A Hyperenzymemia Model as a Means of Studying the Development of Metabolic Disorders

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Hyperenzymemia is a characteristic feature of a number of congenital and acquired diseases [3,7, 13]. The increased activity is known to be caused either by activation of the enzyme in the blood serum due to its structural rearrangement, changes in the conditions controlling the function of a particular catalytic protein, or additional release from tissues due to a process leading to impaired permeability and destruction of cell and subcellular membranes [10]. For a pathogenetic evaluation of hyperenzymemia, elucidation of the cause-effect relationship between the increased enzyme activity and multiple metabolic shifts, and for investigation of the possibility of active intervention in the metabolic processes by administration of enzyme, hyperenzymemia was modeled using exogenous lactate dehydrogenase (LDH).

MATERIALS AND METHODS

Experiments were carried out on outbred gray rabbits weighing 3.7-4.1 kg (15 animals). Hyperenzymemia was induced by i.v. injection of 1 ml mammalian LDH (EC 1.1.1.27) in a dose of 5000 IU/kg body weight. The enzyme was obtained in the form of a homogenous preparation as described elsewhere [1]. Tissue metabolic parameters were determined under standard conditions 60 min after enzyme injection. The rabbits were decapitated. Mitochondria and microsomes were isolated by sequential centrifugation in 0.25 M sucrose. The cytosol fraction was obtained by centrifugation at 105,000 g. Mitochondria were solubilized with Triton X-100. The activity of the following enzymes was determined in subcellular organelles and in the cytosol fraction: LDH [11], malate dehydrogenase [9], glucose-6-phosphate dehydrogenase [6], glutamate dehydrogenase [8], β-hydroxybutyrate dehydrogenase [12], alanine and aspartate transaminases with Lachema kits, and NADH and HADPH oxidase [2]. The protein concentration was measured using the biuret method [5]. The content of malate, oxaloacetate, glutamate, α-ketoglutarate, α-phosphoglycerate, lactate, and pyruvate as described elsewhere [4], and glucose and urea with Lachema kits was measured in protein-free extract. The data were processed statistically using the Student t test.

RESULTS

Intravenous administration of LDH resulted in intensified glucose glycolytic degradation in the liver. Aldolase activity rose by 74% ($p<0.001$) and LDH by 26% ($p<0.001$) (Table 1). These changes are characteristic for cytosolic water-soluble enzymes. In the mitochondrial fraction from the liver the activity of membrane LDH was markedly increased, while the aldolase activity remained unchanged.

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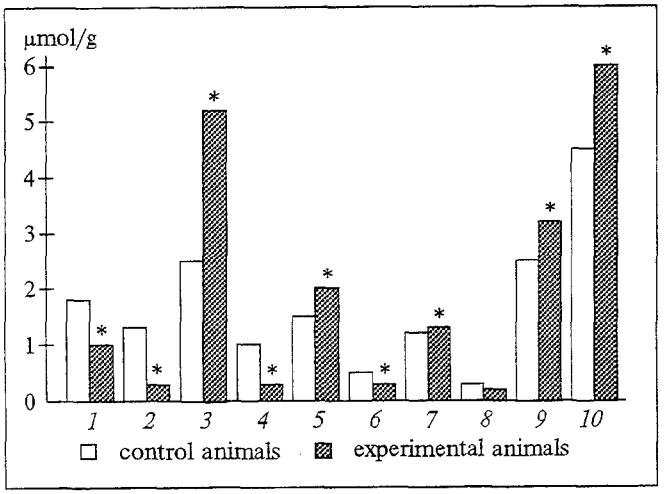

Fig. 1. Content of metabolites in the liver tissue after intravenous administration of lactate dehydrogenase. 1) malate; 2) oxaloacetate; 3) lactate; 4) pyruvate; 5) α–phosphoglycerate; 6) dihydroxyacetone phosphate; 7) glutamate; 8) α–ketoglutarate; 9) urea, mmol/liter; 10) glucose, mmol/liter.

