Cytochemical Characteristics of the Brain of Alcohol-Nonpreferring Wistar Rats with Different Learning Abilities

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 2, pp. 180-182, February 1999
Original article submitted April 10, 1998

Quantitative cytochemical analysis of the activities of glucose-6-phosphate dehydrogenase, aminopeptidase, and glutamate dehydrogenase in the brain of Wistar rats with different learning abilities revealed a difference in the level of hippocampal aminopeptidase activity. Alcohol-nonpreferring rats showed lower activity of glucose-6-phosphate dehydrogenase in the nucleus accumbens compared with intact rats.

Key Words: *Wistar rat brain; learning ability; alcohol-nonpreferring animals; enzymes of glucose and protein metabolism*

It has been shown that in Wistar rats the effects of information load on alcohol preference depend on the type and nature of their cognitive activity [10, 11].

Taking into consideration the shifts in glucose and amino acid metabolism during alcoholization [4,7-9] and the specificity of alcohol-induced reactions in different brain regions [12], we investigated activity of glucose and protein (amino acid) metabolizing enzymes in the brain of nonpreferring Wistar rats with different learning abilities. Enzyme activity was assayed by the methods of quantitative cytochemistry and concentrated on brain regions involved in the realization of purposeful behavior, such as the sensorimotor cortex, caudate nucleus, nucleus accumbens, and hippocampus.

MATERIALS AND METHODS

The initial reactions of the animals to alcohol were tested under conditions of free choice between 10% ethanol and water for 7 days [1]. The rats whose alcohol intake did not exceed 0.1 of the total volume of fluid were considered as alcohol-nonpreferring and used for further experiments. Their learning abilities were tested in an operant conditioning paradigm with food reinforcement [2,6].

Cytochemical indices were measured in 10 male Wistar rats weighing 250 g. The rats were decapitated under light ether anesthesia, their brains were frozen and sectioned at -8°C in a Crio-Cut microtome. Sections (20-μ) from the control (intact) and experimental (conditioned) brains were mounted together. The following structures were examined: sensorimotor cortex (layers III and IV), caudate nucleus (the motor system), nucleus accumbens (the mesolimbic system), and the CA3 area of the hippocampus (the limbic system). The activities of the following enzymes were measured: glucose-6-phosphate dehydrogenase (G-6-PDH), the key enzyme of the pentosophosphate shunt [13], glutamate dehydrogenase (GDH), enzyme of glutamate metabolism [13], and aminopeptidase hydrolyzing proteins by splitting out N-terminal amino acids [3]. Enzyme activity was assayed photometrically on a LUMAM-IZ microscope (2.5 μ probe diameter) at 589 nm (for G-6-PDH and GDH) and 550 nm (for aminopeptidase) and expressed in arbitrary units. Enzyme activity was measured in 100 neurons in each brain structure.

The data were treated statistically with Student's *t*-test.

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0007-4888/99/0002-0163$22.00

RESULTS

The rat population was divided into 3 groups according to the type of cognitive activity [5]: 1) rats with high cognitive activity (10%) which belong to a subdominant type according to zoopsychological criteria; 2) rats with latent cognitive activity (30%, dominant type); 3) rats with suppressed cognitive activity (60%, subordinate type).

Cytochemical data showed that hippocampal aminopeptidase activity in rats of groups 1 and 2 was higher than in the control (unconditioned) rats by 15% and 9%, respectively. Group 2 rats exhibited increased aminopeptidase activity in the nucleus accumbens (Tables 1-3). In group 3 rats aminopeptidase activity did not differ from the control. The GDH activity was the same in all rats. Irrespective of the learning ability, alcohol-nonpreferring rats displayed significantly (by 27-44%) lower activity of G-6-PDH in the nucleus accumbens, which is considered to be the key structure in functional integration of the limbic and striatal regions and realization of inherent forms of motor activity, such as food-procuring behavior. G-6-PDH activity in their neocortex, caudate nucleus and hippocampus did not differ from the control (Tables 1-3).

