Increased mitochondrial creatine kinase in chronically stimulated fast-twitch rabbit muscle

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Fractional extraction and isozyme electrophoresis revealed the presence of small amounts (2.5 % of total cellular activity) of mitochondrial creatine kinase (CK) in rabbit fast-twitch muscle. Chronic nerve stimulation resulted in a decrease of extramitochondrial MM-CK to 60% of its normal value but induced an approx. 4-fold increase in mitochondrial CK. This increase occurred in parallel with the rise in enzyme activities of terminal substrate oxidation.

Creatine kinase isozyme *Mitochondria* *Cytosolic* *Skeletal muscle* *Chronic nerve stimulation*

1. INTRODUCTION

Total activities of creatine kinase (CK) are higher in fast- than in slow-twitch rabbit muscles [1]. Specifically, CK activity in soleus muscle (slow-twitch) amounts to approx. 50% of that in tibialis anterior muscle (fast-twitch). Since chronic indirect stimulation of tibialis anterior muscle induces fast-to-slow transitions (review [2]), it is not surprising that the total activity of CK decreases in parallel with the reduction in glycolytic enzyme activities [3].

Conversely, chronic stimulation of fast-twitch muscle induces 4–6-fold increases in mitochondrial enzyme activities of terminal substrate oxidation [3,4]. Therefore, it was of interest to investigate whether or not chronic stimulation also affects the mitochondrial isozyme of CK and leads to a rearrangement of the CK isozyme pattern.

2. EXPERIMENTAL

Paired electrodes were implanted laterally to the common peroneal nerve of the left hindlimb in adult, male white New Zealand rabbits as described [3]. Stimulation (10 Hz, 0.15 ms single pulse duration) was performed by a telestimulation system [5]. Animals were stimulated 12 h/day with alternating periods of 1 h stimulation and 1 h rest. After different time periods, animals were killed and tibialis anterior muscles of both stimulated and unstimulated legs were dissected and passed through a cooled micro-meatgrinder [6]. Aliquots were homogenized in 20 mM Na-phosphate (pH 7.2) to give 2.5% (w/v) homogenates. Measurements of total enzyme activities and isozyme electrophoreses were performed on the supernatants after centrifugation at 105 × *g*. Approx. 400 mg of the minced muscle were subjected to fractional extraction [6–8] to elute separately extra- and intramitochondrial enzymes. The first 2 extractions in a sucrose medium (0.3 M sucrose, 10 mM triethanolamine HCl, 2 mM EDTA, pH 7.2) accomplish an almost complete elution of soluble cytosolic enzymes. Two successive steps in 0.1 M K,Na-phosphate (pH 7.2) liberate glycolytic enzymes associated with myofibrillar proteins [8] and additionally extract soluble enzymes, especially mitochondrial CK [7,9] from the outer mitochondrial compartment. The final step in 0.1 M K,Na-phosphate (pH 7.2) uses mechanical disintegration with subsequent extraction in the

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presence of 0.5% Triton X-100 to elute soluble intramitochondrial enzymes. Activity measurements for creatine kinase (EC 2.7.3.2), malate dehydrogenase (MDH, EC 1.1.1.37) and citrate synthase (CS, EC 4.1.3.7) were performed as described [10]. CK isozyme electrophoresis was performed on cellulose acetate strips (Cellogel, Chemetron, Milan, Italy) using a 0.04 M Na barbital buffer, pH 8.8. Enzyme activity was visualized using the phenacine methosulfate/nitro blue tetrazolium system. Quantitative evaluation was performed with an LKB UltraScan Laser densitometer.

3. RESULTS

Using both isozyme electrophoresis (fig.1) and fractional extraction we were able to demonstrate that normal fast-twitch tibialis anterior (TA) muscle contains, in addition to a large amount of cytosolic MM-isozyme, small amounts of mitochondrial CK. The fraction of mitochondrial CK amounts to approx. 2.5% of the total cellular CK activity (table 1). Chronic nerve stimulation induced a pronounced increase in mitochondrial CK (fig.1). After 60 days stimulation, the mitochondrial CK increased to 14.3% of the total activity (table 1). Conversely, the cytosolic CK activity decreased to approx. 60% of its original value and total cellular CK activity was reduced to about 70% of its normal value. Thus, the increase in mitochondrial CK did not compensate for the reduced cytosolic activity and the rise in relative activity of mitochondrial CK was higher than its absolute increase.

The increase in mitochondrial CK was progressive with the duration of stimulation (fig.2). A 2-fold elevation resulted after 12 days of stimulation and the activity had increased 4-fold after 60 days. The time course and extent of these changes correlated with similar increases in enzymes of the citric acid cycle, such as citrate synthase and malate dehydrogenase (fig.2). The citrate synthase increased almost 7-fold and mitochondrial malate dehydrogenase 4-fold after 60 days stimulation.

The decrease in cytosolic CK activity (fig.2) can be attributed to a reduced level of the M-subunit, as only minute amounts of MB-CK were observed in the stimulated muscles. MB-CK was not detectable in the unstimulated, contralateral muscles.

