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Dynamics of Changes of AChE Contents in Some Hypothalamic Nuclei after Different Periods of Food Deprivation and Regime

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The contents of acetylcholinesterase were determined by quantitative cytophotometric method in neuron organization of lateral nucleus (LN), ventro-medial nucleus (VMN), paraventricular nucleus and supraoptic nucleus of hypothalamus after different periods of food deprivation and restoration of food regime. The obtained results have shown that cholinergic mediator system of LN is responsible for the perception and switching on the central regulation mechanisms of food motivation. The neurotransmitter system of VMN is necessary for gradual realization of energetic sources of the organism.

Key Words: Food deprivation, Homeostasis, AChE.

INTRODUCTION

Acetylcholinesterase (AChE) is an ellipsoidal molecule and approximately 45 × 60 × 65 Å in dimensions. It consists of a 12 stranded central mixed β-sheet surrounded by 14 α-helices[1]. The biological role of AChE is the termination of impulse transmissions at cholinergic synapses within the nervous system by rapid hydrolysis of the neurotransmitter of acetylcholinesterase[2].

The main focus for the group is acetylcholinesterase (AChE), an enzyme of fundamental interest for its biological activity and its special biophysical properties[3]. It acts very rapidly to stop neurotransmission at cholinergic synapses like those found in the brain and neuromuscular junctions—consistent with the need for fast responses in the neuromuscular system[4]. AChE has practical importance in medicine as a target for drugs for the management of alzheimer and other diseases and in agriculture as a target for pesticides[5].

The problems of realization of the goal-directed reactions of organism are regarded to be the most actual ones in the modern neurophysiology. One of the most important forms of these reactions is food deprivation of animals. It is known that the goal-directed reactions for the food of animals are realized with the participation of a number of cortical and subcortical structures of brain[6, 7]. The central place in these processes belongs to the hypothalamic structure[8]. The intrinsic, mainly hum oral, need is supposed to transform into specific nervous excitation-feeding motivation just here[9, 10]. Activation of these or other CNS structures in unequal levels of motivated excitation is known to be connected with the chemism of different brain

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structures, with the peculiarities of mediation of nervous impulses realized through acetylcholine, noradrenalin and other neurotransmitters or corresponding biological active substances[11]. Acetylcholinesterase is a neurotransmitter of nervous impulses in cholinergic neurons. The specific enzyme, cholinacetyltransferase (CHAT) catalyzes the transfer of acetyl residue from coenzyme A to choline[12]. Inactivation of excreted AChE is realized through acetylcholiesterase (AChE) that has double localization in neurons on the surface of axon membrane and axoplazm[13]. The enzymes are distributed inequally in endoplasmic net, pre- and postsynaptic membranes and Ranvier isthmus[14]. Last and Greenfield[15] demonstrated the distribution of cholinesterase in cholinergic neurons with electron microscope. According to Checler and Vincent[16] there is a high enzymatic activity between axolemma and membranes of glial cells. The pre- and terminal axons varicose have the highest AChE activity. About 50% of total protein is synthesized here[17].

Studying of neurochemical organization of hypothalamic regulation centres of food intake allowed some authors[18, 19] to propose that the mechanism of food behaviour regulation of animals has adrenergic nature. But other authors[20, 21] consider that neurochemical regulation of food behaviour has cholinergic nature. It is also assumed that in the regulation of food behaviour both choline and adrenoreactive brain structures take part[22]. However, these views are not confirmed by corresponding morphoneurochemical studies.

A number of advantages of histochemical methods give an opportunity to study the morphochemical reactions of separate hypothalamic nuclei in food behaviour formation that, perhaps, will allow elucidating the central mechanisms of the regulation of food-directed reactions.

The purpose of the present study is to reveal the brain enzyme system AChE in dynamics of sequential changes of an organism's homeostasis as well as the revealing of neurochemical status of energy turnover in different brain structures under different levels of food motivation.

