Creatine Metabolism in Skeletal Muscles During Hypokinesia

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Reduction of creatine and creatine-phosphate level was revealed in skeletal muscles of mice during 28-day tail suspension test. Addition of a mixture of amino acids-precursors of creatine and branch-chained amino acids in the ratio 1:1 to ration prevented creatine and creatine-phosphate loss in muscles under the used experimental conditions.

Key Words: *hypokinesia; skeletal muscles; creatine; creatine phosphate*

Decreased locomotor load to the muscular system is a typical situation for traumotological patients. Under conditions of hypokinesia, skeletal muscles lose protein nitrogen and nonprotein nitrogen tissue components, *e.g.* creatine [3]. The search for effective means for prevention and correction of these metabolic disturbances in muscles is in progress [8,9]. The efficacy of oral intake of individual amino acids was proven: this leads to inhibition of muscle protein degradation [4,7] or to stimulation of its synthesis under conditions of reduced locomotor activity [5,6]. However, little is known about the capacity of amino acids to prevent the loss of nonprotein nitrogen components in skeletal muscles under conditions of hypokinesia.

The aim of the study was to investigate creatine metabolism in skeletal muscles of mice and to estimate the efficacy of peroral administration of amino acid precursors of creatine for correction of its impairment under conditions of gravity unload (tail suspension test).

MATERIALS AND METHODS

Experiments were performed on 54 male CBA mice weighing 25-30 g. Hindlimb muscle hypokinesia was obtained by removal of the support (tail suspension model) [1]. The animals were divided into 3 experimental groups depending on their ration: group 1 (n=18) received normal balanced vivarium ration; group 2 (n=18) received isocaloric protein-depleted carbohydrate ration; in group 3 (n=18), protein deficit was replenished with amino acid mixture (leucine, isoleucine, arginine, and methionine, 1:1:1:1) in amount equal to the total amine nitrogen content in the standard ration. The animals drank distilled water. The mice were decapitated on days 3, 7 and 28. All manipulations were performed according to the Rules for research using experimental animals (appendix of USSR Ministry of Health Order № 755, August 12, 1977). The experiment was approved by local ethic committee.

The content of creatine in hindlimb skeletal muscles was measured by the reaction with diacetyl, creatine phosphate (CP) by phosphorus content in nonprotein extract [2], glycogen content by indirect anthrone method. For more complete analysis of changes in the creatine—creatine phosphate system, two additional coefficients were calculated: creatine phosphate/creatine ratio reflecting shifts in creatine kinase reaction and a product of creatine phosphate by creatine content (CP×creatine) reflecting their total content in tissues.

The results obtained for animals of the experimental groups were compared with parameters in intact group (n=16) using nonparametric Wilcoxon W test for independent samples.

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RESULTS

Significant decrease in creatine content in skeletal muscles of group 1 mice was detected on day 7 of suspension; the content of CP decreased insignificantly. The CP/creatine ratio remained within the normal (Tables 1 and 2). Significant 1.9-fold decrease in CP×creatine (p=0.001) in mice from this group developed by experimental day 28 due to sufficient loss of CP against the background of normal creatine level (Table 2). These changes occurred against the background of depleted glycogen stores in tissues (Table 3).

Protein deficit in the ration led to earlier decrease in CP content in muscles of group 2 mice (Table 1): on days 3 and 7 of suspension. However, creatine content sufficiently increased against the background of depletion of CP pool. Nevertheless, the decrease in CP content was more pronounced than the increase in creatine level, which was seen from reduction of CP×creatine during this period. These changes suggest that that decrease in CP content in skeletal muscles during suspension against the background of protein deficit can be achieved via two pathways: due to a right shift in the CP—creatine equilibrium or due to CP dissociation to creatine. The decrease in CP concentration could also be associated with sufficient accumulation of other energy substrate, glycogen, in muscles of these mice (Table 3). This assumption is supported by the fact that the decrease in its level in muscles on day 28 of tail suspension was followed by recovery of the CP pool. This observation demonstrates inverse correlation between glycogen and CP concentrations in skeletal muscles.

Compensation of protein deficit with amino acids in animals from group 3 was followed by a significant increase in tissue creatine content at all terms of the experiment. This led to a significant increase in CP concentration in tissues by day 28 of the experiment, when the total CP×creatine content 1.3-fold surpassed this parameter in intact animals (p=0.01). During this period CP/creatine ratio was also restored.

Thus, gravity unload of skeletal muscles significantly modulated creatine metabolism in tissues: at early stages of tail suspension, we observed a right shift in the CP/creatine system aimed at maintenance of sufficient amount of CP, while at later terms, the CP level decreased due to its degradation to creatine. More profound changes developed during tail suspension against the background of protein deficit, when

TABLE 1. CP and Creatine Content in Skeletal Muscles of Mice after Various Periods of Hypokinesia (Median; Interquartile range)

Index, μmol/g tissue	Group	Intact animals	Time of observation, days		
			3	7	28
CP	1	22.7 (21.1±24.4)	25.3 (23.0±26.1)	19.3 (17.4±22.4)	11.5[d] (11.0±13.1)
	2		16.2[d] (15.7±16.7)	17.9[a] (17.6±18.8)	20.8 (18.1±23.5)
	3		20.5 (19.6±22.9)	19.8 (18.1±21.9)	27.0[c] (23.2±31.0)
Creatine	1	59.5 (57.3±61.9)	58.4 (55.9±62.1)	54.1[e] (49.5±54.6)	59.2 (59.0±60.0)
	2		74.0[d] (72.9±75.6)	65.4[a] (61.2±69.4)	78.6[a] (72.6±87.4)
	3		67.9[d] (66.3±73.2)	68.3[a] (65.3±73.2)	65.1[b] (62.5±67.30)

Note. [a]p<0.01, [b]p<0.02, [c]p<0.03, [d]p<0.001, [e]p<0.004 compared to intact animals.

TABLE 2. CP and Creatine Proportions in Skeletal Muscles of Mice after Various Periods of Hypokinesia (Median)

Index	Group	Intact animals	Time of observation, days		
			3	7	28
CP/creatine	1	0.38	0.41	0.38	0.19***
	2	0.38	0.22***	0.28***	0.25***
	3	0.38	0.31***	0.28***	0.42
CP×creatine	1	1331	1456	995*	689***
	2	1331	1207**	1191**	1640
	3	1331	1390	1424	1719*

Note. *p<0.01, **p<0.05, ***p<0.001 compared to intact animals.

TABLE 3. Glycogen Content (mg/%) in Skeletal Muscles of Mice after Various Periods of Hypokinesia (Median; Interquartile Range)

Group	Intact animals	Time of observation, days		
		3	7	28
1	0.34 (0.26±0.45)	0.33 (0.27±0.42)	0.15** (0.11±0.22)	0.26 (0.19±0.31)
2	0.34 (0.26±0.45)	0.46 (0.32±0.54)	1.06*** (0.80±1.33)	0.38 (0.29±0.99)
3	0.34 (0.26±0.45)	0.29 (0.21±0.52)	0.60* (0.45±2.90)	0.25 (0.19±0.48)

Note. *p<0.02, **p<0.003, ***p<0.005 compared to intact animals.

CP loss also occurred due to a shift in creatine—CP equilibrium to the left. Protein replenishment with amino acid mixture (leucine, isoleucine, amino acids inhibiting protein protein degradation and activating protein synthesis in muscles; arginine and methionine, are substrates for creatine synthesis) prevented CP and creatine loss in skeletal muscles under conditions of gravity unload.

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