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MYOSIN LIGHT CHAIN PHOSPHORYLATION AND PHOSPHORYLASE A ACTIVITY IN RAT EXTENSOR DIGITORUM LONGUS MUSCLE

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SUMMARY

Phosphorylation of the 18,500 dalton light chain of myosin and conversion of phosphorylase b to a were examined in relation to isometric tension development. Following a 1 sec tetanic contraction, light chain phosphate content increased from a pre-tetanic value of 0.10 to 0.75 mol phosphate/mol at 7 sec; phosphorylase a activity (ratio of activity -5'AMP/+5'AMP) increased from 0.03 to 0.42 at 4 sec and decreased to control values within 20 sec. Light chain phosphate content, however, declined much more slowly and correlated to post-tetanic potentiation of peak twitch tension. Our results suggest light chain phosphorylation is not obligatory for contraction but may play a role in post-tetanic potentiation.

INTRODUCTION

Phosphorylation of the 18,500 dalton light chain subunit[1] of fast-twitch skeletal muscle myosin by a specific calcium-dependent kinase and its dephosphorylation by a specific phosphatase have been well characterized in vitro (1,2,3). Calcium activation of myosin light chain kinase is thought to be mediated by the reversible binding of the calcium regulatory protein, calmodulin, at calcium concentrations similar to those required for myofibrillar ATPase activation (1,2). Calmodulin also appears to be a subunit of phosphorylase kinase and probably accounts for the similar calcium dependency of this enzyme's activity (4). However, calmodulin does not dissociate from phosphorylase kinase in the absence of calcium as it does with myosin light chain kinase. The precise effects of calcium on calmodulin-mediated phos-

[1] Abbreviations are: 18,500 dalton light chain subunit, LC2; extensor digitorum longus muscle, EDL; pre-tetanic twitch tensions, P_t; post-tetanic twitch tension, P_t^*; maximum potentiated post-tetanic twitch tension, $P^*_{t,max}$.

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phorylase kinase and myosin light chain kinase activities may then differ due to differences in the physical interactions of calmodulin with these enzymes. In order to gain some insight into the intracellular actions of calcium and calmodulin, we have examined the kinetics of myosin LC2 phosphorylation and phosphorylase a formation in rat EDL muscle. Since a potential role for light chain phosphorylation in regulating actomyosin ATPase activity has not been clearly defined (3), we have also examined the relationship between myosin LC2 phos phorylation and isometric tension development. It has been possible from this type of analysis to infer, and to exclude, certain roles for myosin LC2 phosphorylation in mammalian skeletal muscle contraction.

METHODS

Sprague-Dawley rats (50-90 g female) were anesthetized with sodium pentobarbital (50 mg/kg). EDL muscles were excised and mounted vertically in oxygenated Ringer solution maintained at 23° by a circulating water jacket. Attachments were made with silk suture to a fixed muscle holder and a Hewlett-Packard FTA-100 force transducer. This assembly was independent of the surrounding muscle bath. Muscles were stimulated by either a transverse electrical field or focal-type stimulation. Isometric tension development was recorded by a Hewlett-Packard 7754A recorder. Muscle lengths were adjusted so that pre-tetanic peak twitch tensions were maximal. Twitch stimulation frequency was 0.05 c/s. The time of initiation of tetanic stimulation (200 c/s; 1 sec) was defined as t = 0. Following tetany, twitch contractions were reinitiated at a frequency of 0.05 c/s so that post-tetanic peak twitch tension could be examined. To obtain samples for biochemical analyses, the baths were lowered and muscles were frozen by clamps pre-cooled in liquid N_2. For pre-tetanic resting values, muscles were frozen 10 min following the last twitch contraction. Frozen muscles were powdered by percussion at -180° and portions weighed at -60°(15 mg) were homogenized directly into 1 ml of 5M guanidine-HCl, 50 mM potassium phosphate pH 6.8, 1 mM EDTA and 15 mM 2-mercaptoethanol at -15°. The homogenate was then warmed to 0° and a light chain fraction obtained as described (5). The yield of LC2 was 1.7 μg/mg muscle powder. Ths phosphate content of LC2 was determined by measuring the relative amounts of phosphorylated and nonphosphorylated forms separated by polyacrylamide gel electrophoresis in 8 M urea at pH 8.6 (5,6). Phosphorylase activity in muscle extracts was measured in the presence and absence of 5' AMP as previously described (7).