Utilization of glucose through the pentose cycle corresponded to that in the control. Activated glycolytic degradation of glucose was accompanied by accelerated utilization of dihydroxyacetone phosphate (-39%, $p<0.001$) and pyruvate (-83%, $p<0.001$), as well as accumulation of lactate (+111.7%, $p<0.001$) in the liver. The content of α-phosphoglycerate was increased by 32% ($p<0.001$), probably due to its hindered utilization in oxidative and anabolic processes. The prevalent shifts in the glycolytic enzyme-substrate system without changes in the mitochondrial enzymes or triggering enzyme of the pentose cycle suggest that the metabolic shifts in the liver are caused by LDH-induced hyperenzymemia. The structural similarity of LDH from different sources may be responsible for the *in vivo* interaction of exogenous enzyme with a glycolytic metabolite. The activities of glutamate dehydrogenase and β-hydroxybutyrate dehydrogenase in the mitochondria were virtually unchanged. The activity of alanine and aspartate transaminases also remained within the control values. In contrast, the activity of cytosol transaminases was markedly decreased. In the liver during modeled hyperenzymemia the activation of anaerobic oxidative metabolism was accompanied by a relatively high level of detoxication processes, directed toward ammonium elimination, which is indicated from the increased urea content (Fig. 1). Anabolic processes were also intensive: the depletion of the oxaloacetate and 2-oxoglutarate pools is probably related to their consumption for glucose synthesis.

The absence of glutamate dehydrogenase and glucose-6-phosphate dehydrogenase activity in the blood implied an unchanged membrane permeability.

A characteristic feature was a 2-fold increase of the total microsomal NADPH oxidase activity, indicating the acceleration of oxidative detoxication processes mediated by NADPH. Consequently, exogenous LDH under the given experimental conditions activated the microsomal oxidative detoxication.

Thus, modeled hyperenzymemia may be a factor inducing multiple metabolic changes at different metabolic levels. In the cytoplasm, mitochondria, and microsomes both anaerobic and aerobic oxidative processes are activated, due to possible protein-protein interaction and the involvement of common substrates. The active participation of exogenous lactate dehydrogenase in metabolic processes in experimental animals offers promise for its use as an enzyme therapeutic agent.

TABLE 1. Enzymatic Activity (mmol NADH/mg protein/min) in Different Infrastructures of Rabbit Liver Tissues after Intravenous Administration of Lactate Dehydrogenase ($M \pm m$)

Parameter	Structures	Control	Experiment
Malate dehydrgenase	supernatant	1.232±0.092	1.017±0.068*
	mitochondria	0.547±0.041	0.454±0.028*
Lactate dehydrgenase	supernatant	0.380±0.041	0.480±0.053*
	mitochondria	0.033±0.006	0.082±0.007*
Aldolase	supernatant	0.039±0.002	0.068±0.004*
	mitochondria	0.011±0.001	0.014±0.001
Alanine Transaminase, mmol/ml/h	supernatant	2.149±0.095	1.801±0.085*
	mitochondria	3.139±0.138	3.386±0.098
Aspartate transaminase, mmol/ml/h	supernatant	2.052±0.067	1.645±0.082*
	mitochondria	3.499±0.179	3.816±0.104
Glutamate dehydrgenase	mitochondria	0.057±0.003	0.075±0.004
β–hydroxybutyrate dehydrgenase	mitochondria	0.044±0.005	0.049±0.005
Glucose–6–phosphate dehydrgenase	supernatant	0.016±0.0001	0.015±0.0006
NADH oxidase	microsomes	0.003±0.0007	0.006±0.001*
NADPH oxidase	microsomes	0.003±0.0007	0.006±0.001*

Note. *: reliable differences from control

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Comparison of the Effects of 5-Hydroxyeicosatetraenoic Acid and Hepoxilin on Cholinoreceptor Plasticity of *Helix lucorum* Neurons

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Natural polyunsaturated fatty acids (dihomo-γ-linolenic, arachidonic and thymnodonic) are components of cell membrane lipids. Enzymatic transformation of these compounds under the action of lipoxygenases (LO), cytochrome P_{450}, and cyclooxygenase results in the formation of low-molecular-weight oxygenized metabolites - eicosanoids which are universal regulators of cell metabolism [5,15]. Since eicosanoids freely penetrate through the cell membrane, they may act both as intracellular (secondary mediators) and as extracellular (retrograde messengers) signal molecules and participate in transsynaptic regulation of neuron activity as local neuromodulators [5,6,10-15].

Among lipoxygenase eicosanoids, three main groups formed under the influence of 5-, 12-, and 15-LO can be distinguished [15]. In studying the role of eicosanoids in the regulation of cholinoreceptor (ChR) plasticity of *Helix lucorum* neurons, we used polyacethylene analogs of dihomo-γ-linolenic, arachidonic, and thymnodonic acids, which are known to inhibit different lipoxygenases [2]. The results allowed us to suggest the main regulatory role of 5-lipoxygenase eicosanoids including those from the 15-lipoxygenase pathway. On the other hand, involvement of 12-lipoxygenase derivatives in this regulation seemed most unlikely. 15-Hydroxyeicosatetraenoic acid (15-HETE) regulates ChR plasticity [1]. In the present study we tested

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