Kinetic Parameters of Liver Aldehyde Dehydrogenase in Rats with Cold Injury

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We studied the kinetics of liver aldehyde dehydrogenase in rats with cold injury. Low-temperature exposure was followed by a decrease in activity and catalytic efficiency of aldehyde dehydrogenase in rat liver mitochondria. Atypical changes in kinetic characteristics of aldehyde dehydrogenase were found in the cytoplasmic fraction during cold injury.

Key Words: *kinetics; aldehyde dehydrogenase; liver; cold injury*

Among a variety of traumatic diseases, the incidence of thermal injuries of different etiology remains relatively high. Much attention was paid to the effect of high-temperature exposure on the organism. However, little is known about the influence of low temperature. The effect of cold injury on various enzyme systems, including hepatic biotransformation enzymes, remains unknown.

Aldehyde dehydrogenase (ALDH, EC 1.2.1.3) is one of the enzymes of phase I biotransformation. ALDH is present in various organs and tissues (*e.g.*, kidneys, lungs, spleen, and brain); maximum ALDH activity was found in liver cells. Molecular forms of ALDH with different kinetic parameters were revealed in various fractions of subcellular organelles, including the mitochondrial fraction. Two major forms of the enzyme differ in low and high affinity for acetaldehyde [4].

This enzyme has an important role in the protection of cells from endogenous and exogenous aldehydes. Therefore, studying the enzyme properties under pathological conditions is of considerable importance. Various disorders and, particularly, cold injuries are accompanied by an increase in the metabolism and activation of lipid peroxidation (LPO). The concentration of toxic aldehydes (alkanals, alkenals, 4-hydroxyalkenals, malonic dialdehyde, *etc*.) increases under these conditions [9,11].

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Here we studied the kinetic parameters of liver ALDH in experimental rats under conditions of low-temperature exposure.

MATERIALS AND METHODS

Experiments were performed on 25 Wistar rats weighing 160-180 g. The hindlimb was epilated up to the level of the hip joint (under ether anesthesia). Cold injury with dry ice was produced by the contact method (dry ice, -63°C; circularly up to the upper third of the femur). These animals were decapitated on day 3 of the subacute experiment. The mitochondrial and cytoplasmic fractions were isolated from rat liver homogenate by differential centrifugation [5]. ALDH activity was measured as described elsewhere [2]. Protein concentration was estimated by the method of Lowry with modifications [7]. Kinetic parameters of ALDH (Kt, half-reaction time of the enzyme substrate; V_{max}, maximum rate of reaction product accumulation under complete consumption of the substrate; and V_{max}/Kt, coefficient of the catalytic efficiency for the enzyme reaction) were evaluated from primary data [8]. Kinetic parameters were recorded [3]. The results were analyzed by Student's *t* test (BIOSTAT software) [1].

RESULTS

Cold injury was followed by a significant decrease in specific activity of ALDH in rat liver mitochondria

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TABLE 1. ALDH Activity in Rat Liver under Normal Conditions and during Cold Injury (nmol NADH/mg protein/min)

Liver fraction	Intact animals (*n*=25)	Cold injury (*n*=25)
Mitochondria	127.42±17.43	97.21±1.02*
Cytoplasmic fraction	76.70±4.81	73.81±10.12

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

crease in the content of cold-stress proteins and activation of LPO in the liver suggest that cytoplasmic ALDH is characterized by significant conformational changes in the subunit composition. It results in the formation of abnormal enzymes with high affinity for acetaldehyde and low catalytic efficiency. The observed variations in kinetic parameters (Kt and V_{max}) in the cytoplasmic fraction from treated animals (compared to intact rats) reflect non-coordinated inhibition of enzyme activity.

Kinetic properties of ALDH in the mitochondrial and cytoplasmic fractions of the liver during cold injury

TABLE 2. Kinetic Parameters of Rat Liver ALDH under Normal Conditions and during Cold Injury

Parameter	Mitochondria (*n*=25)		Cytoplasmic fraction (*n*=25)	
	normal	cold injury	normal	cold injury
Kt, min	5.79±1.12	9.00±0.78*	3.16±0.66	1.75±0.01*
V_{max}, μmol NADH/min	11.58±1.02	3.94±0.01*	15.81±1.73	2.86±0.13*
V_{max}/Kt, μmol NADH/min^2	2.00±0.15	0.44±0.01*	5.02±0.30	1.64±0.06*

(by 24% compared to intact animals, Table 1). Enzyme affinity for the reaction product, maximum rate, and catalytic efficiency were reduced by 1.6, 2.9, and 4.5 times, respectively (Table 2). Liver ALDH is a tetramer, whose catalytic activity and kinetic properties depend strongly on the interaction between enzyme subunits [6,10]. Cold exposure is probably accompanied by redistribution of enzyme activity between molecular forms with high and low affinity for the reaction product in the liver mitochondrial fraction. Conformational changes in the subunit composition of enzyme macromolecules are followed by an increase in the ratio of ALDH with low affinity for the substrate. ALDH activity decreases under these conditions. Conformational changes in molecular forms of liver ALDH are probably associated with the fact that cold injury leads to an increase in the amount of cold-stress proteins. These proteins determine the direction of metabolic processes (*e.g.*, in the mitochondrial fraction). This leads to activation of LPO in cell membranes followed by an increase in the amount of free intracellular aldehydes that serve as enzyme inhibitors. The type of changes in kinetic parameters (Kt and V_{max}) of ALDH in liver mitochondria under cold conditions reflects a mixed-type inhibition of this enzyme (Table 2).

ALDH activity in the cytoplasmic fraction of the liver remained practically unchanged during cold injury (Table 1). Kinetic properties of liver cytosolic ALDH were characterized by an increase in enzyme affinity for the substrate (by 1.8 times), decrease in the maximum reaction rate (by 5.5 times), and reduction of catalytic efficiency of ALDH (by 3.1 times, Table 2). The increase are probably associated with conformational changes in the subunit composition of the tetrameric enzyme.

We conclude that low-temperature exposure significantly decreased ALDH activity in rat liver mitochondria. The catalytic efficiency of ALDH and half-reaction time of the enzyme substrate decreased during cold injury. Cold injury caused a mixed-type inhibition of ALDH in liver mitochondria. Atypical changes in kinetic characteristics of ALDH were found in the cytoplasmic fraction of rat liver during cold injury.

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