ACTIVITY OF ISOZYMES OF NAD- AND NADP-DEPENDENT MALATE DEHYDROGENASES IN THE MYOCARDIUM OF RABBITS WITH ALLOXAN DIABETES

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Isozymes of NAD- and NADP-dependent malate dehydrogenases (NAD- and NADP-MD) isozymes in rabbit heart muscles were detected by disk electrophoresis in polyacrylamide gel. In alloxan diabetes a significant decrease in mitochondrial NADP-MD activity (in the malate decarboxylation reaction) was observed, but its activity in the cytoplasm was increased. NAD-MD activity (in the oxaloacetate reduction reaction) also was lowered in the myocardium in diabetes, especially in the mitochondria. Insulin restored the ratio between the activities of the NAD- and NADP-MD isozymes, disturbed in diabetes, in the subcellular structures of the myocardium.

KEY WORDS: malate dehydrogenase; heart-muscle isozymes in diabetes.

Malate dehydrogenases (MDs) play an important role in the coupled energy processes in the mitochondria and cytoplasms. NADP- [4, 5, 8, 9] and NAD-dependent [1, 3] malate dehydrogenases (NADP-MD and NAD-MD) are represented in heart muscles in two molecular forms – mitochondrial (M) and cytoplasmic or soluble (S). Mitochondrial and cytoplasmic MDs help to maintain a definite ratio between oxidized and reduced potentials in the subcellular structures, which is regulated by the transfer of malate between the mitochondria and cytoplasm.

Data on the hormonal regulation of MD activity in the heart muscle are few in number. Activity of malic enzyme (NADP-MD) in the mitochondria and cytoplasm of the myocardium in rats, by contrast with the adipose tissue and liver, is unchanged by the action of thyroid or a high-carbohydrate diet [8, 9]. Likewise, Stroev [1] found no significant changes in NAD-MD activity in the heart-muscle mitochondria of rats in thyrotoxicosis; NAD-MD activity of the nonmitochondrial fraction was increased under these circumstances. Insulin, given to these animals, led to a marked decrease in NAD-MD activity in the cytoplasm and a small increase in its activity in the mitochondria of the myocardium.

This paper describes the results of determination of activity of NADP- and NAD-MD isozymes in the myocardium of rabbits with alloxan diabetes before and after insulin therapy.

EXPERIMENTAL METHOD

Diabetes was produced in rabbits kept on a standard diet by intravenous injection of alloxan (130-150 mg/kg body weight) after the animals had been deprived of food for 48 h. Tests were carried out 1 month after administration of alloxan. The blood glucose level of the experimental rabbits was not below 300 mg%. Insulin was injected daily in a dose of 2 units/kg body weight. Weighed samples of myocardial tissue were minced in a homogenizer with Teflon pestle in medium containing 0.25 M sucrose, 10 mM triethanolamine (pH 7.4), and 2 mM EDTA (ratio of tissue to medium 1 : 5). The mitochondria were isolated by differential

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TABLE 1. Activity of NAD- and NADP-MD Isozymes in Heart Muscle of Rabbits with Alloxan Diabetes

Experimental conditions	NADP-MD		NAD-MD	
	cyto-plasm	mito-chondria	cyto-plasm	mito-chondria
Control	2,27 (12)	34,0 (12)	4,18 (18)	17,6 (18)
Diabetes	4,29 (12)	19,0 (12)	3,92 (16)	12,6 (16)
P	<0,05	<0,001	>0,05	<0,01
Diabetes + insulin	1,95 (7)	29,3 (7)	4,05 (8)	21,7 (8)
P	>0,05	>0,05	>0,05	<0,05

Legend. Number of experiments in parentheses.

centrifugation. To determine the activity of the enzymes the mitochondrial membranes were destroyed by treatment with 0.5% Triton X-100. Activity of the enzymes of the soluble fraction of cytoplasm was determined in the supernatant obtained after centrifugation of the homogenates at 45,000 g for 1 h. NAD- and NADP-MD isozymes in the cellular fractions of the myocardium were detected by disc electroprophoresis in polyacrylamide gel on the "Reanal" apparatus with the reagents and under the conditions recommended for that purpose. Activity of the enzymes was determined spectrophotometrically from the change in optical density [8]: NAD-MD – in the reaction of reduction of oxaloacetate in 0.1 M phosphate buffer (pH 7.6), NADP-MD in the malate decarboxylation reaction in 0.1 M Tris–HCl buffer (pH 7.4). The quantity of enzyme catalyzing the conversion of 1 μmole substrate in 1 min per milligram protein was taken as the unit of activity.

EXPERIMENTAL RESULTS

Activity of NAD- and NADP-MD isozymes in the myocardium of the control and diabetic animals, and also of the rabbits treated with insulin, is given in Table 1. In diabetes the activity of mitochondrial NADP-MD was reduced, whereas in the cytoplasm it was increased. Injection of insulin into rabbits with alloxan diabetes was followed by restoration of the disturbed ratio between NADP-MD activities in the subcellular structures of the myocardium. Similar results regarding a decrease in mitochondrial NADP-MD activity on the 14th-18th day of diabetes in rat liver were obtained by Tsoncheva [2].

Considering that the activity of the mitochondrial isozyme of NADP-MD is controlled primarily by the NADP/NADP·H_2 ratio, the writer considers that the decrease in the malic enzyme activity in diabetes is evidence of the low energization of the mitochondria. Young et al. [7] found reduced malic enzyme activity in homogenates of the liver and adipose tissue of rats after starvation for 48 h.

Mitochondrial NAD-MD activity in the myocardium, measured by the rate of conversion of oxaloacetate into malate, also was reduced in diabetes (Table 1). Changes in the cytoplasmic NAD-MD activity were similar in character to those of the mitochondrial isozyme, but were less marked. The decrease in NAD-MD activity in the myocardial mitochondria in diabetes must evidently facilitate the appearance of an additional block in the tricarboxylic acid cycle and a disturbance of hydrogen transport to the mitochondrial membranes. Insulin treatment of rabbits with alloxan diabetes leads to an increase in MD activity in the subcellular structures of the myocardium.

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