Decrease in myosin light chain kinase activity of rabbit fast muscle by chronic stimulation

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Analysis of myosin light chain kinase (MLCK) activity in tibialis anterior muscles of the rabbit revealed that chronic stimulation at a frequency of 10 Hz for 24 h per day reduced the enzyme activity in a time-dependent manner. Since fast twitch muscle contains significantly more myosin light chain kinase than slow twitch muscle, the observed reductions are consistent with the type of fast-to-slow transformation observed for other type-specific muscle characteristics. The present data also indicate that the stimulation-induced decrease in MLCK activity precedes the fast-to-slow conversion of the myosin molecule as judged by pyrophosphate-polyacrylamide gel electrophoresis.

Myosin light chain kinase *Isomyosin* *Chronic stimulation* *Fast-to-slow conversion* *(Skeletal muscle)*

1. INTRODUCTION

It has been shown that a fast-to-slow conversion of skeletal muscle occurs in the rabbit as a result of chronic low frequency stimulation (review [1]). This conversion does not affect the muscle fiber as an entity, as its main functional and structural elements are converted sequentially. The Ca^{2+}-sequestering system is altered early during stimulation [2,3], whereas changes in the myofibrillar proteins require long periods of stimulation [4]. Asynchronous transitions in myosin light [5,6] and heavy chains [6] have been observed indicating that subunits of the same molecule are affected differentially.

Myosin light chain kinase (MLCK) activity is greater in fast twitch muscle than in slow twitch muscle [7]. In view of the previously mentioned alterations in the myosin molecule produced by chronic stimulation, it was of interest to examine the effect of increased contractile activity upon MLCK activity. The relationship between myosin structure and MLCK activity may be important as the enzyme-catalyzed phosphorylation of the 18 kDa light chain (P-light chain) of myosin has been correlated to twitch potentiation in fast skeletal muscle [7–10] in vivo and was shown to increase both the actin-activated ATPase activity of purified myosin [11] and the tension in skinned muscle fibers [12].

2. EXPERIMENTAL

Chronic stimulation was performed by surgically implanting electrodes as described [3,13]. The protocol consisted of 24 h stimulation at 10 Hz (single pulse duration, 0.15 ms). Following cessation of stimulation, the animals were anesthetized with pentobarbital and biopsy samples were taken from the tibialis anterior (TA) muscles of the stimulated and contralateral legs. The tissue was

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pulverized under liquid nitrogen and stored at −80°C until analyzed for MLCK activity.

MLCK activity was determined in tissue extracts according to [14]. Protein concentrations were measured by the method of Bradford [15].

Isomyosin patterns of stimulated and contralateral muscles were evaluated by pyrophosphate-polyacrylamide gel electrophoresis [16] to monitor the extent of the transformation of myosin that resulted from stimulation.

3. RESULTS AND DISCUSSION

The activity of MLCK measured in the TA muscles of the contralateral leg of the animals in this study averaged 41 (± 3 SE) nmol phosphate incorporated · min^{-1} · mg^{-1} protein, a value similar to that reported for other fast twitch, white muscles [7]. Fast twitch, white skeletal muscle of the rabbit contains approx. 3.5-times greater kinase activity than slow twitch muscle [7]. Thus, a reduction in enzyme activity resulting from chronic stimulation would be consistent with the fast-to-slow conversions that occur with regard to other fiber type-specific characteristics [1].

The effect of chronic stimulation upon MLCK activity is illustrated in fig.1. Values are expressed as the ratio of the absolute activity in the stimulated muscle divided by that of the unstimulated contralateral leg. It is clear that stimulation for 24 h at 10 Hz for periods between 2 and 73 days reduced the enzyme activity in a time-dependent manner. The data also show that the reductions in enzyme activity occurred already after 5 days of stimulation. The kinase activity after long periods of chronic stimulation (33–73 days; $\bar{x}$ = 18 nmol phosphate incorporated · min^{-1} · mg^{-1} protein) approached that of slow skeletal muscle [7].

