ACETYLCHOLINESTERASE ACTIVITY IN SOME HYPOTHALAMIC NUCLEI AND THE CEREBRAL CORTEX DURING CHANGES OF FEEDING ROUTINE

G. G. Gasanov, É. R. Sadykhova, and V. N. Anders

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KEY WORDS: brain; acetylcholinesterase; feeding routine

Data in the literature on the neurochemical mechanism of the feeding behavior of animals are very contradictory [3, 4, 7, 8]. The view is generally accepted that activation of various structures of the CNS in animals at different levels of motivation excitation is connected with the special character of mediation of nervous impulses in the corresponding brain formations. Structural-chemical interaction in such cases has received little study, for until now mainly electrophysiological and biochemical methods of investigation have been used, and these give only integrative results for relatively large (compared with micro- and ultrastructures) regions of brain tissue.

In the investigation described below changes in acetylcholinesterase (AChE) activity in the hypothalamic nuclei and cerebral cortex were studied by methods of quantitative light-optical histochemistry and electron cytochemistry.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar albino rats weighing 150-180 g. The experimental animals were deprived of food for 1, 3, and 5 days. They were then divided into two groups: the animals of group 1 were killed after starvation for the above periods, whereas feeding of the animals of group 2 was resumed, given food according to their needs, then killed at various times (2 and 24 h). The control animals remained on the ordinary animal house diet. The lateral (HL) and ventromedial (VM) hypothalamic nuclei and the sensomotor cortex were investigated. To assess the neurochemical specificity of these brain structures at the cellular and subcellular levels, the method of histochemical and electron-cytochemical detection of AChE activity of Karnovsky and Roots (1964) was used in the modification of Anders and Sal'nikov [1] for nerve tissue. Cytospectrophotometry of the optical histochemical preparations was carried out on an MPM-0.5 photometer (from Opton, West Germany) at a wavelength of 486 nm, with a 40 objective, and with probes 8 and 80 μ in diameter in the plane of the preparation. The parameters of measurement used were selected on the basis of data in the literature [1, 2, 5]. In each version of the experiments 120-150 photometric measurements were obtained in the cytoplasm of the neurons and the same number in the neuropil. Ultrathin sections for electron microscopy were cut on a Reichardt (Austria) ultratome and examined in the EM-210 electron microscope (from Philips, The Netherlands).

EXPERIMENTAL RESULTS

The results of cytospectrophotometry of the optical histochemical preparations in the control and experimental groups of animals are given in Table 1 and Fig. 1.

On the first day of starvation of the animals an increase in the intensity of the reaction for AChE was clearly observed in the cytoplasm of cholinergic neurons of hypothalamic nucleus HL and in their surrounding neuropil. A very small increase in enzyme activity also was observed in the neuropil of neurons of hypothalamic nucleus VM and in the upper layers

Laboratory of Neurohistology and Histochemistry, Institute of Physiology, Academy of Sciences of the Azerbaijan SSR, Baku. Laboratory of Experimental Pathology and Pathomorphology of the Brain, Institute of Psychiatry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 2, pp. 61-63, February, 1982. Original article submitted March 7, 1981.

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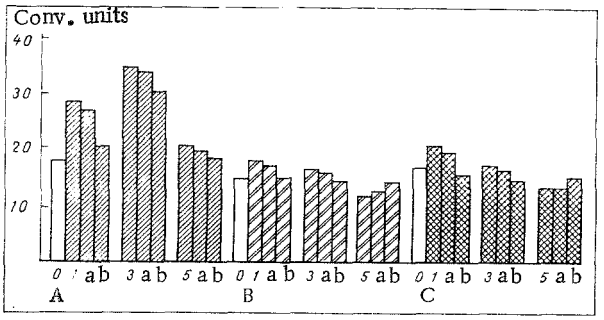

Fig. 1. Dynamics of AChE activity in cytoplasm of neurons of hypothalamic nuclei HL (A) and VM (B) and in sensomotor cortex (C) following changes of diet. 0) Normal AChE activity, 1) deprivation for 1 day, 3) for 3 days, 5) for 5 days. a) 2 h, b) 24 h after resumption of feeding.

