Temperature Dependence of Acetylcholine Esterase Activity in Synaptic Membranes from Rat Brain during Hypothermia

N. K. Klichkhanov, R. A. Khalilov, and I. S. Meilanov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 3, pp. 326-328, March, 2000
Original article submitted August 17, 1998

The degree of substrate inhibition of acetylcholine esterase activity decreases in plasma synaptosome membranes isolated from the cerebral cortex of rats subjected to short-term and prolonged hypothermia at 20°C, while activation energy and breakpoint of the temperature curve in the Arrhenius coordinates remain unaffected. The observed changes are supposed to compensate the inhibitory effect of hypothermia on enzyme activity.

Key Words: *acetylcholine esterase; temperature dependence; synaptic membranes; hypothermia; rats*

Even a short-term drop of core temperature to 20°C in homeothermic animals induces adaptive changes in the enzymatic apparatus in the brain [3]. A peak appears on the temperature plot of activity of the glutaminase isolated from synaptosome brain fraction, which corresponds to body temperature and suggests activation of temperature compensation of synaptic transmission, because glutaminase catalyzes synthesis of excitatory transmitter glutamate in glutamatergic synapses. The same changes probably take place in other neurotransmitter cerebral systems during hypothermia. Acetylcholine esterase (ACE) is a key enzyme in cholinergic cerebral synapses. Here we studied the temperature dependence of activity of this enzyme in rats subjected to 20°C hypothermia.

MATERIALS AND METHODS

The study was carried out on 54 random-bred male albino rats weighing 170-200 g. The animals were divided into three groups. Group 1 rats were control, group 2 rats were subjected to 20°C hypothermia, and group 3 rats were subjected to a long-term hypothermia for 2 h. Hypothermia was modeled in a special chamber, where rats were cooled to 20°C during 60 min. Core temperature was measured. Synaptosomes were isolated from the gray substance of the cerebral hemispheres by differential centrifugation [5], and plasma

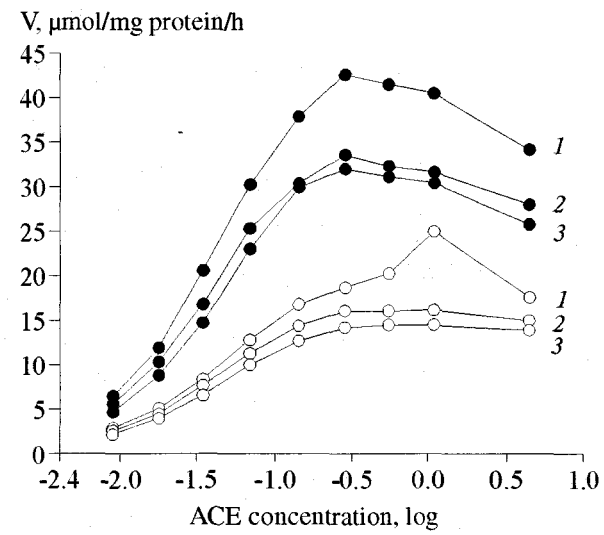

Fig. 1. The rate (V) of acetylthiocholine (ATC) hydrolysis in synaptic membranes isolated from the gray substance of the cerebral cortex of rats subjected to 20°C hypothermia. Incubation at 37°C (filled circles) and 15°C (open circles). Here and in Fig. 2: *1*) control; *2*) short-term hypothermia 20°C; *3*) long-term (2 h) hypothermia 20°C.

Laboratory of Ecological Biochemistry, Laboratory of Biophysics and Physiology, Institute of Biology, Dagestan State University, Makhachkala

0007-4888/00/0003-279$25.00

TABLE 1. Effect of Hypothermia (20°C) on ACE Activity in Synaptosomal Membranes from Gray Substance of Cerebral Cortex in Rats ($M\pm m$, n=6-8)

Index		Control	Hypothermia 20°C	
			short-term	long-term
V_m, μmol/mg protein/h	37°C	55.4±2.1	44.1±1.8*	43.5±2.4*
	15°C	23.8±0.7	19.9±1.0*	19.9±0.7*
K_m, mmol	37°C	0.064±0.003	0.060±0.002	0.069±0.002
	15°C	0.063±0.002	0.059±0.002	0.062±0.003
Breakpoint, °C		24.8±0.9	24.9±0.4	25.6±0.7
Activation energy, kJ/mol				
above breakpoint		22.7±0.6	23.4±0.6	21.5±0.5
below breakpoint		34.0±0.7	35.2±1.0	33.8±0.4

Note. *p<0.05 compared to the control.

