DOI 10.1007/s10517-017-3797-8

Effect of Hypothermia on Kinetic Characteristics of Lactate Dehydrogenase in Rat Brain under Conditions of Global Ischemia and Reperfusion

R. A. Khalilov, A. M. Dzhafarova, and S. I. Khizrieva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 3, pp. 313-317, March, 2017
Original article submitted May 18, 2016

We studied activity and kinetic characteristics of lactate dehydrogenase (LDH) in rat brain under conditions of incomplete global ischemia followed by reperfusion against the background of mild hypothermia. It was found that hypothermia leads to a decrease in LDH activity in the ischemic brain; the maximum velocity of the enzyme-catalyzed activity decreased and Michaelis constant increased, due to which the efficiency of catalysis decreased to the level observed in control rats. Ischemia against the background of hypothermia was accompanied by a decrease in the inhibition constant and narrowing of effective pyruvate concentration range. Blood flow resumption in the ischemic brain against the background of mild hypothermia led to an increase in LDH activity, the maximum reaction velocity increased, and Michaelis constant decreased, which lead to a significant increase in the efficiency of catalysis. This was accompanied by an increase in enzyme inhibition constant and a shift of the optimum on the concentration curve towards lower pyruvate concentrations.

Key Words: *rats; hypothermia; ischemia; reperfusion; lactate dehydrogenase*

Ischemic stroke, the most common and severe cerebral vascular disorder, is the main cause of death and disability not only in Russia, but also all over the world [1]. Circulation disturbances in the brain during ischemia initiate a cascade of biochemical reactions that underlie tissue damage. The main mechanisms of neuronal damage include depletion of energy resources against the background of brain tissue acidosis, ion homeostasis disturbances, excessive accumulation of excitatory amino acids producing a neurotoxic effect, and enhanced production of ROS inducing oxidative stress [10,11]. Blood flow resumption in the ischemic brain (reperfusion) can produce severe damaging effect by manifold increasing the concentration of free radicals [5].

Hypothermia can be an effective method for brain protection from ischemic and reperfusion injury [3,9]. The protective effect of hypothermia is mediated by diverse mechanisms: inhibition of the synthesis and secretion of transmitters, especially extracellular glutamate, decrease in the blood-brain barrier permeability, and suppression of free-radical processes [4]. The main physiological mechanism of hypothermia-induced neuroprotection is metabolic reduction that decreases oxygen and glucose demands of the tissues [9]. Hypothermia decelerates metabolic processes thus maintaining the content of high-energy phosphates and blood pH. It was shown that ischemia against the background of moderate hypothermia is not associated with significant changes in the brain content of glucose, ATP, and lactate, the main trigger of the "ischemic cascade" [6]. The level of lactate in cells is determined by the catalytic efficiency of lactate dehydrogenase (LDH), the key enzyme of anaerobic glycolysis. LDH, a crossroad enzyme of carbohydrate metabolism, participates in the regulation of finely balanced catabolism and anabolism, anaerobic and aerobic glycolysis. We have previously described sig-

Department of Biochemistry and Biophysics, Dagestan State University, Makhachkala, Republic of Dagestan, Russia. ***Address for correspondence:*** albina19764@mail.ru. A. M. Dzhafarova

nificant changes in activity and kinetic characteristics of LDH in various brain structures during ischemia, reperfusion, and hypothermia of different depth and duration [2,7]. However, combined effect of hypothermia and ischemia on this enzyme remains poorly studied.

We analyzed the effect of mild (33°C) hypothermia on the activity and kinetic characteristics of LDH in the brain of rats with global ischemia and reperfusion.

MATERIALS AND METHODS

The experiments were conducted on white outbred male rats weighing 150-200 g. Brain ischemia was modeled by complete ligation of both carotid arteries under anesthesia (thiopental, 40 mg/kg) for 60 min. For creation of the reperfusion model of ischemic damage, both common carotid arteries were clamped for 60 min and then blood flow was resumed (60 min) to achieve reperfusion of previously ischemic tissue. The body temperature was maintained at the physiological level (37°C) during ischemia and reperfusion. To simulate ischemia/reperfusion with mild hypothermia, the body temperature of the animals was reduced to 33°C before the occlusion of the carotid arteries. Sham-operated rats served as the control.

LDH activity was assessed spectrophotometrically by a decrease of NADH concentration in the reaction mixture as a result of enzymatic reduction of pyruvate into lactate. The reaction mixture: 2.4 ml 0.1 M phosphate buffer (pH 7.4), 0.3 ml sodium pyruvate solution (Sigma), 0.3 ml 1 mM $NADH_2$ solution (Sigma), and 0.05 ml tissue extract containing 25 μg protein. LDH activity was studied in pyruvate concentration range from 0.1 to 25.6 mM and expressed in nanomoles of NADH oxidized in the enzymatic reaction per 1 min per 1 mg protein (nmol/min×mg protein). Kinetic characteristics — maximum velocity (Vm), Michaelis constant (Km), and inhibition constant (Ki) were calculated from the concentration dependence of NADH oxidation rate by the least squares procedure using Statistica software. Haldane equation was used in the "nonlinear estimation" option.