EXPERIMENTAL

The research was conducted on male albino Wistar rats in the same age and 170–200 g in weight. The experimental animals were not given food during 1, 3 and 5 days accordingly, but they had a free water access. The animals were culled into 2 subgroups. The experimental animals were decapitated by hilotine knife after different periods of food deprivation (FD). The first subgroup of animals were sacrificed after pointed periods of food deprivation, while in the second subgroup of animals the food regime was restored (FR) and they were sacrificed after different periods of restoration, after 2, 6, 24 h, 3 and 5 days. During these periods the experimental animals received food *ad libidum*. The controls were kept on the usual food regime. For each variant of experiments five albinos of total 70 rats have been used. The lateral nucleus (LN), ventro-medial nucleus (VMM), paraventricular nucleus (PVN) and supraoptical nucleus (SON) of the hypothalamus were studied.

The brains of experimental animals were extracted and the hypothalamus with adjacent areas was placed on microtome table. On the cryostat MK-25 fresh frozen sections in 18-um thicknesses from hypothalamic nuclei were obtained. The Karnovsky-Rutch ferrocyanide method modified for nervous tissues was used to reveal histochemical activity of the AChE[23].

This method is based on recovering ferrocyanide potassium which is realized as a result of hydrolysis of acetylcholine. Ferrocyanide with Cu^{2+} ions gives yellowish-brownwish precipitate of Cu^{2+}, whose intensity of colouring gives ground to evaluate the activity of the enzyme precipitate.

The cytospectrophotometry of preparations was carried out on the photometer on 486 nm wavelength for AChE. The diameter of the sonde[19, 23] on the surface of preparation was 8 μm. There were 120–150 indicators of photometry in neurons in each experiment.

RESULTS AND DISCUSSION

The photometric data have shown that AChE contents in separate hypothalamic nuclei of control animals were not equal. The results of photometric evaluation of cholinergic neurons indicate the high AChE level in SON and PVN. The results of photometric studies of separate hypothalamic nuclei in standard and in different periods of feeding behaviours are presented in Tables 1–3 and Figs. 1–3.

The increase of the reaction to AChE activity in the cytoplasm of LN cholinergic neurons in the first days of food deprivation was observed (161% as compared to control). The marked increase of AChE activity in LN neurons in the early periods of FD indicates that the cholinergic system of this structure is more reactive to the perception of initial food deficiency. In favour of this assumption, the data of predominance of sensitivity of cholinergic structures in LN has been shown. The dominant role in the perception of initial food deficiency belongs to the cholinergic systems which are believed to be more informative than monoaminergic ones, so far as the polysinapticity of its switching in the rising system has been established while the monoaminergic paths are monosynaptic[19, 24].

The correctness of such assumption is also supported by the observation in saturated animals at the background of administration of adrenergic substances in VMN in which the increase of food uptake has been observed[25, 26].

The increase of food deprivation duration for 3 days is characterized by prominent motivational behaviour of animals (increased motor exploratory activity) and further increase of reactions to AChE in lateral nucleus (201% in comparison to controls). It should be noticed that along with the behavioural activity, the simultaneous reactivity of the organism's homeostasis which is regulated by the vagal and hypothalamic-hypophysal system is also necessary[27, 28]. It follows that the increased enzymatic activity of LN observed during the expressed and motivated behaviours of animals reflects the involvement of cholinergic afferent neurons of the studied brain structures, which provide rapid rearrangement in the homeostasis of organism.

Strong reduction of AChE activity in almost all studied hypothalamic nuclei on the fifth day of food deprivation appears to be related to the weakening of the regulator system of brain cholinergic system in this period. A strong reduction of enzymatic activity of nervous cells is related to their increased functional activity[29].

