Coordinate expression of alkali and DTNB myosin light chains during transformation of rabbit fast muscle by chronic stimulation

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Chronic nerve stimulation induces an almost complete exchange of the fast myosin light chains (LC) LC1f and LC2f to the slow light chains LC1s and LC2s. LC3f decreases only by 30%. The exchange of the DTNB LCs (LC2f to LC2s) precedes that of LC1f to LC1s. However, the equimolar ratio of total alkali to total DTNB LCs is maintained. This points to a hitherto unknown regulation that preserves this ratio independent of whether alkali and DTNB LCs are expressed in fast or slow isotypes.

Chronic stimulation *Alkali and DTNB myosin light chain* *Stoichiometry*

1. INTRODUCTION

Indirect stimulation of fast-twitch rabbit muscle with a frequency of 10 Hz induces a progressive replacement of fast by slow myosin [1–6]. This transformation affects the various light chains differentially. It has been suggested that the replacement of fast by slow myosin light chains is asynchronous since light chains LC2f and LC3f were found to disappear in chronically stimulated muscles earlier than LC1f [2,3]. In view of these observations we decided to investigate in more detail the time course of the slow and fast myosin light chain transitions.

2. MATERIALS AND METHODS

Paired electrodes were implanted in the left hindlimb laterally to the common peroneal nerve in adult male rabbits (White New Zealand strain). Stimulation (10 Hz frequency, 0.15 ms single pulse duration) was performed by means of a recently described telestimulation system [7]. Two stimulation protocols were used: (i) continuous stimulation (24 h a day); (ii) intermittent stimulation in which the animals were stimulated 12 h a day in such a way that stimulation was in effect 1 h on, 1 h off. The total stimulation times were as specified in section 3. After the animals were sacrificed, stimulated and contralateral tibialis anterior muscles were quickly dissected and frozen in melting isopentane (−160°C). Muscles were stored at −70°C until they were further processed.

Myosin was purified according to [8] and myosin light chains according to [9]. Electrophoresis was performed under denaturing conditions according to [10] with 12.5% (w/v) polyacrylamide slab gels with 3% stacking gels containing sodium dodecylsulfate (SDS). After staining with Coomassie-blue, amounts of protein were evaluated densitometrically with a LKB UltraScan Laser Densitometer. Molar concentrations of the light chains were calculated after correcting for their different staining intensities. Calibration curves for staining intensity were obtained by electrophoresis of varying amounts of purified light chains. The following staining coefficients were determined: LC1s, 1.0; LC1f, 0.77;

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LC2s, 1.0; LC2f, 1.0; LC3f, 0.79. The following M_r-values [11] were used: LC1s, 27000; LC1f, 21000; LC2s, 19000; LC2f, 18000; LC3f, 16000.

3. RESULTS AND DISCUSSION

The time course of the exchange of fast to slow myosin light chains in muscles undergoing continuous (24 h/day) stimulation is shown in fig.1. The values were obtained from single animals. In unstimulated tibialis anterior muscle LC1f, LC2f and LC3f are present in the following molar ratios 1.63:2.0:0.32, respectively. Slow myosin light chains LC1s and LC2s are hardly detectable.

It is evident that the light chain pattern of fast-twitch tibialis anterior muscle (TA) is almost completely transformed after 150 days' stimulation. Its myosin light chain complement is now characterized by the coexistence of fast and slow myosin light chains in the following molar ratios LC1s:LC1f:LC2s:LC2f:LC3f of 1.40:0.36:1.91:0.09:0.23. Fast myosin light chains LC1f and LC2f have thus been replaced mainly by their slow counterparts LC1s and LC2s. Despite these alterations, LC3f displays only small changes as its relative concentration decreases by only one third. The persistence of LC3f in long-term stimulated muscles was confirmed by two-dimensional electrophoresis (not shown). This finding is in contrast to earlier studies [2,3] in which no LC3f could be detected in chronically stimulated tibialis anterior muscles. This discrepancy is probably due to the previous use of less refined analytical methods. The stimulation-induced transformation of the myosin LC pattern is therefore incomplete, at least after continuous stimulation for 150 days. As had occurred in nerve cross-union experiments [12], slight changes were also seen in the LC pattern of the unstimulated contralateral TA. These changes (not shown) consisted in the appearance of small amounts of LC1s and LC2s (0.1 mol/mol myosin) after stimulation periods >60 days.

