

Effect of Hypothermia on Kinetic Characteristics of Acetylcholine Esterase in Rat Erythrocyte Membranes

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 7, pp. 56-58, July, 2004
Original article submitted June 11, 2003

We studied kinetic and thermodynamic characteristics of acetylcholine esterase in rat erythrocyte membrane after whole-body hypothermia (20°C) of different duration. Hypothermia increased the degree of substrate inhibition for acetylcholine esterase, maximum rate, and Michaelis constant. The temperature dependence of acetylcholine esterase activity remained practically unchanged.

Key Words: *hypothermia; erythrocytes; membranes; acetylcholine esterase; temperature dependence*

Recent studies demonstrated structural role of acetylcholine esterase (AChE) not associated with its catalytic activity [9,10]. AChE was found in erythrocyte membranes (700-800 molecules per erythrocyte) [8]. The physiological role of the enzyme in erythrocytes remains unknown. Clinical observations indicate that AChE is absent in erythrocyte membranes of patients with paroxysmal nocturnal hemoglobinuria. In rat erythrocytes AChE is presented by dimers anchored to phosphatidylinositol in the outer membrane leaflet [8] and is similar by its kinetic characteristics to AChE from mammalian brain [12].

Osmotic resistance of rat erythrocytes decreases during hypothermia, which produces an increase in extraerythrocytic hemoglobin content [7]. These changes led intensification of lipid peroxidation in the blood and organs [6]. The mechanism underlying changes in osmotic resistance of erythrocytes during hypothermia is poorly understood. They can be related to structural changes in erythrocyte membrane at low temperature. Here we studied kinetic characteristics of AChE from rat blood erythrocytes during hypothermia (20°C). Under these conditions AChE plays a role of

the reporter molecule that reacts to microenvironmental changes.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 180-200 g. Intact animals were kept in a vivarium at room temperature and served as the control. To produce hypothermia the rats were cooled in Plexiglas chambers. Cold water (10°C) circulated through a case of the chamber. Body temperature (measured in the rectum) progressively decreased from 38 to 20°C over 55-60 min. The animals were decapitated immediately (short-term hypothermia, SH, series I) or after 2 h at 20°C (prolonged hypothermia, PH, series II). Then the rats were decapitated. Erythrocyte membranes were isolated by hemolysis in hypotonic Tris-HCl buffer (0.1 M, pH 7.4) [2]. Ghosts were washed with a buffer containing 1.5 mM ethylenediamine-tetraacetic acid to remove hemoglobin. AChE activity in the suspension of erythrocyte membranes was measured as described elsewhere [4] using acetylthiocholine iodide (ATCh) as the substrate. We measured the rate of ATCh hydrolysis (0.008-4.000 mM) during incubation at 37 and 20°C. Concentration dependences are presented in coordinates of the reaction rate against substrate concentration logarithm (mmol). The temperature dependence of AChE activity was estimated

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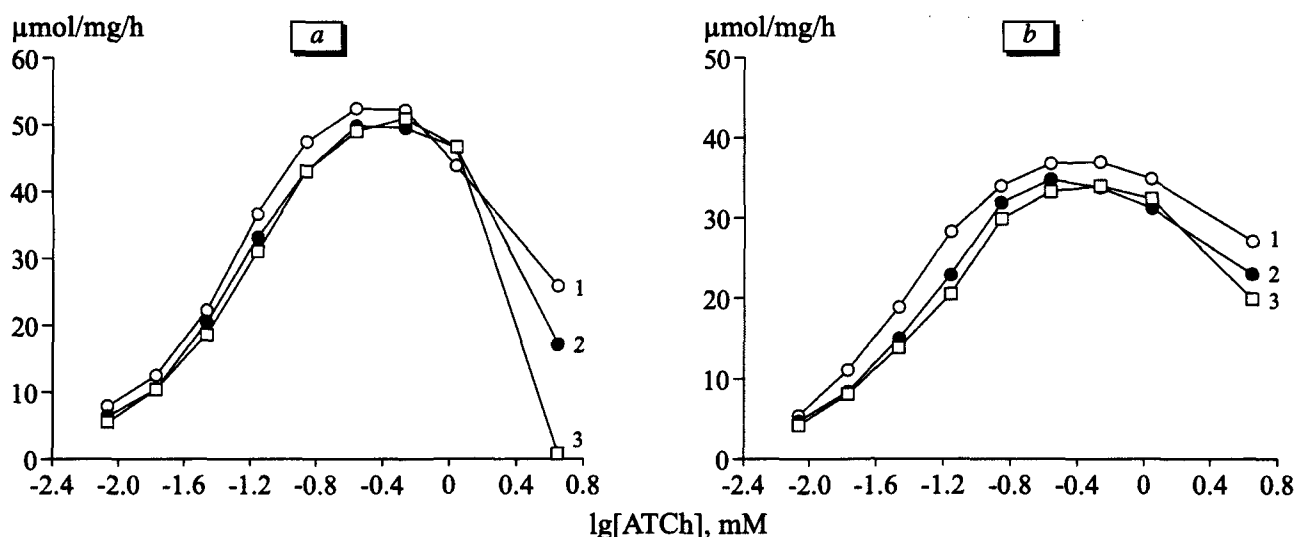


Fig. 1. Concentration dependences for the rate of acetylthiocholine iodide (ATCh) hydrolysis by erythrocyte membrane acetylcholine esterase at various temperatures. Incubation at 37 (a) and 20°C (b). Here and in Fig. 2: control (1) and short-term (2) and prolonged hypothermia (3).

ted in the presence of 0.5 mM ATCh at 5–40°C and presented in Arrhenius coordinates.