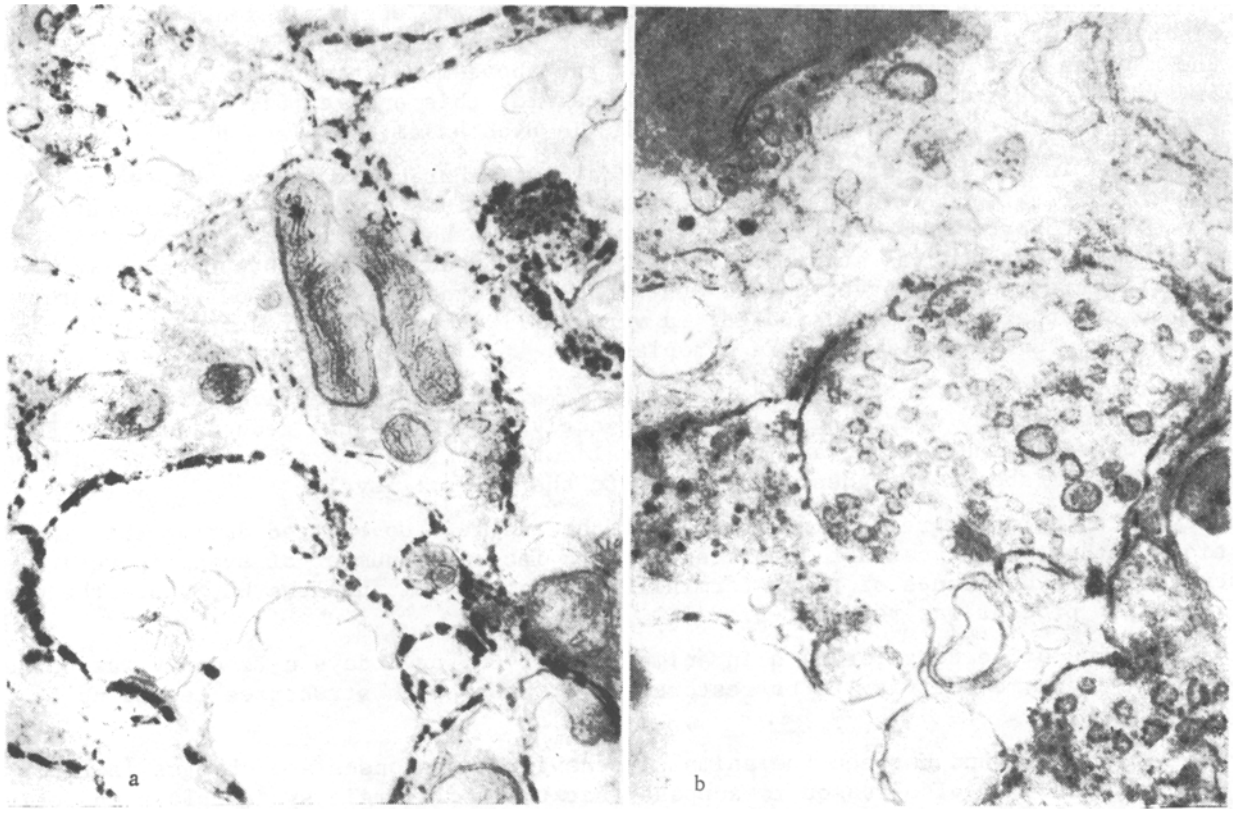

Fig. 2. Distribution of reaction product for AChE in nerve tissue of hypothalamic nucleus HL during changes in diet. a) Increase in deposition of reaction product in membranes of nerve terminals afer food deprivation for 3 days, 20,000 ×; b) decrease in deposition of AChE reaction product in synaptic membranes and shortening of active zone of contact in places with an increased number of synaptic vesicles after resumption of feeding in animals deprived for 3 days, 30,000 ×.

(II, III) of the cortex. An increase in the period of starvation to 3 days, when the animals' motivational behavior was expressed most clearly, with searching movements, was accompanied by a further increase in the intensity of the reaction for the enzyme in hypothalamic nucleus HL, where an increase in the number of AChE-positive neurons was found together with an increase in AChE activity in the cytoplasm of the neurons and in the neuropil. An increase in the intensity of the reaction for AChE also was observed in the neuropil of the lower layers (V, VI) of the cortex. In this period a tendency for AChE activity to fall was observed in the cytoplasm of neurons of hypothalamic nucleus VM.

TABLE 1. AChE Activity in Hypothalamic Nuclei (HL, VM) and Sensomotor Cortex after Various Periods of Food Deprivation (data of cytospectrophotometry, in conventional units)

Brain structure	Control	Duration of starvation, days		
		1	3	5
HL:				
neurons	17,8±0,3	28,7±0,3*	35,7±0,5*	18,9±0,4
neuropil	7,5±0,2	12,3±0,2*	15,9±0,4	10,2±0,4*
VM:				
neurons	14,8±0,4	16,1±0,3*	15,5±0,4	12,3±0,3*
neuropil	6,1±0,1	7,4±0,3	7,9±0,6*	6,9±0,5
Cortex:				
II, III layers	16,4±0,2	18,7±0,2	17,5±0,7*	15,1±0,4*
V, VI layers	12,2±0,1	12,4±0,3*	14,8±0,4*	13,5±0,5

*P < 0.001.

