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Isoenzyme Profile of Lactate Dehydrogenase in the Cranial Cervical Sympathetic Ganglion under Normal Conditions and during Synaptic Blockade

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The isoenzyme profile of lactate dehydrogenase in the cranial cervical sympathetic ganglion of rabbits was studied under normal conditions and during blockade of nicotinic cholinergic synapses. Under normal conditions this profile was presented by 5 isoforms of the enzyme (lactate dehydrogenases 1, 2, 3, 4, and 5). Activity of H-isoforms prevailed. Blockade was accompanied by heterotropic allosteric inhibition of lactate dehydrogenase isoforms. H- and M-isoforms underwent simultaneous changes. Activity of H-isoforms sharply decreased. However, the ratio between lactate dehydrogenases 1 and 2 during complete or partial blockade did not differ from that observed in experiments with the intact ganglion. M-isoforms (lactate dehydrogenases 4 and 5) disappeared during partial blockade. Activity of hybrid lactate dehydrogenase 3 significantly decreased and was undetected during partial and complete blockade, respectively. Our results indicate that enzyme activity and isoenzyme profile of lactate dehydrogenase are determined by function of nicotinic synapses.

Key Words: *blockade; ganglion; lactate dehydrogenase; nicotinic cholinergic synapses*

Much attention is paid to synaptic regulation of metabolism in the nervous tissue [2-4]. Among a variety of neurotransmitters systems, the nicotinic cholinergic (N-CL) system plays a special role, because processes occurring in N-CL synapses serve as the initial stage of other synaptic process (at least in autonomic ganglia) [2,6]. Taking into account the functional significance of N-CL synapses, it is important to evaluate their role in the metabolism in nervous tissue. This problem can be solved in experiments on the model of dosed synaptic blockade with nicotinic receptor antagonists, which allows adequate evaluation of the role of N-CL synapses in biochemical systems of the nervous tissue.

Here we studied the enzyme system of lactate dehydrogenase (LDH) under conditions of partial or complete blockade of N-CL synapses in the cranial cervical sympathetic ganglion (CCSG). LDH is a major enzyme of energy metabolism that plays a role in various processes of cell metabolism [5,11]. It is important to determine the molecular mechanisms of modulation of the LDH system during synaptic blockade.

MATERIALS AND METHODS

Experiments were performed on 14 adult Chinchilla rabbits. Blockade was induced by a nicotinic re-

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TABLE 1. Isoenzyme Profile of LDH in CCSG under Normal Conditions and during Synaptic Blockade

Experiment		Ratio of H-subunits in the total activity of LDH, %	Ratio of isoenzymes in the total activity of isoforms, %				
			1*	2*	3*	4*	5*
Control (n=5)		63	30	25	21	13	10
Blockade	partial (n=4)	83	46	40	14	—	—
	complete (n=5)	89	55	45	—	—	—

Note. n, number of animals. Here and in Table 2: *numerical designations are given according to the common nomenclature of LDH isoenzymes.

ceptor antagonist dimecoline. The mechanisms of bimecoline-produced changes were studied on rabbits [8]. A ganglionic blocker was injected subcutaneously (10 or 50 mg/kg), which resulted in partial or complete blockade of synaptic transmission. The samples were taken in the period corresponding to the maximum blocking effect (1 h after treatment).

LDH isoenzymes in CCSG homogenates were separated in the reaction with nitroblue tetrazolium after disc electrophoresis in polyacrylamide gel (micromethod with modifications of Panavene) [7]. Activity of LDH isoenzymes was estimated by the method of photographic photometry and expressed in relative units. Electrophoretograms were photographed at the maximum formazan absorption wavelength (569 nm). An aerial film allowed us to select the exposure time at which the integral optical density of each isoenzyme band on the electrophoretogram was proportional to optical density of the negative image. The images of isoenzyme bands were densitometried on an IFO-451 microphotometer. The results were analyzed by Wilcoxon—Mann—Whitney U test (significance level [α]=1%).

The composition of LDH was estimated by the relative activity of isoenzymes (%) and calculated as follows: $B_i=100\% A_i/\Sigma A_j$, where i and j are the numbers of LDH isoenzymes according to numerical nomenclature (1, 2, 3, 4, and 5) [5,12]; B_i is the ratio of each isoenzyme in the total activity of LDH; and A_i is isoenzyme activity (rel. units).