TABLE-1

ACHE ACTIVITY IN CYTOPLASM OF NEURONS IN SOME HYPOTHALAMIC NUCLEI AFTER DIFFERENT PERIODS OF FOOD DEPRIVATION (FD)

Hypothalamic nuceli	Control (M ± m)	Duration of FD								
		1 day			3 day			5 day		
		M ± m	p	%	M ± m	p	%	M ± m	p	%
LN AChE	17.8 ± 0.3	28.7 ± 0.3	< 001	16.1	35.7 ± 0.5	< 0.001	20.1	18.9 ± 0.4	> 0.050	10.6
VMN AChE	14.8 ± 0.4	16.1 ± 0.3	> 0.05	10.9	15.5 ± 0.4	> 0.050	10.5	13.3 ± 0.3	< 0.001	8.3
PVN AChE	21.3 ± 0.2	24.1 ± 0.1	> 0.05	11.3	28.4 ± 0.2	< 0.010	13.3	20.5 ± 0.4	< 0.010	9.6
SON AChE	22.5 ± 0.4	22.1 ± 0.4	< 0.01	9.8	24.2 ± 0.1	> 0.050	10.8	21.7 ± 0.3	< 0.100	9.6

TABLE-2

ACHE ACTIVITY IN THE CYTOPLASM OF NEURONS IN SOME HYPOTHALAMIC NUCLEI DURING RESTORATION OF FD AFTER 3 DAYS OF FD

Hypothalamic nuceli	Control (M ± m)	3 days (M ± m)	The periods of restoration of FR after 3 days of FD								
			2 h			6 h			24 h		
			M ± m	p	%	M ± m	p	%	M ± m	p	%
LN AChE	17.8 ± 0.3	35.7 ± 0.5	39.4 ± 0.2	< 0.01	19.6	33.6 ± 0.1	> 0.050	18.8	32.5 ± 0.3	> 0.05	18.3
pVN AChE	21.3 ± 0.2	28.4 ± 0.2	27.9 ± 0.2	< 0.01	13.1	26.7 ± 0.1	< 0.001	12.5	25.8 ± 0.2	> 0.05	12.1
SON AChE	22.5 ± 0.4	24.2 ± 0.1	24.4 ± 0.6	> 0.50	11.4	23.8 ± 0.1	< 0.020	10.6	22.9 ± 0.4	< 0.05	10.1

TABLE-3

ACHE ACTIVITY IN THE CYTOPLASM OF NEURONS OF SOME HYPOTHALAMIC NUCLEI DURING RESTORATION OF FR AFTER 3 DAYS OF FD

Hypothalamic nuclei	Control (M ± m)	3 days of FD			The periods of restoration					
					3 days			5 days		
		M ± m	p	%	M ± m	p	%	M ± m	p	%
LN AChE	17.8 ± 0.3	30.2 ± 0.8	< 0.001	169	24.2 ± 0.41	> 0.001	135	18.8 ± 0.9	< 0.001	10.6
VMN AChE	14.8 ± 0.4	16.7 ± 0.3	< 0.001	119	18.1 ± 0.4	> 0.050	122	16.3 ± 0.3	> 0.050	11.0
PVN AChE	21.3 ± 0.2	19.2 ± 0.6	< 0.001	90	20.7 ± 0.4	> 0.050	90	17.3 ± 0.6	< 0.010	8.1
SON AChE	22.5 ± 0.4	22.2 ± 0.7	< 0.001	99	25.8 ± 0.7	> 0.001	115	22.6 ± 0.6	< 0.001	10.0