The results were processed by one-way ANOVA using Statistica software. The significance of differences was determined using Fisher's test at significance level p=0.05. Each curve on the graphs of the concentration dependence of the oxidation rate of NADH is the average of 8 independent experiments.

RESULTS

Occlusion of the carotid arteries in rats for 1 h did not affect the shape of the concentration dependence of LDH activity (Fig. 1), but enzyme activity increased at all studied pyruvate concentrations, in particular, by 40.3% at the optimum concentration (Fig. 2).

Blood flow resumption in the brain during reperfusion produced an opposite effect: LDH activity in the brain at the optimum pyruvate concentration decreased by 37.6% in comparison with the control and the optimum was shifted towards higher substrate concentrations.

Occlusion of carotid arteries against the background of mild hypothermia was accompanied by a decrease in LDH activity at all studied pyruvate concentrations to a level observed in control rats. At optimum concentration of pyruvate, the decrease in activity was 33% in comparison with ischemic animals (Fig. 2). Recovery of cerebral blood flow after ischemia at hypothermia was accompanied by a significant increase in LDH activity at all the studied concentrations of pyruvate, however, the values of activity were lower than the control values. Thus, with an optimum pyruvate concentration, the increase in LDH activity was 28% in comparison with non-hypothermic animals after reperfusion, but this value was by 20.5% below the control. The decrease in rat body temperature to 33°C during cerebral reperfusion led to a shift in the optimum on activity curve towards lower pyruvate concentrations (corresponding to those in control rats).

Since the concentration dependence of LDH clearly demonstrates the phenomenon of substrate inhibition, Haldane model can be used to describe the kinetics and to determine the kinetic parameters of the enzyme at saturation and fixed concentrations of cofactor (NADH) [15].

Using this model, we calculated kinetic characteristics of LDH (Table 1). We found that Vm increased by 30% during ischemia, while Km decreased by 16.3%. This increase in the reaction rate with slight decrease in Km leads to a significant (by 58.2%) increase in the Vm/Km ratio reflecting the catalysis efficiency at physiological concentrations of substrate. Carotid artery occlusion against the background of hypothermia led to a decrease in Vm (by 27.6%) and an increase in Km (by 19.1%) of LDH in comparison with non-hypothermic animals that led to a significant (by 38.7%) decrease in Vm/Km ratio. According to Haldane model, it is possible to calculate the optimum point of *S*opt:

$$Sopt=\sqrt{Km\times Ki}.$$

Occlusion of carotid arteries did not affect Sopt and only slightly increased Ki. Hypothermia reduced Ki (by 20.4%), Sopt, and, hence, the range of effective pyruvate concentrations (calculated as the difference between Ki and Km (Δ=Ki-Km).

During reperfusion, Vm decreased by 33.3% in comparison with the control, while Km, on the con-

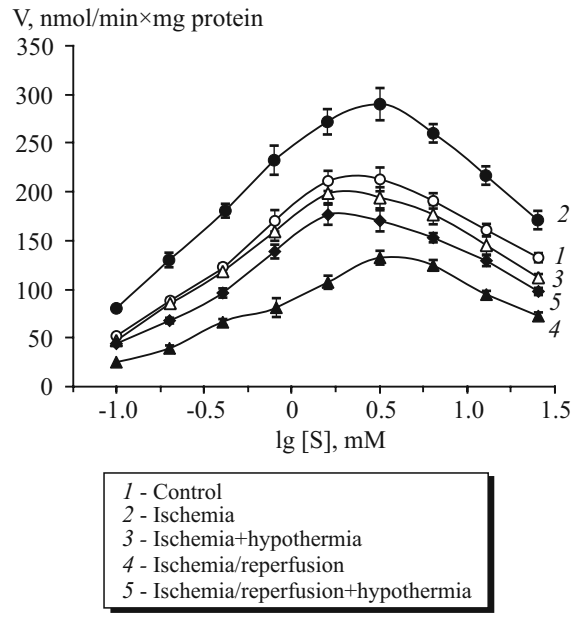

Fig. 1. Concentration dependence of LDH activity in rat brain at ischemia/reperfusion with hypothermia.

