Kinetic Characteristics of Myosin ATPase in Dog Skeletal Muscles after Shin Bone Fracture

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Kinetic characteristics of myosin from dog anterior tibial and gastrocnemius muscles after shin bone fracture were studied. Myosin affinity for ATP increased in muscles of the injured and contralateral limbs.

Key Words: *myosin; enzymatic kinetics; skeletal muscles; injury*

Contractile characteristics of skeletal muscles of the damaged segment decrease after limb bone fractures; recovery of their functions remains incomplete not only by the moment of treatment discontinuation, but even during delayed periods after it [3,4]. It is known that at the molecular level the contractile capacity of skeletal muscles is determined by the work of the actin-myosin complex, its intensity depending on myosin capacity to ATP hydrolysis (myosin ATPase activity). Hence, we think that changes in the kinetic characteristics of contractile proteins, specifically, myosin, underlie disorders in skeletal muscles contractile function after injury. In addition, the kinetics of myosin as an ATPase is little studied.

Here we studied kinetic characteristics of myosin ATPase in different muscles of dogs after shin bone fracture.

MATERIALS AND METHODS

Shock comminuted fracture of the right shin bones was induced in 10 adult mongrel dogs by a stroke of a 5-kg load dropped from 1.5 m height onto the lateral surface of the limb. The shin was then immobilized with a splint and after 24 h closed osteosynthesis by Ilizarov's device was carried out (surgical interventions were performed by M. A. Stepanov, Cand. Med. Sci.). Group 1 animals (n=5) were sacrificed at the end of osteosynthesis (duration of fracture healing varied from 35 to 49 days), group 2 (n=5) animals were sacrificed 3 months after the end of treatment. The anterior tibial muscle (ATM) and *m. gastrocnemius* (MG) of the injured and contralateral limb segments were collected for analysis. The results in experimental group were compared with the parameters in intact animals (n=7). All manipulations on animals were carried out in accordance with the European Convention for Protection of Vertebrates Used for Experimental and Other Purposes and Regulations of Studies on Experimental Animals (Supplement to the Order of the Ministry of Health of the USSR No. 755 of August 12, 1977). The experimental study was approved by the Ethics Committee of G. A. Ilizarov Center.

Myosin was isolated by a modified method based on solubility of myosin and actin in saline solutions of different ionic strength and consisting in repeated successive precipitation and dissolving of myosin in potassium chloride solutions of different concentrations. After isolation and re-precipitation the resultant myosin preparation was lyophilized.

Before studies of the kinetic characteristics 2 mg myosin was dissolved in 1 ml 0.5 M KCl. The purity of myosin preparation was evaluated by electrophoresis on a Paragon Beckman system on plates of the same firm. Electrophoresis showed that the resultant protein had a solitary clearly seen track, indicating the purity of the preparation. Enzyme activity was evaluated by the amount of inorganic phosphorus formed in myosin reaction with ATP in the presence of Ca^{2+}.

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The composition of incubation mixture was as follows: 1 ml 0.05 M Tris-HCl (pH 7.6 in 0.1 M KCl), 0.2 ml $CaCl_2$ (0.1 M), 0.3 ml ATP solution (of different concentrations), and 0.3 ml water. The reaction was started by adding myosin (0.2 ml containing 2-3 mg protein). The mixture was incubated at 26°C for 5 min. The reaction was stopped by adding 1 ml 7.5% trichloroacetic acid. Inorganic phosphorus formed as a result of enzymatic cleavage of ATP was measured in solution by reaction with ammonium molybdate. In order to measure the pre-formed phosphate, a control test was carried out in which trichloroacetic acid was added to the incubation mixture before adding myosin. Enzyme activity of myosin ATPase was calculated per mg protein in a sample, protein was measured by the method of Lowry.

The results were statistically processed using nonparametric Wilcoxon *W* test for independent samples.