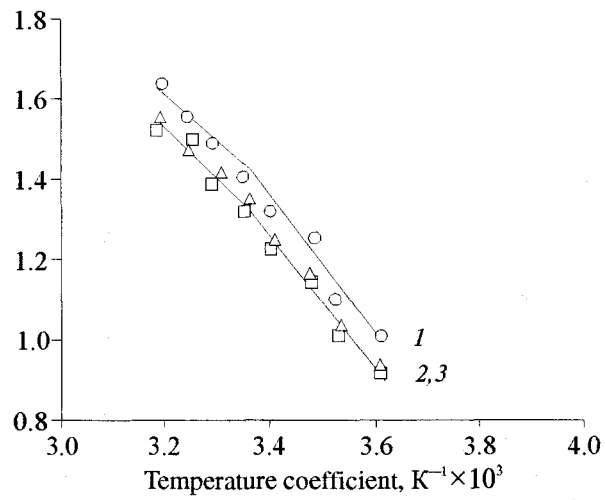

Fig. 2. Activity of acetylcholine esterase in synaptic membranes isolated from the cerebral gray substance of rats subjected to 20°C hypothermia. Ordinate: logarithm of the reaction rate.

membranes were obtained by sedimentation after osmotic shock. Activity of ACE in the synaptosome membrane fraction was determined by modified [2] method of Ellman. Acetylthiocholine was used as the substrate. Activity of ACE was recorded at 5-40°C (substrate concentration 0.5 mM), and the rate of acetylthiocholine hydrolysis (9.10×10^{-6}-4.4×10^{-3} M) was measured at 37°C and 15°C.

RESULTS

At the initial stages of synaptic transmission, the concentration of neurotransmitter is 0.5-5.0 mmol [1], which is biologically important and corresponds to the ACE substrate inhibition concentrations.

The measurements of concentration dependence of enzymatic reaction at the temperatures above and below breakpoint on the Arrhenius plots showed that the degree of substrate inhibition is smaller at low temperatures (Fig. 1). ACE activity is known to depend on lipid composition and phase state of the matrix [4], so deactivation of substrate inhibition at low temperatures can be explained by phase changes of membrane lipids.

A short-term hypothermia reduced the degree of ACE substrate inhibition (Fig. 1), and this effect was observed at both incubation temperatures. Michaelis constant K_m remained unchanged, but the maximum rate of reaction V_m decreased by 25% (Table 1).

The study of temperature dependence of ACE activity revealed two linear fragments in the Arrhenius plots (Fig. 2) and a breakpoint corresponding to 24.8°C. The character of temperature dependence and breakpoint did not depend on the duration of hypothermia. These data indicate that hypothermia produces no significant changes in the lipid microenvironment of ACE in synaptic membranes. A significant decrease in V_m and substrate inhibition was observed, which is evidently related to some changes in the enzyme molecule. However, these changes do not affect the affinity of the enzyme to its substrate, since K_m did not change.

REFERENCES

1. R. N. Glebov and G. M. Kryzhanovskii, *Functional Biochemistry of Synapses* [in Russian], Moscow (1978).
2. M. N. Maslova and L. V. Reznik, *Ukr. Biokhim. Zh.*, **48**, No. 4, 450-454 (1976).
3. I. S. Meilanov and M. V. Avshalumov, *Fiziol. Zh.*, No. 9, 102-106 (1997).
4. M. Brzin, J. Sketelj, and B. Klinar, in: *Handbook of Neurochemistry*, Ed. A. Laitha, Vol. 4, New York (1983), pp. 251-292.
5. F. Hajos, *Brain Res.*, **93**, No. 3, 484-489 (1975).