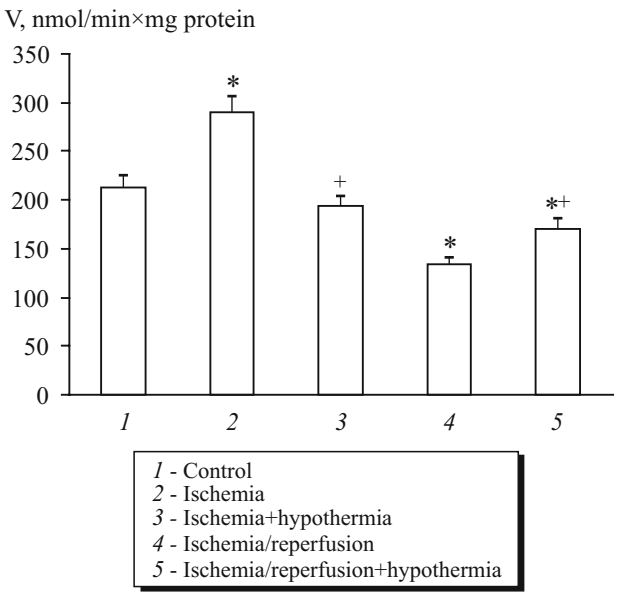

Fig. 2. LDH activity in rat brain during ischemia/reperfusion with hypothermia. $p<0.05$ in comparison with *control, +with ischemia and ischemia/reperfusion (concentration of pyruvate in the incubation medium 3.2 mM).

trary, increased by 79%. Due to this, the efficiency of catalysis (Vm/Km) dramatically decreased (by 67.2%; Table 1). Blood flow recovery in the brain of ischemic rats under conditions of mild hypothermia, on the contrary, increased Vm of LDH by 31.4% in comparison with non-hypothermic animals, while Km decreased by 38%. This increase in Vm and decrease in Km significantly (by 2.4 times) improved the efficiency of LDH catalysis that was by 19.9% below the control level. Reperfusion was accompanied by a decrease in Ki by 29.5% and a shift of the optimum on the concentration curve towards higher concentrations. The range of effective substrate concentrations was narrowed by 31.6% in comparison with the control. This attests to more significant contribution of Km to changes in Sopt. Hypothermia of reperfused animals led to a slight increase in Ki (by 15.2%) in comparison with non-hypothermic rats. In this case, a shift of Sopt toward lower (corresponding to control) pyruvate concentrations was observed, and the range of effective concentrations of pyruvate increased by 17.8% in comparison with that at reperfusion.

Thus, hypothermia led to a significant decrease in the catalytic efficacy of LDH in rats with ischemia. This negative modulation of LDH activity probably maintains low level of glycolytic lactate in the brain under conditions of ischemia and thereby contributes

TABLE 1. Kinetic Characteristics of Rat Brain LDH in the Normal and under Conditions of Deep, Mild, and Moderate Prolonged Hypothermia (n=8; $M\pm m$)

Experimental condition	V_m	K_m	K_i	V_m/K_m	S_{opt}	$D=K_i-K_m$
Control	271.05±11.32	0.431±0.032	21.85±1.07	620.25±26.3	3.09±0.08	21.42±1.03
Ischemia	352.86±15.81*	0.360±0.020	23.21±0.82	980.17±24.9**	2.89±0.11	22.85±0.45
Ischemia+ hypothermia	255.75±9.70^{+}	0.441±0.014^{+}	18.48±0.61	600.35±20.70^{+}	2.80±0.09	18.05±0.58
Ischemia/ reperfusion	180.72±7.91^{*++}	0.770±0.032^{**++}	15.42±0.80^{*+}	204.17±11.02^{***+++}	3.45±0.21^{+}	14.65±0.58^{*+}
Ischemia/ reperfusion+ hypothermia	237.54±9.15^{o}	0.478±0.012^{oo}	17.75±0.42	496.94±15.60^{*ooo}	2.93±0.11	17.27±0.34*

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ in comparison with the control; $^{+}p<0.05$, $^{++}p<0.01$, and $^{+++}p<0.001$ in comparison with ischemia; $^{o}p<0.05$, $^{oo}p<0.01$, and $^{ooo}p<0.001$ in comparison with ischemia/reperfusion.

to neuroprotection. The change in the catalytic efficiency of enzymes can occur due to both Km and Vm. Vm is a value that depends on the concentration of the enzyme and the number of its turnover (kcat). The concentration of the enzyme in the cell, in turn, is determined by the rate of its synthesis and degradation. It has been shown that hypothermia increases the concentration of ubiquitin and thereby can increase the rate of proteosomal degradation of many proteins at ischemia [4], including LDH. However, our data indicate that significant changes affect not only Vm, but also Km at hypothermia-ischemia. This suggests structural modifications of molecules of LDH, and not a decrease in their number. Chaperones can participate in the change in the spatial configuration of LDH [8]. Hypothermia increases the expression of chaperon protein of HSP70 [13]. It is possible that chaperones lead to the spatial modification of LDH with the formation of new conformers of enzyme that differ in kinetic characteristics.