RESULTS AND DISCUSSION

The extents of myosin LC2 phosphorylation and phosphorylase a formation in rat EDL muscles were determined during rest, tetany and following relaxation (Fig. 1,2). The phosphate content of LC2 in pre-tetanic resting muscle was 0.10 ± 0.01 mol phosphate/mol LC2. This value did not change significantly during a one sec tetanic contraction (0.14 mol phosphate/mol LC2 at 950 msec).

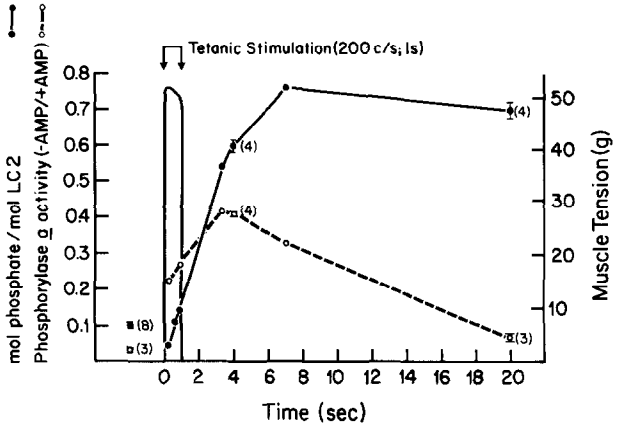

Figure 1. Myosin LC2 phosphate content (●) and phosphorylase a activity (o) during and following a one sec isometric tetanic contraction. Pre-tetanic resting values are shown preceding t = 0. Bars indicate 1 S.E.M. and numbers in parentheses are the number of muscles used for each point.

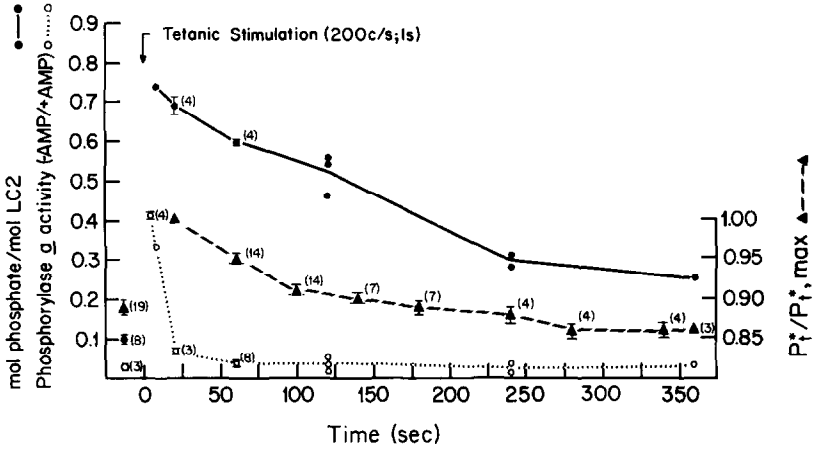

Figure 2. Myosin LC2 phosphate content (●), phosphorylase a activity (o) and ratio of peak twitch tension to maximum peak twitch tension (▲) following a one sec isometric tetanic contraction. Descending phases only are shown. Twitch contractions were initiated 20 sec following tetany at a frequency of 0.05 c/s and muscles were frozen at specified times 20 sec following the previous twitch contraction. Pre-tetanic values are shown preceding t = 0. Bars indicate 1 S.E.M. and numbers in parentheses are the number of muscles used for each point.