The ratio of H-subunits in the total activity of LDH (%) was calculated taking into account the tetrameric structure of this enzyme [5,12]: $B_H=0.25 \Sigma(5\text{-}i)B_i$, where B_H is the ratio of H-subunits in the total activity of LDH (%); and i is the numbers of isoenzymes (1, 2, 3, 4, and 5).

RESULTS

The isoenzyme profile of LDH in the intact ganglion was presented by 5 major isoforms (Table 1). Activity of the anodic fraction (LDH-1) was maximum. The relative activity of other isoenzymes consecutively decreased from LDH-1 to LDH-5. The total activity of anodic fractions LDH-1 and LDH-2 and ratio of H-subunits in the total activity of LDH were 50% of activity of other isoenzymes and M-subunits. These data indicate that activity of cardiac-type LDH isoforms is maximum in rabbit CCSG [5].

Partial and complete synaptic blockade modified the isoenzyme profile of LDH (Table 1). These changes were similar under conditions of partial and complete blockade. It should be emphasized that most changes developed even during partial blockade. Cathodic fractions LDH-4 and LDH-5 were undetected during partial blockade. Under these conditions activity of anodic fractions LDH-1 and LDH-2 and hybrid fraction LDH-3 sharply decreased and was less than 50% of the control level (Table 2). We revealed a 5-fold decrease in the total activity of LDH (Table 2).

LDH-3 disappeared during complete blockade. Activity of anodic fractions LDH-1 and LDH-2 and total activity of LDH decreased more significantly (Table 2; 9 and 16% of the control, respectively).

Changes in the isoenzyme profile and activity of LDH were followed by a progressive increase in the ratio of H-subunits in the total activity of LDH (Table 1).

Synaptic blockade was accompanied by a decrease in activity of anodic fractions. Therefore, the LDH-1/LDH-2 ratio under conditions of partial or complete blockade did not differ from normal (1.2). Cathodic fractions underwent simultaneous chan-

TABLE 2. Decrease in Activity of LDH and Isoenzymes during Synaptic Blockade (% of the control)

Blockade	LDH	LDH isoenzyme		
		1*	2*	3*
Partial	81	71	70	87
Complete	91	84	84	—

Note. All changes are significant ($p \leq 0.05$).

ges and disappeared from the isoenzyme profile of LDH during synaptic blockade. The data suggest that high degree of homology in the subunit composition (predominance of H- or M-subunits) determines the intermolecular interaction between isoenzymes of each fraction. It explains the stereotypic response to cholinoceptor blockade. In available literature we found no data on the similarity of changes in cathodic and anodic LDH fractions under various experimental conditions. Our findings indicate that blockade of N-CL transmission modulate activity of various LDH isoenzymes by a similar molecular mechanism. The observed differences in inactivation of cathodic and anodic fractions are associated with variations in the charge distribution in H-LDH and M-LDH under basal conditions [12].

It could be hypothesized that blockade manifested in allosteric inhibition of LDH. Our previous studies under similar experimental conditions showed that partial blockade is followed by a significant increase in the concentration of neuronal RNA [4]. These opposite changes in enzyme activity of LDH and RNA contribute to the disappearance of genetic regulation of LDH during blockade. Our results are consistent with published data on allosteric regulation of LDH isoenzymes [5, 9-12]. The resistance to nonspecific factors (variations in temperature and pH; ultraviolet irradiation; *etc*.) consecutively decreases from LDH-1 to LDH-5 [1,5, 10,13]. Similar changes in activity of isoenzymes were revealed during blockade of N-CL. These data suggest that allosteric inhibition of LDH during synaptic blockade is mediated by a heterotropic mechanism. Taking into account the mechanism of homotropic regulation [5], similar changes in activity of anodic and cathodic fractions exclude the possibility of substrate inhibition. However, dysfunction of ionotropic N-CL synapses that accompanies blockade is followed by changes in ion homeostasis [6]. Published data show that LDH is highly sensitive to pH of the medium [5]. Moreover, catalytic activity of the enzyme is determined by electrostatic forces [12]. These data suggest that changes in ion homeostasis contribute to variations in the isoenzyme profile of LDH.

Our results indicate that partial and complete blockade of N-CL synapses excludes the involvement of LDH in energy supply and other metabolic processes in CCSG. The CCSG-specific isoenzyme profile and activity of LDH are maintained by normal function of N-CL synapses. Our results are consistent with published data that various processes occurring in N-CL synapses modulate several enzyme systems (*e.g.*, in CCSG) [3].

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