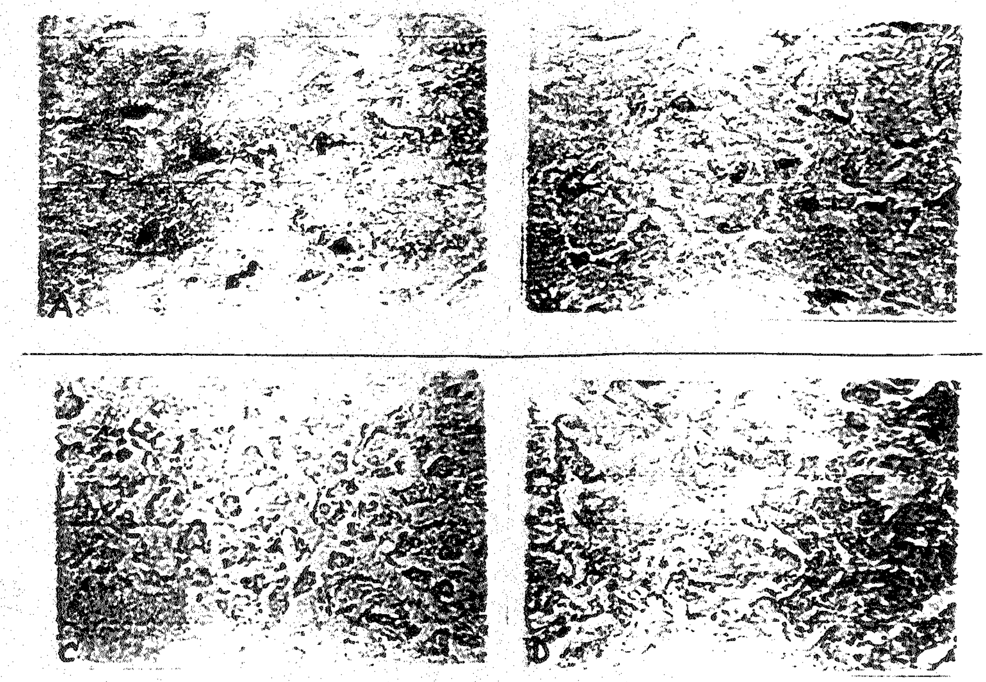

Fig. 1. Activity of acetylcholinesterase in front (A) and medial (B) parts of LN, SON (C), PVN (D) of the hypothalamus of control animals x400

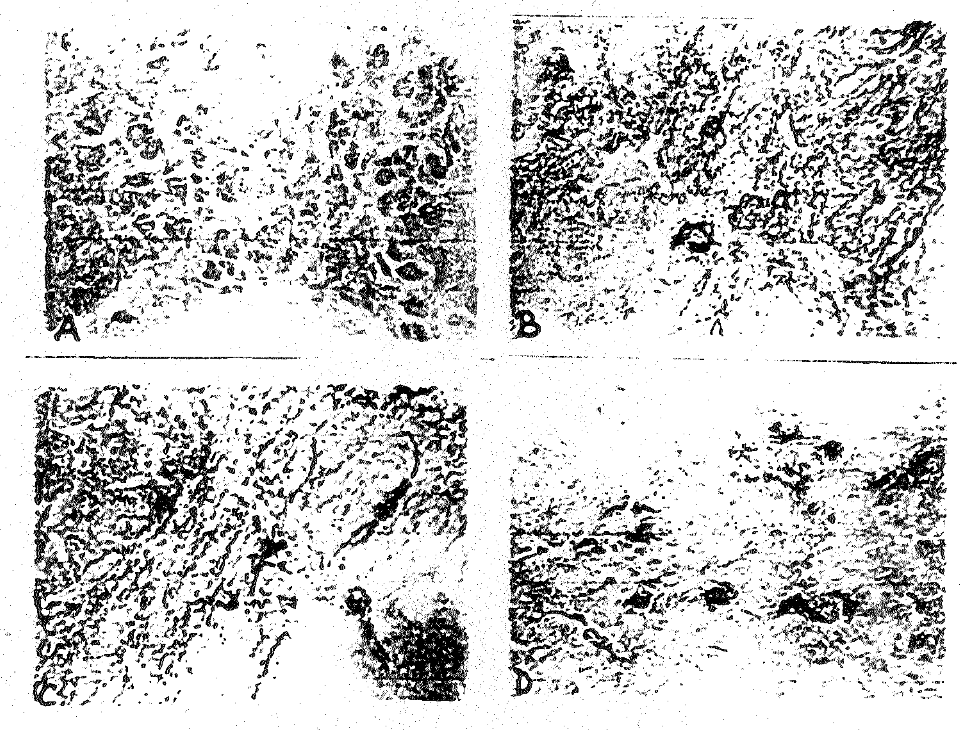

Fig. 2. Activity of acetylcholinesterase in SON (A), exterior-lateral zone (B), perifornical region (C), LN (D) of the hypothalamus after one day food deprivation x400