However, within 10-20 sec following muscle relaxation the phosphate content had increased to a range of 0.65 to 0.75 mol phosphate/mol LC2. Subsequent values declined slowly in a single exponential manner ($k = 0.007\ sec^{-1}$). There was no significant difference in the pattern of phosphorylation of LC2 with or

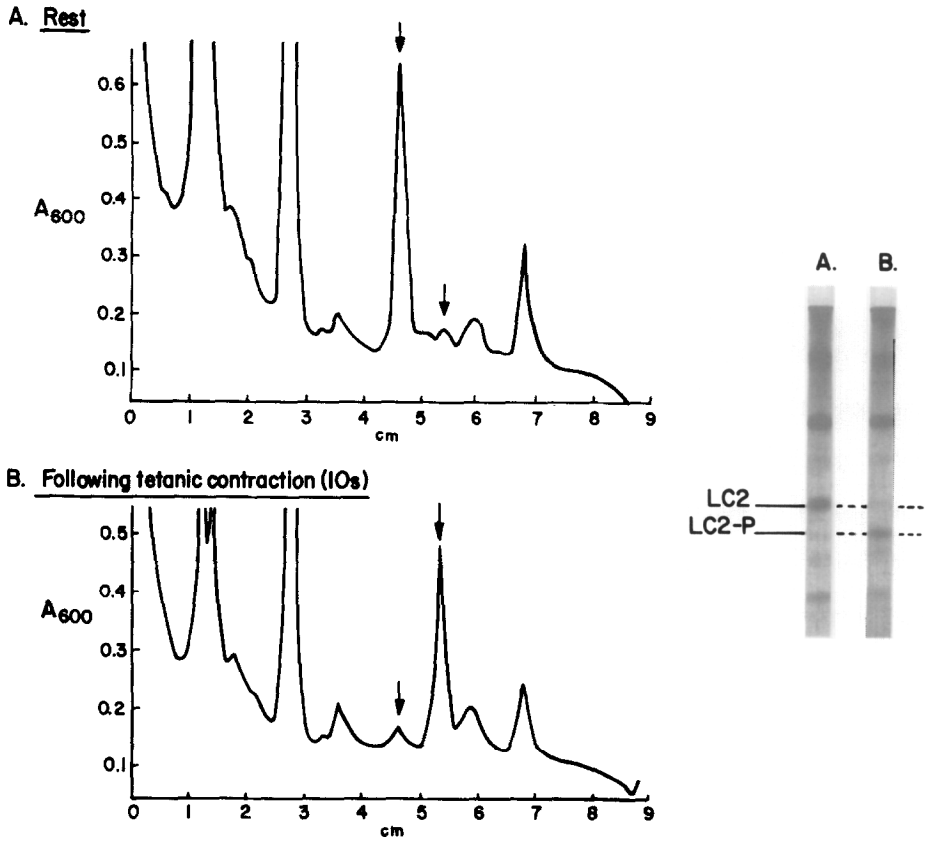

Figure 3. Densitometric profiles of myosin light chain fractions electrophoresed in the presence of 8 M urea at pH 8.6. Light chain fractions were obtained from rat EDL muscles frozen at (A) rest (pre-tetanic) and (B) 10 sec following onset of continuous tetanic stimulation (200 c/s). Arrows indicate peaks corresponding to phosphorylated and nonphosphorylated forms of LC2.

without twitches after tetany (not shown). With prolonged tetanic stimulation the phosphate content of LC2 increased to 0.92 mol phosphate/mol LC2 (Fig. 3).

Phosphorylase a activity was low in resting muscle (ratio of activities in absence and presence of 5' AMP = 0.03). Upon initiation of tetanic stimulation, activity increased in a time-dependent manner and attained maximum values 2-3 sec after muscle relaxation (ratio of activities = 0.42). Subsequent activity decreased rapidly to pre-tetanic values ($k = 0.11\ sec^{-1}$).