Thus, we revealed certain relationships between learning abilities and the intensity of protein metabolism (aminopeptidase activity) in the hippocampus (the limbic system) of Wistar rats. Irrespective of learning abilities, the alcohol-nonpreferring rats showed local changes in G-6-PDH activity, the key enzyme of the pentosophosphate shunt, in the nucleus accumbens (the mesolimbic system).

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TABLE 1. Enzyme Activities in the Brain of Group 1 Rats ($M\pm m$)

Enzyme	G-6-PDH		GDH		Aminopeptidase	
	control	experiment	control	experiment	control	experiment
Layer III	0.404±0.002	0.434±0.004 (107.4)	0.612±0.008	0.613±0.010 (100.2)	0.302±0.002	0.290±0.002 (96)
Layer V	0.573±0.006	0.578±0.008 (100.8)	0.817±0.009	0.788±0.015 (96.5)	0.418±0.004	0.410±0.009 (98.1)
Caudate nucleus	0.373±0.004	0.342±0.005 (91.7)	0.763±0.006	0.781±0.012 (102.4)	0.360±0.003	0.383±0.004 (101.4)
Nucleus accumbens	0.471±0.005	0.344±0.004 (73)*	0.762±0.009	0.805±0.013 (105.6)	0.385±0.003	0.383±0.004 (99.5)
Hippocampus	0.608±0.005	0.624±0.004 (102.6)	0.771±0.008	0.773±0.012 (100.4)	0.424±0.003	0.489±0.004 (115.3)*

Note. Here and in Tables 2 and 3: percent of control is given in parentheses; *$p<0.05$ compared with control.

TABLE 2. Enzyme Activities in the Brain of Group 2 Rats ($M\pm m$)

Enzyme	G-6-PDH		GDH		Aminopeptidase	
	control	experiment	control	experiment	control	experiment
Layer III	0.381±0.003	0.396±0.003 (103.9)	0.738±0.008	0.769±0.005 (104.2)	0.277±0.002	0.274±0.002 (98.9)
Layer V	0.514±0.004	0.479±0.003 (93.2)	0.987±0.007	1.011±0.007 (102.4)	0.389±0.004	0.398±0.003 (102.3)
Caudate nucleus	0.439±0.003	0.437±0.004 (99.5)	0.938±0.007	0.975±0.007 (103.9)	0.370±0.002	0.367±0.003 (99.1)
Nucleus accumbens	0.590±0.005	0.333±0.005 (56.4)*	0.944±0.007	0.992±0.006 (105.1)	0.351±0.002	0.384±0.003 (109.4)*
Hippocampus	0.548±0.003	0.544±0.002 (99.2)	0.923±0.009	0.903±0.006 (97.8)	0.390±0.003	0.427±0.004 (109.5)*

TABLE 3. Enzyme Activities in the Brain of Group 3 Rats ($M\pm m$)

Enzyme	G-6-PDH		GDH		Aminopeptidase	
	control	experiment	control	experiment	control	experiment
Cortical layer III	0.404±0.002	0.426±0.005 (105.4)	0.612±0.008	0.631±0.010 (103.1)	0.302±0.002	0.291±0.002 (96.4)
Cortical layer V	0.573±0.006	0.533±0.005 (93)	0.817±0.009	0.829±0.013 (101.5)	0.418±0.004	0.405±0.009 (96.9)
Caudate nucleus	0.373±0.004	0.344±0.007 (92.2)	0.763±0.006	0.789±0.014 (103)	0.360±0.003	0.366±0.003 (101.7)
Nucleus accumbens	0.471±0.005	0.312±0.005 (66.2)*	0.764±0.009	0.825±0.014 (108.2)	0.385±0.003	0.394±0.004 (102.3)
Hippocampus	0.608±0.005	0.657±0.004 (108.1)	0.771±0.008	0.788±0.014 (102.2)	0.424±0.003	0.422±0.004 (99.5)

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