

EFFECT OF HYDRATION AND DEHYDRATION OF ANIMALS  
ON BIOENERGETICS AND MEDIATOR PROCESSES IN  
NEUROSECRETORY CELLS OF THE ANTERIOR HYPOTHALAMUS

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UDC 611.814.1:612.015.11]  
.12:612.015.31

Histochemical methods were used to study the activity of oxidoreductases and enzymes inactivating mediators in the neurosecretory cells of the anterior hypothalamus during hydration and dehydration in rabbits. Enzymes of the Krebs cycle and of the electron transport system were shown to respond by increased activity to dehydration and by reduced activity to hydration. Activity of  $\alpha$ -glycerophosphate dehydrogenase and glucose-6-phosphate dehydrogenase was increased compared with the control in both cases. Monoamine oxidase activity was reduced during dehydration but increased during hydration; changes in acetylcholinesterase activity were in the opposite direction.

KEY WORDS: hypothalamus; enzymes; hydration; dehydration.

A topical problem in the structural and functional organization of the hypothalamus is the distribution of the activity of enzymes which play an important role in the supply of energy in neurosecretory cells. Meanwhile the functional activity of these cells is regulated by the nervous system through both adrenergic and cholinergic fibers [1, 8]. In the accessible literature only fragmentary and contradictory information could be found on the activity of certain oxidoreductases [3, 4], and enzymes inactivating mediators [12, 14] in the hypothalamic-pituitary neurosecretory system.

The object of the present investigation was accordingly to study the effect of hydration and dehydration on the neurosecretory cells of the supraoptic (SON) and paraventricular (PVN) nuclei and the neurohypophysis (NH) with respect to enzymes of the following systems: glycolysis) lactate dehydrogenase (LD); the Krebs cycle) succinate dehydrogenase (SD) and malate dehydrogenase (MD); the electron transport system) NAD- and NADP-diaphorases, cytochrome oxidase (CO); the pentose phosphate cycle) glucose-6-phosphate dehydrogenase (G6PD); the system oxidizing fatty acids)  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ GPD), and enzymes participating in hydrolysis of mediators) acetylcholinesterase (AChE) and monoamine oxidase (MAO).

#### EXPERIMENTAL METHOD

Experiments were carried out on 18 male chinchilla rabbits with a mean weight of 2 kg. The animals were divided into three groups (six rabbits in each group); the animals of group 1 received water by injection into the gastrointestinal tract in a dose of 5% of body weight (hydration), the animals of group 2 received 5% NaCl solution (dehydration), and the intact rabbits of group 3 served as the control. The animals were decapitated on the 7th day of the experiment. Tissue sections of the hypothalamus and NH were cut in a cryostat at between  $-15$  and  $-18^{\circ}\text{C}$  immediately after sacrifice. The enzymes NAD- and NADP-diaphorases, CO, and  $\alpha$ GPD were determined after Nachlas [7], G6PD, FD, MD, and LD according to the instruction in [6], MAO after Glenner [10], and AChE after Karnovsky [13]. To study AChE a modified Davidenko's method [5] was used on cryostat sections fixed in calcium-formol; in addition, a method on slides also was used in order to preserve topographic relationships. If butyrylthiocholine iodide was used in the reaction medium practically no staining of nervous structures took place, indicating the absence of pseudocholinesterase in the tissue studied. In 24 cells in SON, PVN, and NH (the necessary number of cells was determined by Ashmarin's method [2]), activity of the enzymes was assessed by the method of Astaldi and Verga [11], and the results were subjected to statistical analysis [9].

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Department of Histology, Khar'kov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 86, No. 12, pp. 734-737, December, 1978. Original article submitted April 4, 1978.

