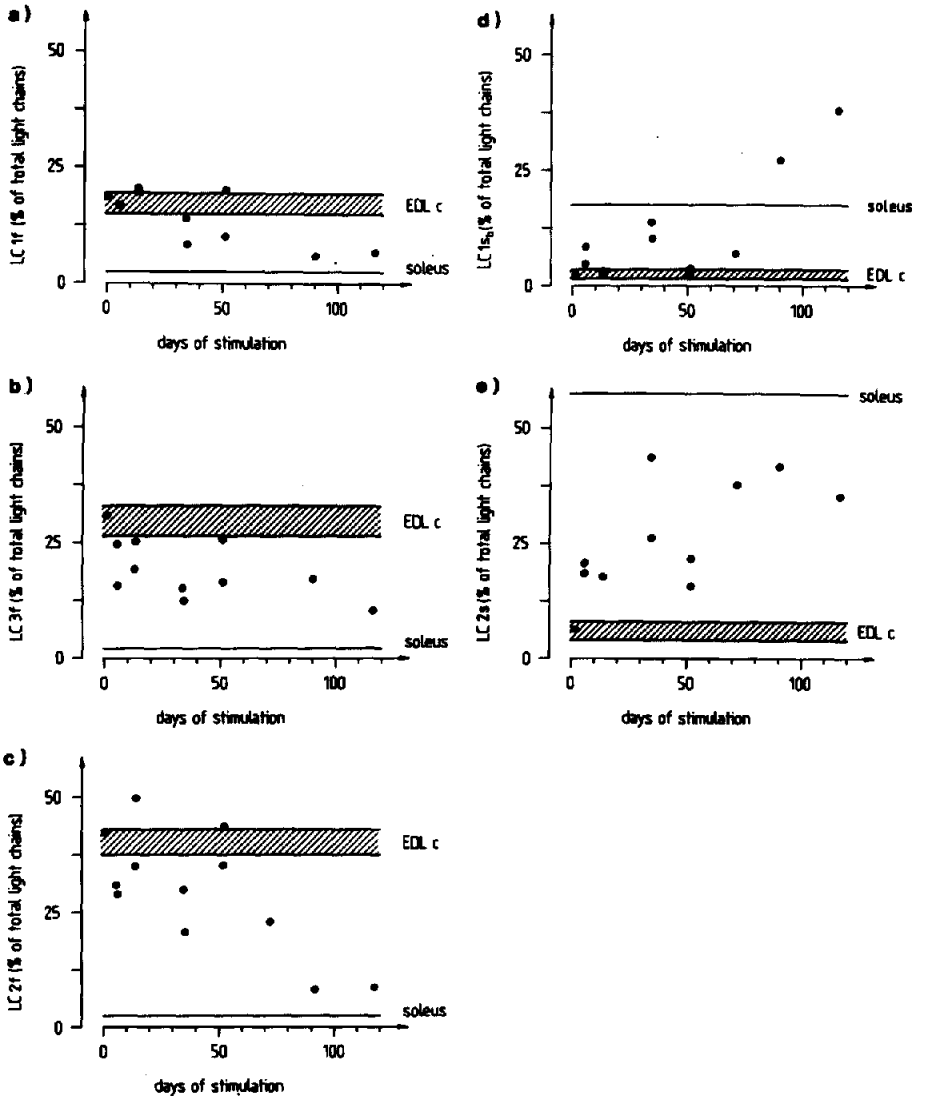

Fig.1. Quantitative evaluation of radioactivity incorporated by in vitro translation into fast and slow myosin light chains of chronically stimulated extensor digitorum longus muscle of the rabbit. The radioactive spots corresponding to the different myosin light chains were excised from dried polyacrylamide gels after two-dimensional electrophoresis and the incorporated radioactivity was measured in a liquid scintillation counter. The measured radioactivity was divided by the methionine content of the individual light chains (mol met/mol light chain). Values are expressed as percentages of total light chain content. The hatched bars represent means ± SD (n = 11) of the control values (EDLc) in unstimulated contralateral muscles.

substantially in the stimulated fast-twitch muscles. Long-term stimulated muscles eventually contained higher amounts of the $LC1s_b$ mRNA than normally present in slow-twitch soleus muscle (fig.1d). Interestingly enough, the slow $LC1s_a$ mRNA remained at very low levels in the stimulated muscles (not shown). Consequently, in comparison with soleus muscle with approximately equal $LC1s_a$ and $LC1s_b$ mRNA contents [4], long-term stimulated fast-twitch muscles displayed extremely low $LC1s_a/LC1s_b$ mRNA ratios.

