ACTIVITY OF ENZYMES OF THE MALATE AND LACTATE SHUNTS IN THE SARCOPLASM AND MITOCHONDRIA OF DENERVATED MUSCLES

G. A. Zamoskovskaya

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The ratio between the activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) was determined in the soluble fraction of the cytoplasm and the mitochondria of intact and denervated soleus (red) and gastrocnemius (consisting mainly of white fibers) muscles of rabbits. LDH isozymes of the H type predominated in the cytoplasm of the intact soleus muscle and of the M type in the gastrocnemius muscle. The ratio between MDH_{cyt}/LDH_{cyt} and MDH_{mit}/MDH_{cyt} activities was higher in the soleus than in the gastrocnemius muscle. During the period of maximal development of neurogenic muscular atrophy (5 weeks after division of the sciatic nerve) the isozyme spectra were similar and the ratios between MDH_{cyt}/LDH_{cyt} and MDH_{mit}/MDH_{cyt} activities were equalized. During recovery of the normal structure of the muscle cells (7th-24th weeks after denervation) the activity and isozyme composition of LDH and MDH in the cell structures of both muscles gradually approximated the control level.

The red (tonic) skeletal muscles are characterized by a high rate of metabolism linked with tissue respiration. In the white (phasic) skeletal muscles the chief source of energy is anaerobic glycolysis. Since energy metabolism differs in the red and white muscles, these muscles differ in the ratio between the activities of certain mitochondrial and cytoplasmic enzymes [6] and in their lactate dehydrogenase (LDH; E. C. 1.1.1.27) isozyme spectra [3, 5]. Denervation of the red and white muscles considerably reduces the difference between them both in the character of their metabolism and in the isozyme composition of the cytoplasmic LDH [3, 7, 10].

LDH and malate dehydrogenase (MDH; E. C. 1.1.1.37), located in the soluble fraction of the cells, evidently compete for glycolytic $NAD \cdot H_2$ [4], the hydrogen of which is transported in the composition of malate [8] and also, perhaps, of lactate [5] through the mitochondrial membrane.

The dynamics of changes in LDH and MDH activity in the sarcoplasm and mitochondria of the soleus and gastrocnemius muscles was studied at various times after division of the sciatic nerve.

EXPERIMENTAL METHOD

Male rabbits weighing 2.5-3 kg were used. The methods of denervation, determination of activities of LDH and MDH and their isozymes, and determination of the protein content were described previously [2].

EXPERIMENTAL RESULTS AND DISCUSSION

LDH activity in the sarcoplasm of the intact gastrocnemius muscle is higher, and in the soleus muscle lower than MDH activity (Table 1). The mean ratio between the specific activities of MDH/LDH in the gastrocnemius and soleus muscles was 0.68 (10.5 : 15.5) and 1.68 (5.48 : 3.26), respectively. In the gastroc-

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TABLE 1. Activity of LDH and MDH (in E*) in the Sarcoplasm and Mitochondria of Skeletal Muscles at Various Times after Denervation (M ± m)†