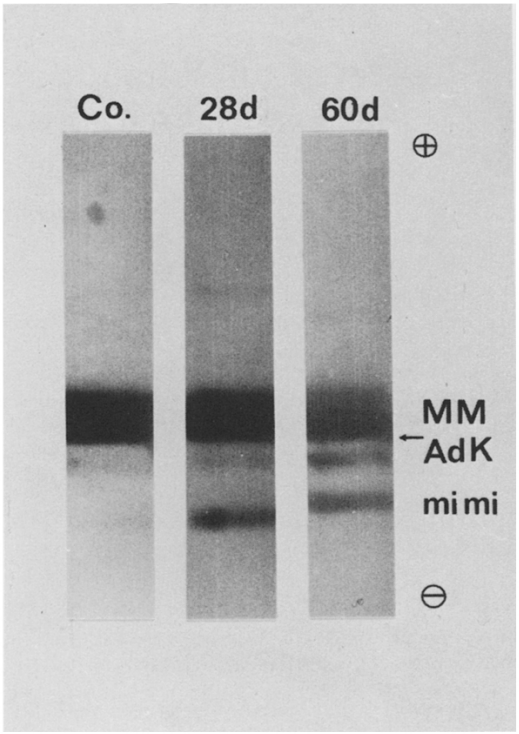

Fig.1. Electrophoreses of creatine kinase isozymes in control (Co), 28 and 60 days stimulated (12 h/day) rabbit tibialis anterior muscles. Arrow marks the point of sample application. AdK, adenylate kinase; MM, cytosolic CK; mimi, mitochondrial CK.

Table 1

Activities of total, cytosolic and mitochondrial creatine kinase in control and chronically stimulated tibialis anterior muscles of the rabbit

Stimulation (days)	Creatine kinase activity (U/g muscle)		
	Total	Cytosolic	Mitochondrial
0	600	585	15
8	558	536	22
14	479	452	27
28	434	396	38
60	419	359	60

Cytosolic and mitochondrial isozyme activities were calculated from total activities and the electrophoretically determined percentages of the 2 isozymes

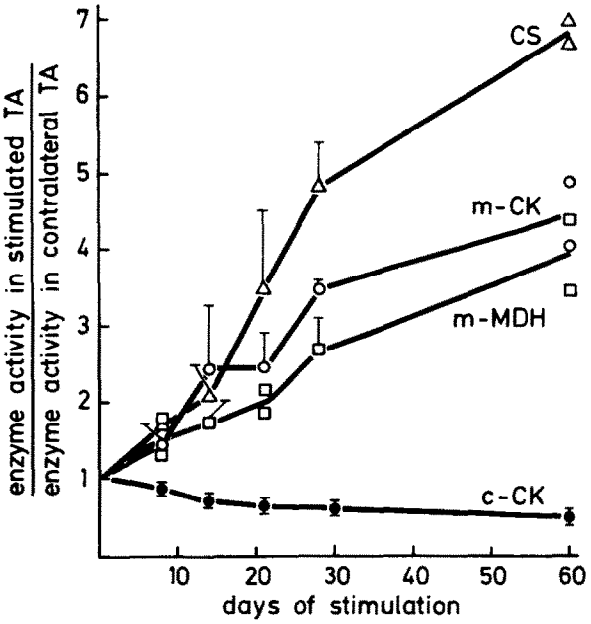

Fig.2. Time course of stimulation-induced changes in mitochondrial (m-CK) and cytosolic (c-CK) creatine kinase, mitochondrial malate dehydrogenase (m-MDH), and citrate synthase (CS). Enzymes were measured after fractional extraction from extra- and intramitochondrial compartments [6,7]. Activities in the stimulated tibialis anterior were compared to their values in the unstimulated, contralateral muscles. The values represent means ± SD for 3 animals at each time point, except for m-MDH at 28 and 60 days and m-CK at 60 days where individual values are given.

At present it is not known whether the appearance of small amounts of the MB-heterodimer is related to the stimulation-induced fast-to-slow transition or reflects regenerative processes following fiber damage [11].

4. DISCUSSION

Using 2 independent methods, this study has shown the existence of small amounts of mitochondrial CK in normal fast-twitch rabbit muscle and revealed a several-fold increase in chronically stimulated muscle. Pronounced increases in mitochondrial CK have also been found in muscles of marathon runners [12]. It has been recently demonstrated that chronic stimulation of fast-twitch rabbit muscle induces parallel increases in enzyme activities of terminal substrate oxidation and total mitochondrial volume [4]. These increases result in values that exceed those normally found in slow-twitch 'red' muscles [4]. The quantitatively similar increases in enzyme activities of the citric acid cycle seen in this study, suggest that the absolute amount of mitochondrial CK correlates with the aerobic-oxidative potential of the muscle. These results indicate an important role of mitochondrial CK in the intra-extramitochondrial transfer of high energy phosphate (creatine phosphate shuttle [13,14]) not only in heart but also in fatigue-resistant red skeletal muscle. It is remarkable that this metabolic change correlates with the reduced capacity of glycolytic substrate phosphorylation (decrease in glycolytic enzyme activities) in the chronically stimulated muscle.

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