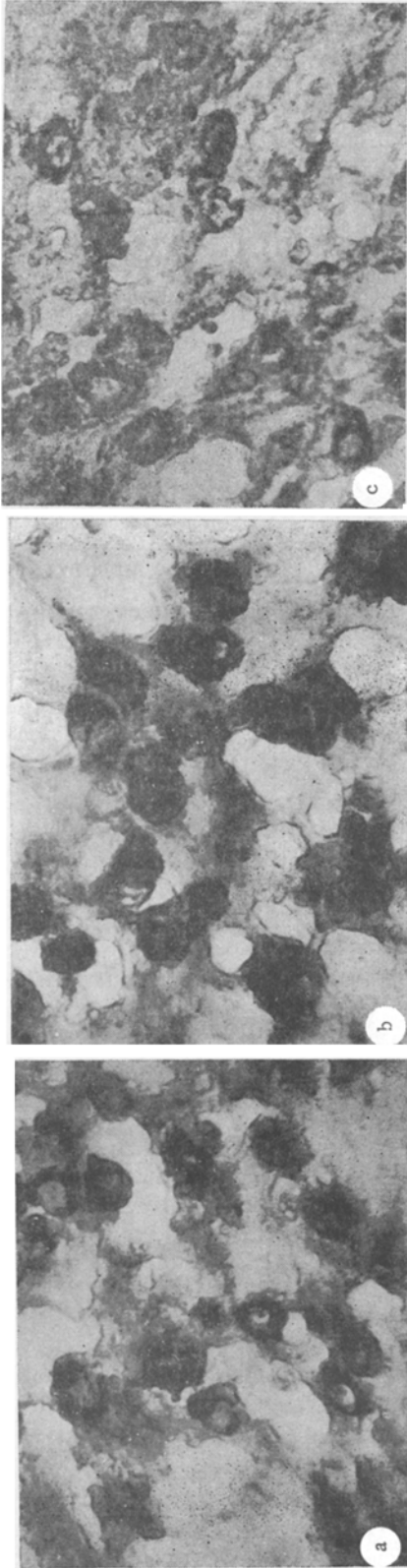


Fig. 1. AChE activity in neurosecretory cells on rabbit supraoptic nucleus: a) intact animals (control); b) during dehydration; c) during hydration. Staining by Karnovsky's method, 400  $\times$ . Extension of bellows 50 cm.

TABLE 1. Distribution of Enzyme Activity in Neurosecretory Cells of Anterior Hypothalamus and Neurohypophysis of Rabbits during Hydration and Dehydration