Muscle	Time after denervation (in weeks)	LDH				MDH			
		sarcoplasm		mitochondria		sarcoplasm		mitochondria	
		per mg protein	per gram tissue	per mg protein	per gram tissue	per mg prot.	per g tissue	per mg prot.	per g tissue
Gastrocnemius	0	15,50±1,10	669±33	1,11±0,03	0,90±0,07	10,5±0,65	435±13	5,59±1,03	2,70±0,94
	1	14,25±0,80	611±45	0,80±0,05	0,50±0,01	11,4±1,03	473±13	5,75±0,60	3,51±0,43
	3	4,46±0,27	156±2,6	0,90±0,06	0,97±0,01	4,6±0,17	176±7	5,42±0,04	3,51±0,02
	5	3,35±0,28	68,4±5,3	1,04±0,03	1,44±0,05	4,25±0,43	87±4	5,60±0,16	4,05±0,25
	12	3,96±0,94	101±14	0,90±0,02	0,60±0,01	5,5±0,67	133±4,5	5,60±0,45	2,67±0,36
	24	16,17±0,40	329±20	0,92±0,02	0,70±0,09	11,9±0,50	223±30	3,10±0,04	2,97±0,06
	36	12,00±0,97	393±20	1,07±0,04	0,92±0,12	9,5±0,22	310±38	4,92±0,37	4,34±0,60
Soleus	0	3,26±0,30	122,5±4,6	0,092±0,047	0,085±0,004	5,48±0,27	174,7±6,5	9,90±1,09	8,09±1,09
	1	2,97±0,34	119,0±7,0	0,137±0,030	0,110±0,020	4,76±0,60	155,2±14,0	6,49±0,47	5,76±0,47
	3	1,62±0,07	56,6±3,3	0,122±0,018	0,097±0,070	1,79±0,20	71,0±3,0	2,70±0,04	3,35±0,01
	5	1,17±0,11	26,3±2,5	0,102±0,020	0,064±0,008	1,18±0,12	44,6±3,0	2,66±0,28	2,37±0,19
	12	1,44±0,24	29,1±1,0	0,119±0,022	0,095±0,039	2,00±0,17	50,4±1,7	3,55±0,28	3,22±0,30
	24	3,83±0,24	88,0±2,3	0,134±0,084	0,092±0,030	5,66±0,40	83,2±5,0	2,70±0,01	2,50±0,06
	36	3,58±0,16	105,0±15,1	0,100±0,010	0,125±0,027	4,70±0,62	116,4±25	6,00±1,45	3,00±0,71

*The quantity of enzyme catalyzing the oxidation of 1 μmole NAD · H_2 at 25° C was taken as the unit of enzyme activity (E).

†Mean values from six to 10 experiments are shown.

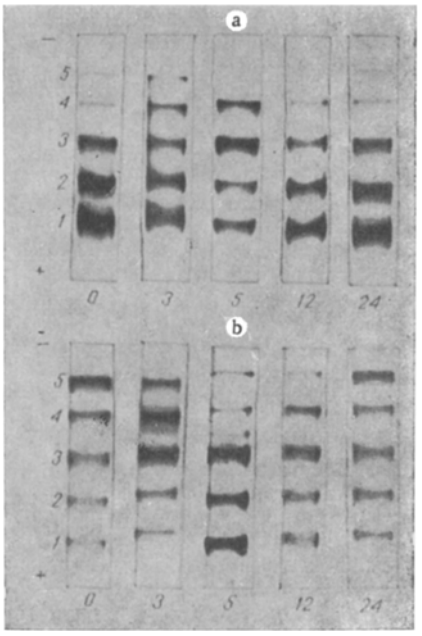

Fig. 1. Effect of denervation on isozyme spectrum of lactate dehydrogenase (LDH) in soleus (a) and gastrocnemius (b) muscles of the rabbit. Abscissa, time after denervation of muscles (in weeks); ordinate, LDH isozymes.

nemius muscle glycolytic NAD·H_2 is evidently utilized chiefly for the reduction of pyruvate, whereas in the soleus muscle it chiefly reduces oxaloacetate into malate. LDH isozymes of the M type (Fig. 1), catalyzing the LDH reaction mainly in the direction of lactate formation [9], in fact predominate in the gastrocnemius muscle. In the intact red muscles, on the other hand, an aerobic LDH spectrum was found and the MDH_{mit}/MDH_{cyt} ratio was higher than in the gastrocnemius muscle, namely 1.81 (9.90 : 5.48) and 0.53 (5.59 : 10.5), respectively. The conditions in the soleus muscle are thus favorable for pyruvate transport into the mitochondria and for transfer of the reduced NAD·H_2 through the mitochondrial membrane by the malate shunt.