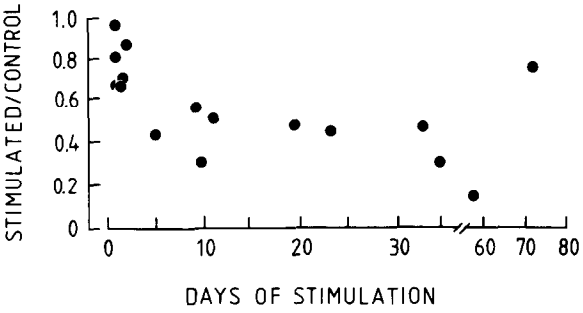

Fig.1. Time course of the effect of chronic low frequency (10 Hz, 24 h/day) stimulation upon the activity of MLCK in rabbit fast twitch TA muscle. Values are expressed as enzyme activity (nmol phosphate incorporated · min^{-1} · mg^{-1} protein) in the stimulated muscle divided by the activity in the contralateral muscle. Each point represents 1 animal.

The reduction in MLCK activity following stimulation was different in both magnitude and time course when compared to the myosin isozyme pattern of the stimulated muscles in pyrophosphate-polyacrylamide gel electrophoreses (fig.2). Lane A of the gel represents the myosin isozyme pattern of an unstimulated TA muscle with the characteristic 3 fast isomyosins. Lane E shows the typical pattern of myosin from the slow twitch soleus muscle and lanes B, C, and D show the isomyosin patterns from muscles stimulated for 10, 20 and 24 days, respectively. A comparison of these data with the MLCK response (fig.1) indicates, that at these periods when the absolute

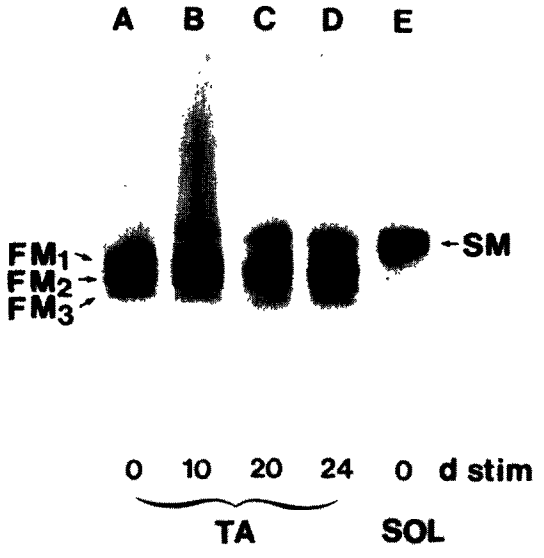

Fig.2. Pyrophosphate-polyacrylamide gel electrophoresis of stimulated and contralateral unstimulated TA muscles. Lanes: A, unstimulated TA muscle showing the characteristic 3 isomyosins FM1, FM2, FM3; E, slow soleus muscle; B–D, TA muscles stimulated for 10, 20 and 24 days, respectively. A comparison of the lanes indicates that chronic 10 Hz stimulation resulted in a gradual synthesis of a myosin isomorph that migrates in the region of the slow species (SM).

value of the MLCK activity in the stimulated muscles was between 33 and 58% of the contralateral muscles, the predominant isomyosins were still those of the fast type.

It has been suggested that the differences in myosin light chain phosphorylation between fast and slow muscles are partly related to the differences in the activities of MLCK and myosin light chain phosphatase [7,8,17]. Since chronic stimulation resulted in decreases in enzyme activity without marked changes in the myosin composition, the model of the stimulated fast twitch muscle undergoing fast-to-slow conversion may be very useful in determining the validity of this hypothesis.

Phosphorylation of the P-light chain of myosin from fast twitch skeletal muscle has been implicated as causing the potentiation of isometric twitch tension that accompanies low frequency [7,9] and/or tetanic stimulation [8]. This hypothesis has been supported by recent demonstrations that phosphorylation of the P-light chain results in an increase in the actin-activation of myosin ATPase activity [11] and in increases in tension of skinned fast twitch fibers [12]. The relationship between P-light chain phosphorylation and potentiation of isometric twitch tension has been questioned by Westwood et al. [18]. However, Moore et al. [19] have pointed to the pitfalls in the previous report [18]. The ability of chronic stimulation to predictably manipulate MLCK activity and possibly myosin phosphorylation could make it a useful model in elucidating the effects of P-light chain phosphorylation upon muscle contraction.

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