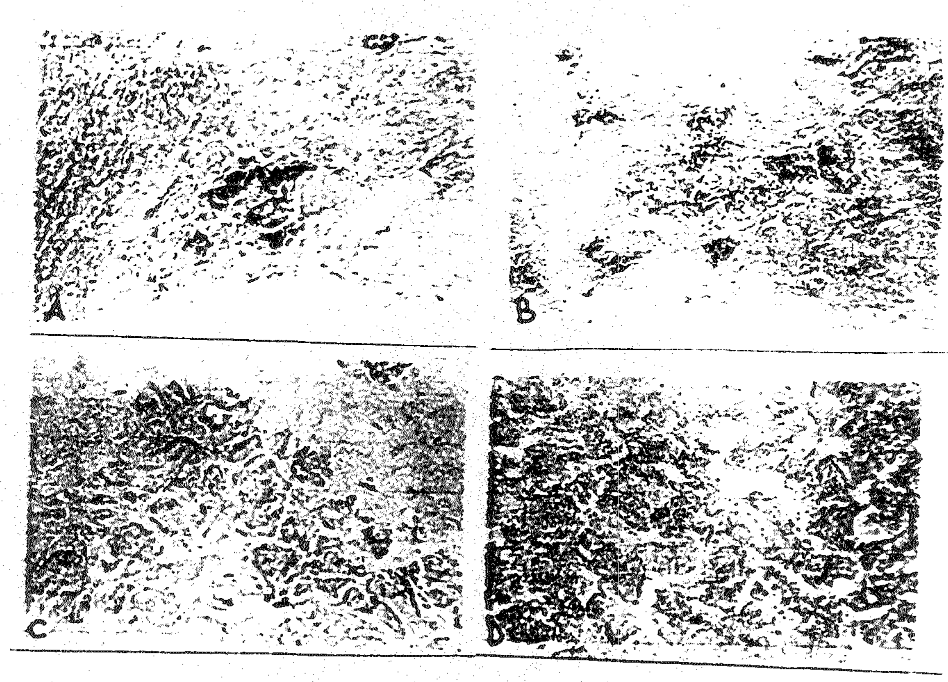

Fig. 3. Activity of acetylcholinesterase in exterior-lateral zone (A), periofonical region (B), SON (C) and PVN (D) of the hypothalamus after three day food deprivation x400

The purpose of the second part of this work was to study the interrelations between these neurotransmitter systems at the background of restoration of food regime after 3 days of food deprivation.

The results of these studies are presented in Tables 2 and 3. The restoration of FD during 2, 6, 24 h, 3 and 5 days was characterized by gradual decrease of the AChE activity in LN neurons and also in other hypothalamic nuclei having increased the AChE activity during starvation.

Thus, the obtained results of the histochemical researches of AChE activity in separate hypothalamic structures on different periods of food deprivation showed that cholinergic system of LN is responsible for perception and switching on the central regulation mechanism of food motivation. Our concept coincides finely with the opinion of Nicolaids[30] who considers that the condition of hunger and satiety is regulated by the rates of intracellular energic production, which depends on the accessibility of food substrates and character of hormonal secretion. The preservation on the high level of neuronal organization of the VMN is necessary for gradual realization of energetic sources of the organism[27].

All these allow us to conclude that the function of LN is closely related to organization of the neuropsychological mechanisms of regulation of the afferent impulses of the organism after various periods of food motivation and after restoration of food regime.

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