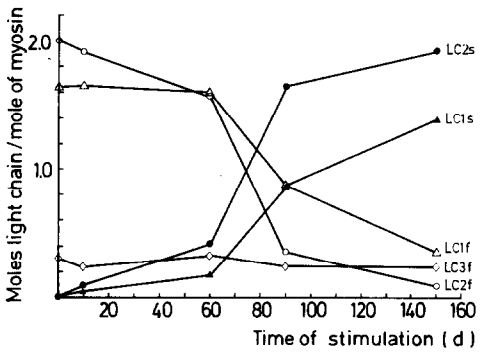

Fig.1. Time-dependent changes in the stoichiometry of fast and slow myosin light chains during chronic (24 h/day) nerve stimulation of fast-twitch tibialis anterior muscle of the rabbit. The individual light chains were determined densitometrically after electrophoresis of myosins purified from muscles stimulated for various periods. Molar concentrations were calculated as in section 2.

The decrease of fast light chains LC1f, LC2f and the increase of slow light chains LC1s, LC2s follow symmetric time courses (fig.1). This observation suggests, that in spite of the fast to slow exchange, the ratio between alkali and DTNB (phosphorylatable) light chains is maintained. The ratio (LC1s + LC1f + LC3f)/(LC2s + LC2f) was calculated for the different time points (table 1) and was found to be about 1 throughout the stimulation period.

The replacement of LC1f and LC2f by LC1s and LC2s is not a continuous process as the most extensive changes occur between 60 and 90 days (fig.1). Taking into account the relatively short half-lives of the myosin light chains [13], it is evident that this rearrangement of the light chain pattern is not in a direct time relationship with their half-lives. It appears that the transformation process is induced after a threshold has been reached. Fig.1 also shows that the replacement of LC2f by LC2s precedes that of LC1f by LC1s and is more pro-

Table 1

Molar ratios of total alkali (LC1s + LC1f + LC3f) to total DTNB (LC2s + LC2f) myosin light chains in chronically (24 h/day) stimulated and unstimulated contralateral tibialis anterior (TA) muscles of the rabbit

Stimulation (days)	Molar ratio	
	Contralateral TA	Stimulated TA
9	1.00	0.96
60	0.97	1.05
90	1.00	1.00
150	0.98	1.00

nounced. This difference in response of DTNB and alkali LC is emphasized by the results shown in fig.2.

Chronic intermittent nerve stimulation (12 h/day) induces smaller changes in the myosin light chain pattern as illustrated (fig.2) by the fact that the amount of LC3f remains unchanged. Only slight increases are seen in LC1s with corresponding decreases in LC1f. However, pronounced alterations occur in the DTNB light chains. Between 50–60 days' stimulation, there is a 6.5-fold increase in LC2s that parallels a decrease in LC2f to half of its original level. The calculated ratio between total alkali (LC1s + LC1f + LC3f) and DTNB (LC2s + LC2f) light chains is 1.1 in the 60-days intermittently stimulated muscle.

A relevant finding of this study is that the phosphorylatable (DTNB) light chains have the lowest threshold and therefore respond earlier and more extensively to alterations in contractile activity. An asynchronous transition in myosin light chains with the exchange of LC2f to LC2s preceding that of LC1f to LC1s has been reported [14].

Despite the differences in the time course, the exchange of the fast and slow isotypes displays a surprising symmetry. This points to a regulation that maintains a constant ratio between alkali and DTNB light chains independent of their fast or slow isotype characteristics. The existence of only fast or only slow alkali and DTNB light chains in normal fast or slow rabbit muscle fibres respectively [15], represents the two extreme possibilities of a large continuum of hybrid combinations which are possible during the induced fibre transformation. The maintenance of an equimolar ratio (table 1) suggests that myosins containing less than 4 light chains do not exist during the transformation. Since it has been shown that the transformation of the myosin LC pattern involves altered transcriptional activity [16], the control mechanism for maintaining the stoichiometry of alkali and DTNB light chains may be operative at the transcriptional level. The unique specificity of the binding of the alkali and DTNB light chains to the S1 subunit of the heavy chain may represent an additional control for the maintenance of the constant ratio between the two LC species.

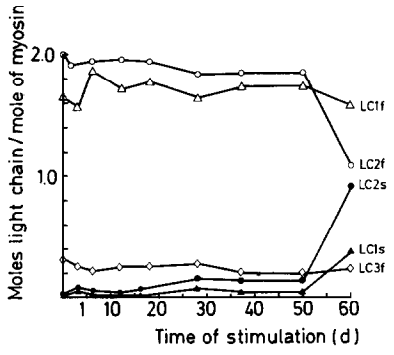

Fig.2. Time-dependent changes in the stoichiometry of fast and slow myosin light chains during intermittent (12 h/day) nerve stimulation of fast-twitch tibialis anterior muscle of the rabbit.

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