The activity of both enzymes in the sarcoplasm of the red and mixed muscles fell (particularly sharply in the latter) during the 5 weeks after division of the sciatic nerve. Differences in the activities of cytoplasmic LDH in the two muscles decreased considerably in the period of maximal development of the atrophic changes [2]. Changes in opposite directions also took place in the LDH isozyme spectrum of the denervated muscles: the content of type-M isozymes decreased in the gastrocnemius and that of type H decreased in the soleus muscle. The LDH spectrum in both muscles was similar 5 weeks after denervation (Fig. 1), in agreement with results obtained by other workers [1, 3, 7]. The MDH activity in the sarcoplasm of both muscles fell to values comparable with their LDH activity; as a result, 5 weeks after denervation the ratio between MDH/LDH activities in the cytoplasm was close to 1.26 for the gastrocnemius and 1.01 for the soleus muscles. The specific activity of mitochondrial LDH was more than one order of magnitude, or if expressed per gram tissue, about three orders of magnitude less than the LDH activity of the sarcoplasm. Despite the low relative percentage of LDH in the mitochondria of the muscles, the mitochondrial LDH isolated from 1 gram gastrocnemius muscle catalyzed the conversion on the average of 0.9 μmole substrate in 1 min at 25° C (Table 1) and it could evidently significantly affect pyruvate metabolism in the mitochondria of that muscle. The LDH activity in the mitochondria of the soleus muscle was 10 times less than in the gastrocnemius and its functional role in the soleus muscle appears negligible. Activity of mitochondrial LDH was not significantly changed in the denervated muscles and, consequently, the ratio LDH_{mit}/LDH_{cyt} increased during the development of neurogenic muscular atrophy.

The activity in the mitochondria of the denervated gastrocnemius muscle remained at the control level, while in the soleus muscle it was 3.7 times lower than the control by the 5th week after denervation. The ratio between MDH_{mit}/MDH_{cyt} activities in the gastrocnemius muscle rose from 0.53 (5.59 : 10.5) to 1.63 (5.60 : 4.25), in agreement with views regarding the increased intensity of respiratory metabolism in this muscle after denervation. MDH activity in the mitochondria of the soleus muscle was reduced much more in the mitochondria than in the sarcoplasm (Table 1), indicating a decrease in its respiratory metabolism. Meanwhile the MDH_{mit}/MDH_{cyt} ratio was higher in the red muscle 5 weeks after denervation. Consequently, no correlation was found between the MDH_{mit}/MDH_{cyt} ratio and the intensity of respiratory metabolism in the denervated red muscle. The difference between the specific activities of MDH located in the mitochondria and sarcoplasm is evidently a more important indicator of the functional activity of the malate shunt in the two muscles. Denervation of the muscles led to disappearance of the difference between the mitochondrial and cytoplasmic MDH activities in the two muscles.

During regeneration of the nerve fibers and restoration of the normal structure of the muscle cells (7th-24th week after denervation) [2] the activity and isoenzyme composition of LDH and MDH in the cell structures of the two muscles gradually approximated to the normal level characteristic of intact muscles (Table 1).

The observed changes in the activity and isozyme composition of LDH and MDH observed in these experiments point to stimulation of the function of the respiratory metabolism of pyruvate in the period of

neurogenic atrophy of the gastrocnemius muscle: the sharp decrease in LDH activity together with the relative increase in the content of its "aerobic" isozymes prevents the utilization of glycolytic NAD · H_2 for the reduction of pyruvate into lactate and provides favorable conditions for the reduction of pyruvate in the mitochondrion; the increase in the ratio between MDH/LDH activities in the sarcoplasm leads to the more rapid formation of malate from oxaloacetate in the sarcoplasm, the transport of malate into the mitochondria, the utilization of the transported enzyme, and, consequently, the reducing potential in the reactions of tissue metabolism.

The opposite direction of the changes in MDH and LDH activity in the denervated red muscle prevents the movement of pyruvate and hydrogen of NAD · H_2 into the mitochondria, so that the conditions for stimulation of anaerobic glycolysis are created.

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