Following tetanic stimulation there was a transient increase in peak twitch tension similar to that previously reported for rat EDL (8) and frog

sartorius (9) muscles (Fig. 2). The maximum potentiated response occurred at 20 sec and was 1.13 ± 0.01 times peak twitch tension of pre-tetanic controls. Subsequent twitch responses declined exponentially ($k = 0.011\ sec^{-1}$) at a rate similar to the dephosphorylation of light chain. Twitch responses immediately following tetany were not maximal (e.g., at 4 sec $P^*_t/P^*_{t,max} = 0.94$). Hence, post-tetanic potentiation, similar to LC2 phosphorylation, appeared to consist of a rapid, rising phase followed by a slower, descending phase.

The results obtained with rat EDL muscles are similar in some respects to those recently obtained for frog sartorius and semitendenosus muscles (10) and rabbit gracilis muscles (11). In all studies tetanic stimulation led to phosphorylation of myosin light chain. However, the resting phosphate values obtained from quick-frozen muscles were higher in the previous reports (0.4 to 0.5 mol phosphate/mol LC2) and the net incorporation as a result of tetany was substantially less. The reasons for these differences are unclear but may be related to differences in species, prior manipulations of muscle or techniques used to quantitate LC2 phosphorylation.

The pre-tetanic resting values for myosin LC2 phosphate content and phosphorylase a activity in rat EDL muscle are consistent with biochemical evidence that myosin light chain kinase (1,2) and phosphorylase kinase (12) activities require calcium in concentrations greater than those existing in resting sarcoplasm (1,2). Furthermore, it appeared that stimulation of kinase activities occurred as a result of tetanic stimulation, presumably due to calcium influx to the sarcoplasm (13,14). These activities were evident for at least several seconds following muscle relaxation despite sequestration of free calcium from the sarcoplasm (13). Thus, the inactivation of the calmodulin-dependent phosphorylation reactions appears to be slower than inactivation of contraction. The relative rates of LC2 phosphorylation and subsequent dephosphorylation, 2.1 and 0.035 μmol/min/g tissue, respectively, agree with the measured activities of myosin light chain kinase and phosphatase in rabbit skeletal muscle homogenates (2,3).

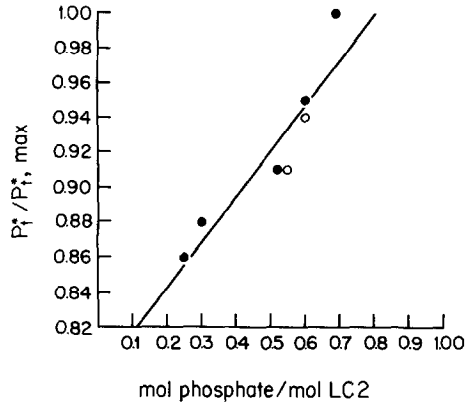

Figure 4. Correlation of post-tetanic potentiation $P_t^*/P_{t,max}^*$ to myosin LC2 phosphate content following a one sec isometric tetanic contraction. Ascending phase (o) denotes values of LC2 phosphate content and $P_t^*/P_{t,max}^*$ immediately following tetany ($t < 20$ sec). Descending phase (●) denotes those values occurring at or following attainment of maximum values ($t \geq 20$ sec). By linear regression (least squares method), $P_t^*/P_{t,max}^* = 0.79 \pm 0.26$ mol phosphate/mol LC2 ($r^2 = 0.85$).

Although our results suggest that myosin LC2 phosphorylation is not an obligatory event in contractile element activation or force generation, the phosphorylation may be related to post-tetanic potentiation. Both processes are characterized by rising and subsequent declining phases of comparable rates. In addition, during both phases the phosphate content of LC2 correlated to the degree of potentiation (Fig. 4). The basis of this relationship is currently being investigated.

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