RESULTS

During incubation at 37°C the slope of the right (inhibitory) shoulder of the concentration dependence curve of ATCh hydrolysis by erythrocyte membrane AChE was more abrupt than that of the left shoulder (Fig. 1). It serves as a criterion of cooperative substrate inhibition. The degree of substrate inhibition was quantitatively characterized as the ratio (*Q*) between the reaction rates at the optimum (0.5 mM) and maximum concentrations of the substrate (4 mM). At 37°C the degree of substrate inhibition in control, SH, and PH groups was 2.0, 2.9, and 63.6, respectively. The degree of substrate inhibition during incubation of samples from control, SH, and PH animals at 20°C was 1.4, 1.5, and 1.7, respectively.

The maximum rate and Michaelis constant were derived from the Lineweaver—Burk plot (Table 1). Incubation at 37 and 20°C showed that the maximum rate increases after SH (by 45 and 26%, respectively) and PH (by 28 and 20%, respectively). We revealed

an increase in the Michaelis constant after SH (by 54 and 63%, respectively) and PH (by 40 and 57%, respectively). Our results indicate that hypothermia modified kinetic characteristics of AChE. It should be emphasized that substrate inhibition underwent most pronounced changes after PH. Various factors can affect substrate inhibition of AChE [11]. The degree of substrate inhibition depends on the composition of lipids interacting with the enzyme, chemical modification of the enzyme molecules produced by reactive oxygen species, *etc.* [3]. Previous studies showed that acetylcholine in concentrations of 10^{-3} – 10^{-2} M induces cooperative structural transition in bovine erythrocyte membranes, which results in substrate inhibition of AChE [1]. These changes in the erythrocyte membrane are probably related to the interaction of acetylcholine with AChE. It can be hypothesized that hypothermia affects the interaction between AChE and lipids, which modifies kinetic characteristics of the enzyme.

Temperature dependences of AChE activity in Arrhenius coordinates can be approximated by two linear regions that are characterized by effective energy of activation (Fig. 2). Hypothermia had no effect on

TABLE 1. Maximum Rate and Michaelis Constant for AChE of Rat Blood Erythrocyte Membranes during Hypothermia ($M \pm m$)

Experimental conditions	Maximum rate, $\mu\text{mol/mg protein/h}$		Michaelis constant, mM	
	37°C	20°C	37°C	20°C
Control ($n=6$)	58.6 ± 4.1	41.1 ± 3.1	0.072 ± 0.005	0.056 ± 0.003
SH ($n=8$)	$85.2 \pm 1.2^*$	$51.7 \pm 2.5^*$	$0.111 \pm 0.003^*$	$0.091 \pm 0.004^*$
PH ($n=12$)	$75.0 \pm 4.7^*$	$49.4 \pm 3.5^*$	$0.101 \pm 0.004^*$	$0.088 \pm 0.007^*$

Note. $^*p \leq 0.05$ compared to the control.

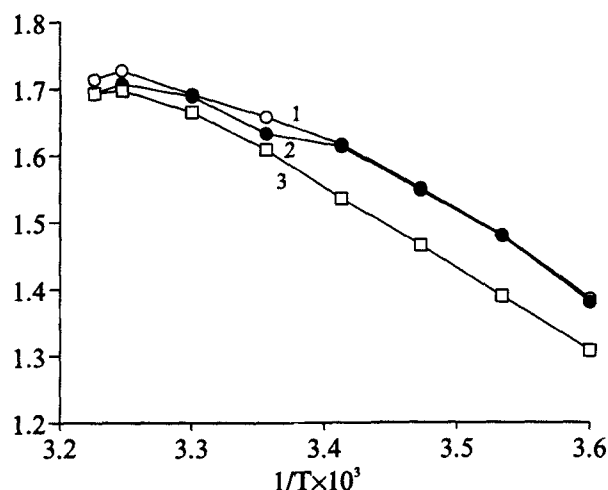


Fig. 2. Temperature dependence (Arrhenius coordinates) of acetylcholine esterase activity from erythrocyte membrane at acetylthiocholine iodide concentration of 0.5 mmol. T, incubation temperature. Ordinate: logarithm of the rate of acetylthiocholine iodide hydrolysis.

TABLE 2. Inflection Point and Activation Energy for AChE of Rat Blood Erythrocyte Membranes during Hypothermia ($M \pm m$, $n=10$)

Experimental conditions	Inflection point, °C	Activation energy, kJ/mol	
		over inflection	under inflection
Control	20.90±0.37	10.43±0.63	23.75±1.00
SH	21.70±0.43	9.26±0.50	23.88±1.30
PH	21.60±0.49	10.81±0.75	25.52±0.75

the temperature dependence of AChE activity. Effective energy of activation for two linear regions and inflection point remained practically unchanged (Table 2). Our previous studies showed that hypothermia (20°C) has no effect on microviscosity of erythrocyte

membrane lipids measured with fluorescent probe pyrene. However, microviscosity of annular lipids increased by 50% under these conditions [7]. Our results are consistent with published data that annular lipids in the erythrocyte membrane can undergo phasic transitions at 20°C [5]. PH produced more significant changes in kinetic characteristics of AChE compared to SH. These changes are probably associated with strengthening of protein-lipid interactions at the site of enzyme localization. The index characterizing substrate inhibition of AChE (Q) can be used as a sensitive indicator of changes in protein-lipid interactions in the erythrocyte membrane.

This work was supported by the "Russian Universities" Program (grant No. 07.01.034).

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