The increases in slow light chain mRNA isoforms compensated the concomitant decreases in their fast counterparts (fig.1a–c). The levels of the LC2f and LC3f mRNAs decayed rapidly, whereas the LC1f mRNA content only decreased with prolonged stimulation. This finding is consistent with previous observations at the protein level, i.e. maintenance of appreciable amounts of LC1f in long-term stimulated muscles [2,3].

3.2. *Stimulation-induced changes in heavy chain mRNAs*

In order to address the question as to the coordinated expression of myosin light and heavy chains, tissue contents of heavy chain mRNAs were assessed in the same RNA preparations used for in vitro translation of the light chains. Under the chosen conditions, the slow HCI mRNA was undetectable in normal and short-term stimulated EDL muscles (fig.2b inset). However, the fast HCIIa mRNA was progressively exchanged with the slow HCI mRNA with prolonged stimulation (fig.2). Low concentrations of HCI mRNA became detectable by 21 days of stimulation. Long-term stimulated muscles ultimately resembled the slow-twitch soleus muscle with HCI mRNA as the predominant isoform (fig.2b). In some long-term stimulated animals, a faint signal for HCI mRNA was also detected in the unstimulated, contralateral muscles. It is noteworthy that the signal for the slow HCI mRNA was only moderately elevated in those muscles which displayed only minor increases in the slow light chain mRNAs (e.g. the 52-day stimulated animals in figs 1 and 2).

The exchange of HCIIa mRNA with HCI mRNA obviously followed the fast-to-slow transitions of the myosin light chain mRNA isoforms in the same muscles. This result is in consonance with

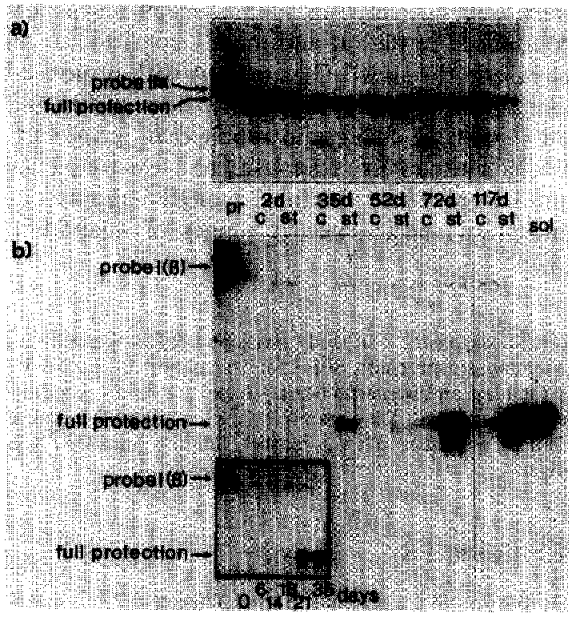

Fig.2. Stimulation-induced changes in fast myosin HCIIa mRNA and slow myosin HCI mRNA as detected by S1-nuclease mapping. Total RNA was extracted from muscles stimulated (st) during different time periods and the unstimulated contralateral (c) muscles. Samples of 20 μg were subjected to S1-nuclease mapping with (a) the fast myosin HCIIa probe (pMHC40) and (b) the slow myosin HCI probe (pUC4H). The pMHC40 probe yielded a fully protected 360 nt fragment, the pUC4H probe produced a 170 nt long RNA-DNA, S1-nuclease-resistant hybrid. In order to detect signals for the slow HCI mRNA in short-term (0–35 days) stimulated muscles (see inset in b), prolonged exposure times had to be used. For comparison, total RNA from normal soleus muscle was also analysed for HCI mRNA. Duration of stimulation is given in days in the panel between a and b. Abbreviations: pr, probe; sol, soleus.

our previous observations in low-frequency stimulated fast-twitch muscles of the rat where the fast-to-slow transition at the level of myosin light chain mRNAs also preceded that of the heavy chain mRNAs [6,7]. However, rabbit and rat differ remarkably with regard to the extent of the stimulation-induced fast-to-slow transitions. Rat fast-twitch muscle responded to low-frequency stimulation mainly with a rearrangement of the fast isomyosin pattern. The major change in rat muscle (stimulated up to 56 days) was an increase in LC1f and a decrease in LC3f at both the mRNA and protein levels. This resulted in a shift from a LC3f homodimer containing fast isomyosin to a LC1f homodimer containing fast isomyosin. The

increase in slow myosin subunit mRNAs was less pronounced in the rat and mainly consisted of an increase in the slow $LC1s_b$ mRNA with prolonged stimulation. The present results show earlier and more extensive changes in rabbit than in rat fast-twitch muscle in response to low-frequency stimulation. These findings strongly support our concept of species-specific ranges of adaptation [15].

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