Electron-cytochemical studies of the lateral hypothalamic region and sensomotor cortex showed the presence of large quantities of precipitate of the enzymatic reaction along the course of the intracellular membranes, in the region of the pre- and postsynaptic membranes, and in the membranes of the synaptic vesicles at the above-mentioned times of food deprivation (Fig. 2). The distribution of AChE was typical for this enzyme [6], but the reaction itself was much stronger than normal, especially in hypothalamic nucleus HL.

An increase in the time of starvation to 5 days was characterized by a decrease in the animals' motor activity, in contrast with the previous periods of deprivation, amounting in some of them to stupor, but by increased aggressivenes in others. A decrease in AChE activity in all brain structures studied (Table 1) corresponded to the above changes in behavior. Differences were observed in the distribution of the reaction product in neuron bodies in the hypothalamic nuclei studied: a very small increase in its concentration in the neuropil and a low concentration in the cytoplasm.

Resumption of feeding for 2 h, and later for 24 h, in animals deprived of food for 3 days was characterized by weakening of searching activity and gradual reduction in AChE activity in hypothalamic nucleus HL and the cerebral cortex (Fig. 1). AChE activity in hypothalamic nucleus VM showed a tendency to return to the control level.

The cytochemical picture of distribution of the enzyme also changed during this period. Contraction of the zone of contact in places with an increased number of synaptic vesicles was observed in the membranes of nerve terminals in hypothalamic nucleus HL, where the quantity of reaction product was reduced (Fig. 2).

On resumption of feeding for 24 h in animals deprived for 5 days a tendency was noted for the level of enzyme activity to be restored in all the brain structures tested up to the control values.

Correlation was found between the animals' behavioral responses and changes in their AChE activity. There is also reason to suppose that the cholinergic system plays an active role in the response to changes of diet. Of all the brain structures studied, the most marked changes under these circumstances took place in hypothalamic nucleus HL.

It can be concluded that the functional role of the cholinergic system of the brain during periods of food deprivation is to provide for motor responses of the animal aimed at maintaining its homeostasis.

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EFFECT OF BUTYROXAN ON ULTRASTRUCTURAL CHANGES IN THE PITUITARY PRODUCED BY TETANUS TOXIN

L. M. Rumbesht, É. A. Bardakhch'yan, and A. I. Polyak

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A previous investigation [3] showed that a unique response of the hypothalamo-hypophyseal neurosecretory system (HHNS) arises to injection of tetanus toxin (TT). During the first 5 h the output of neurosecretion was greatly activated and increased, and after 3 days the accumulation of neurosecretory material was associated with degenerative changes in the pituicytes and structural changes in the endothelium.

In the present investigation ultrastructural changes were studied in the caudal section of the HHNS at different times after injection of TT and administration of pharmacologic agents with predominantly central action. The α-adrenoblocker butyroxan used for this purpose, a Soviet drug which blocks mainly central adrenergic structures [1], statistically significantly increased the length of survival of experimental rats by almost 2 days after injection of 0.67 or 1.0 lethal dose of TT [2]. It can be tentatively suggested that the HHNS plays an important role in the mechanism of this effect.

EXPERIMENTAL METHOD

Experiments were carried out on 17 male albino rats weighing 260-280 g. A lethal dose of TT in 0.25 ml of 0.85% NaCl solution was injected intramuscularly into the left leg. An aqueous solution of butyroxan was injected intramuscularly into the right leg in a dose of 10 mg/kg body weight immediately after the injection of TT, and this was repeated daily until the animal died. Signs of local tetanus appeared in the rats after 36 h, followed by generalized ascending tetanus with spontaneous convulsions after 96-108 h and death of the animals on the 5th-6th day. Control animals received injections of butyroxan only, in the same dose.

Material for electron microscopy was taken 5 h and 3 days after injection of the toxin and butyroxan. Pieces of the posterior lobe of the pituitary were fixed in glutaraldehyde in phosphate buffer, postfixed in osmium tetroxide solution, dehydrated in acetone, and embedded in a mixture of Epon and Araldite and in Epon-812. Ultrathin sections cut on the LKB-8800 ultramicrotome were stained with lead citrate and uranyl acetate and studied in the JEM-100 electron microscope.

EXPERIMENTAL RESULTS

The ultrastructure of the posterior lobe of the pituitary in rats receiving physiological saline did not differ from that of the corresponding region of the brain in intact animals [3].

Only slight activation in the caudal section of HHNS was observed 5 h after injection of butyroxan. The number of fibers filled with elementary granules and empty vesicles, i.e.,

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