Enzyme	SON			PVN			NH		
	hydration	control	dehydration	hydration	control	dehydration	hydration	control	dehydration
G6PD	4.3 $\pm$ 0.08*	4.0 $\pm$ 0.07	4.4 $\pm$ 0.06*	4.0 $\pm$ 0.07*	3.7 $\pm$ 0.05	4.2 $\pm$ 0.07*	4.3 $\pm$ 0.07*	3.9 $\pm$ 0.06	4.3 $\pm$ 0.08*
LD	4.6 $\pm$ 0.07†	4.7 $\pm$ 0.06	4.5 $\pm$ 0.05†	4.5 $\pm$ 0.07†	4.4 $\pm$ 0.07	4.3 $\pm$ 0.06†	4.4 $\pm$ 0.07†	4.3 $\pm$ 0.06	4.5 $\pm$ 0.07†
SD	1.5 $\pm$ 0.07*	2.3 $\pm$ 0.07	3.4 $\pm$ 0.07*	1.0 $\pm$ 0.05*	2.4 $\pm$ 0.07	3.2 $\pm$ 0.05*	1.4 $\pm$ 0.07*	2.2 $\pm$ 0.05	3.4 $\pm$ 0.07*
MD	1.1 $\pm$ 0.02*	3.3 $\pm$ 0.06	4.1 $\pm$ 0.04*	1.2 $\pm$ 0.04*	3.2 $\pm$ 0.05	4.2 $\pm$ 0.05*	1.2 $\pm$ 0.05*	3.2 $\pm$ 0.05	4.1 $\pm$ 0.03*
NAD-diaphorase	1.2 $\pm$ 0.02‡	1.4 $\pm$ 0.07	3.1 $\pm$ 0.02*	1.1 $\pm$ 0.03‡	1.3 $\pm$ 0.06	3.2 $\pm$ 0.04*	1.2 $\pm$ 0.05†	1.1 $\pm$ 0.03	3.0 $\pm$ 0.08*
NADP-diaphorase	1.3 $\pm$ 0.06*	1.9 $\pm$ 0.07	3.5 $\pm$ 0.07*	1.4 $\pm$ 0.07*	1.6 $\pm$ 0.06	3.3 $\pm$ 0.06*	1.1 $\pm$ 0.03†	1.0 $\pm$ 0.01	3.4 $\pm$ 0.07*
CO	1.4 $\pm$ 0.07*	3.2 $\pm$ 0.03	4.1 $\pm$ 0.04*	1.3 $\pm$ 0.06*	3.1 $\pm$ 0.03	3.9 $\pm$ 0.03*	1.4 $\pm$ 0.07*	3.1 $\pm$ 0.03	3.8 $\pm$ 0.05*
$\alpha$ GPD	3.6 $\pm$ 0.07*	1.5 $\pm$ 0.08	3.9 $\pm$ 0.07*	3.8 $\pm$ 0.07*	1.6 $\pm$ 0.08	4.1 $\pm$ 0.08*	3.9 $\pm$ 0.08*	1.7 $\pm$ 0.06	4.0 $\pm$ 0.08*
MAO	3.5 $\pm$ 0.06*	2.8 $\pm$ 0.08	2.4 $\pm$ 0.09*	4.6 $\pm$ 0.07*	3.4 $\pm$ 0.1	2.7 $\pm$ 0.07*	4.6 $\pm$ 0.07*	2.5 $\pm$ 0.07	2.3 $\pm$ 0.09‡
AChE	3.8 $\pm$ 0.1‡	4.1 $\pm$ 0.07	4.8 $\pm$ 0.05*	3.1 $\pm$ 0.1*	3.8 $\pm$ 0.09	4.1 $\pm$ 0.09‡	0	0	0

\*P < 0.001.

†P > 0.05.

‡P < 0.05.

## EXPERIMENTAL RESULTS

As Table 1 shows, enzymes of the Krebs cycle (SD, MD), and the electron transport system (NAD- and NADP-diaphorases, CO) in the structures tested underwent changes synchronized with the changes in neurosecretion, i.e., they responded by increased activity to dehydration and reduced activity to hydration. Meanwhile the activity of enzymes linking carbohydrate metabolism with fatty acid metabolism ( $\alpha$ GPD) and the pentose phosphate cycle (G6PD) increased, compared with the control in both cases. The fact that the activity of these enzymes increased at a time when there was a general decrease in activity of the oxidoreductases must be regarded as a compensatory mechanism aimed at restoring plastic processes in the neurosecretory cells of the anterior hypothalamus.

AChE activity was higher in SON and lower in PVN in the control. During dehydration, AChE activity was increased significantly, whereas during hydration it was reduced in both SON (Fig. 1) and PVN, i.e., the changes in its activity corresponded to changes in the neurosecretory process. In NH, AChE activity could not be detected, although during dehydration isolated intensely stained areas were observed.

MAO activity, which was moderate in the intact animals, was intensified when neurosecretion was depressed (hydration) and weakened when it was enhanced (dehydration) in the various structures studied. The mutually opposite changes in MAO and AChE activity correspond to the principle of enzymic opposition of these enzymes [15].

The suggested model of interaction between enzyme systems linking the energy balance with regulation of the neurosecretory cell, on the one hand, and the production of neurosecretory substance, on the other hand, thus give a more complete picture of the functional structure of neurosecretory cells of the anterior hypothalamus.

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