RESULTS

The relationship between the reaction rate and the substrate concentration for myosin samples isolated from ATM and MG segments of the contralateral and injured limbs was presented by a sigmoid curve. Myosin exhibited no ATPase activity at low ATP concentrations (0.0001-0.001 mol/liter), as the reaction rate did not change with increasing ATP level under these conditions. Therefore, we evaluated myosin ATPase activity for ATP concentrations >0.01 mol. For this interval we calculated the maximum reaction rate (V_{max}) and Michaelis constant (K_M) by the double inverse coordinates method.

By the moment of fracture healing, the maximum rate of myosin-catalyzed ATP hydrolysis was significantly higher in the contralateral limb ATM segment and in injured limb MG segment than in intact animal muscles (Table 1). Three months after treatment, V_{max} was significantly elevated in ATM of both limbs and exhibited a trend to elevation in MG.

Myosin K_M in ATM and MG of both limbs decreased significantly by the end of treatment, this indicating myosin affinity for ATP (Table 2). Three months after the end of treatment, low K_M values persisted for myosin from injured and contralateral limb MG segments.

TABLE 1. Myosin V_{max} $M \times s^{-1} \times 10^3$ in Dog Skeletal Muscles after Comminuted Fracture of Shin Bones (Me; 25÷75 procentiles)

Group	Anterior tibial muscle		Muscules gastrocnemius	
	contralateral segment	injured segment	contralateral segment	injured segment
Intact	0.245 (0.171÷0.287)		0.259 (0.217÷0.484)	
1	0.838* (0.826÷0.868)	0.229 (0.203÷0.265)	0.234 (0.200÷0.370)	0.957* (0.614÷0.986)
2	0.774* (0.679÷0.983)	0.514* (0.499÷0.726)	0.683 (0.447÷0.965)	0.502 (0.408÷0.717)

Note. Here and in Table 2: *$p \leq 0.05$ compared to intact animals.

TABLE 2. Myosin K_M (mmol ATP) in Dog Skeletal Muscle after Comminuted Fracture of Shin Bones (Me; 25÷75 procentiles)

Group	Anterior tibial muscle		Musculus gastrocnemius	
	contralateral segment	injured segment	contralateral segment	injured segment
Intact	16.35 (15.05÷19.07)		14.70 (13.92÷24.90)	
1	2.38* (1.59÷5.06)	7.37* (5.45÷11.33)	5.85* (4.71÷6.48)	2.45* (2.45÷2.63)
2	11.16 (8.01÷22.04)	13.94 (9.12÷20.18)	7.37* (6.49÷12.85)	7.07* (5.25÷9.20)

Analysis of kinetic characteristics of myosin as an ATPase is rather difficult for several causes. To begin with, as we mentioned, the relationship between the rate of myosin hydrolysis of ATP and substrate concentration was presented by a sigmoid curve indicating polysubstrate nature of myosin reaction with ATP with numerous allosteric centers (or regulators) [1]. Under these conditions the increase of myosin (isolated from the skeletal muscles after bone injury) affinity for the substrate in parallel with acceleration of this enzymatic reaction indicated a decrease in the number of active centers, activity of the remaining centers increasing. The cause of this increase of myosin affinity for ATP is presumably explained by the synthesis of myosin with modified kinetic characteristics during the posttraumatic period in the muscle segments of both the injured and contralateral (!) limbs and/or by modification of mature (functioning) molecules of this contractile protein. This hypothesis explains the high V_{max} and low K_M values of myosin 3 months after the end of treatment (complete cycle of myosin exchange in the skeletal muscles can reach 100 days [2]).

Our data explain why reduction of contractile capacity of skeletal muscles after injuries is paralleled by an increase in myosin affinity for ATP in muscles during the posttraumatic period. Individual myosin ATPase activity *in vitr*o differs significantly from myosin activity in the actin-myosin complex *in vivo*. In addition, other factors are essential for contractile activity of muscles as an organ, for example, neurotrophic, hormonal regulation, *etc.* However, despite these facts, the reduction of contractile capacity of skeletal muscles after traumas is presumably not determined by reduction of myosin affinity for ATP.

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