Modification of LDH can be determined among other things by its oxidation by ROS accumulated during reperfusion. It is shown that in the Fenton medium that generates hydroxyl radicals the activity of the enzyme reduced significantly [12], which was observed by us at reperfusion. Hypothermia reduces immediate blood flow at reperfusion of the ischemic brain [3,14] that leads to a decrease in the production of ROS. Increase of the efficiency of LDH catalysis may be a consequence of a decrease in the modifying effect of oxygen radicals on the enzyme in the brain at ischemia/reperfusion with hypothermia.

The observed effects of hypothermia on kinetic characteristics of LDH suggest that changes in the brain concentration of lactate in animals during ischemia and reperfusion against the background of cooling to 33°C are associated with not only inhibition of metabolic rate and energy-saving mode of cell functioning, but also with changes in the mechanisms of functioning of LDH, the key enzyme of anaerobic glucose oxidation.

REFERENCES

1. Gusev EI, Skvortsova VI, Krylov VV. Decrease of lethality and invalidity from cerebral vascular diseases in the Russian Federation. Nevrol. Vestn. 2007;39(1):128-133. Russian.
2. Khalilov RA, Dzhafarova AM, Dzhabrailova RN, Emirbekov EZ. Analysis of the kinetic characteristics of lactate dehydrogenase from the rat brain during ischemia and reperfusion. Neurochem. J. 2014;8(4):265-270.
3. Alva N, Palomeque J, Carbonell T. Oxidative stress and antioxidant activity in hypothermia and rewarming: can RONS modulate the beneficial effects of therapeutic hypothermia? Oxid. Med. Cell. Longev. 2013;2013. ID 957054. doi: 10.1155/2013/957054.
4. Andresen M, Gazmuri JT, Marin A, Regueira T, Rovegno M. Therapeutic hypothermia for acute brain injuries. Scand. J. Trauma Resusc. Emerg. Med. 2015;23:42. doi: 10.1186/s13049-015-0121-3
5. Eltzschig HK, Eckle T. Ischemia and reperfusion--from mechanism to translation. Nat. Med. 2011;17(11):1391-1401.
6. Erecinska M, Thoresen M, Silver IA. Effects of hypothermia on energy metabolism in Mammalian central nervous system. J. Cereb. Blood Flow Metab. 2003;23(5):513-530.
7. Khalilov RA, Dzhafarova AM, Dzhabrailova RN, Khizrieva SI. The kinetic and thermodynamic characteristics of lactate dehydrogenase in the rat brain during hypothermia. Neurochem. J. 2016;10(2):156-165.
8. Place SP, Hofmann GE. Comparison of Hsc70 orthologs from polar and temperate notothenioid fishes: differences in prevention of aggregation and refolding of denatured proteins. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2005;288(5):R1195-R1202.
9. Polderman KH, Herold I. Therapeutic hypothermia and controlled normothermia in the intensive care unit: practical considerations, side effects, and cooling methods. Crit. Care Med. 2009;37(3):1101-1120.
10. Rodrigo R, Fernandez-Gajardo R, Gutierrez R, Matamala JM, Carrasco R, Miranda-Merchak A, Feuerhake W. Oxidative stress and pathophysiology of ischemic stroke: novel therapeutic opportunities. CNS Neurol. Disord. Drug Targets. 2013;12(5):698-714.
11. Szydlowska K, Tymianski M. Calcium, ischemia and excitotoxicity. Cell Calcium. 2010;47(2):122-129.
12. Tangkosakul T, Tantimongcolwat T, C. Isarankura-Na-Ayudhya C, Mejare M, Bülow L, Prachayasittikul V. Native and chimeric metal-binding lactate dehydrogenase as detection and protection tools for oxidative stress induced by Fenton's reaction. EXCLI J. 2009;8. doi: 10.17877/DE290R-8480.
13. Terao Y, Miyamoto S, Hirai K, Kamiguchi H, Ohta H, Shimojo M, Kiyota Y, Asahi S, Sakura Y, Shintani Y. Hypothermia enhances heat-shock protein 70 production in ischemic brains. Neuroreport. 2009;20(8):745-749.
14. Yenari MA, Han HS. Neuroprotective mechanisms of hypothermia in brain ischaemia. Neuroscience. 2012;13(4):267-278.
15. Zakhartsev M, Johansen T, Pörtner H.O, Blust R. Effects of temperature acclimation on lactate dehydrogenase of cod (Gadus morhua): genetic, kinetic and thermodynamic aspects. J. Exp. Biol. 2004;207(Pt 1):95-112.