CHANGES IN ACETYLCHOLINESTERASE ACTIVITY IN THE PARVOCELLULARNUCLEI OF THE RAT HYPOTHALAMUS DUE TO SUPPRESSION OF PITUITARY ADRENOCORTICOTROPIC FUNCTION

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Acetylcholinesterase activity in the region of the ventromedial and arcuate nuclei was reduced more in the hypothalamus of animals which had received hydrocortisone solution daily for 10 days in order to suppress ACTH secretion than in control animals. The level of enzyme activity was reduced in the neurons of the ventromedial nucleus; most neurons of the arcuate nucleus had lost the whole of their acetylcholinesterase activity.

The important role of the parvocellular nuclei of the hypothalamus in regulating the tropic functions of the adenohypophysis has recently become increasingly evident. The parvocellular nuclei are the place of liberation of the releasing factors (or releasing hormones) which control the synthesis of the corresponding tropic hormones of the pituitary and their liberation into the blood stream [1, 10, 11]. Synthesis of corticotropic releasing factor takes place in the corresponding "tropic" region of the hypothalamus, which includes the ventromedial and arcuate nuclei [9, 12], whose neurons possess anticholinesterase activity [6]. Since the cholinergic mechanism plays an important role in the transmission of nervous impulses, and also in neurosecretion [4], it would be interesting to study the changes in acetylcholinesterase (ACE) activity in the neurons of these nuclei during changes in their functional activity. Insufficient attention has been paid to this problem in the literature. The level of production of ACTH in the pituitary is known to influence synthesis of hypothalamic corticotropic releasing factor by the "short feedback" principle [8], and under these circumstances the functional activity of neurons of the ventromedial and arcuate nuclei is modified.

The object of the present investigation was to study the character of the distribution and activity of ACE in the neurons of the arcuate and ventromedial nuclei of the hypothalamus when ACTH production by the pituitary was suppressed by repeated injections of hydrocortisone.

EXPERIMENTAL METHOD

Experiments were carried out on 24 male albino rats weighing 120-140 g, divided into two groups: experimental and control, with 12 animals in each group. The rats of the experimental group received a subcutaneous injection of 0.6 ml hydrocortisone solution (10 mg hydrocortisone /100 g body weight) daily for 10 days [3, 5]. The control rats received the same volume of physiological saline. Ten days after the beginning of the experiment the animals of both groups were decapitated. The adrenals were removed and weighed on torsion scales. ACE was detected histochemically in the hypothalamic nuclei by the method developed by the writer previously [2]. The hypothalamus was removed and fixed in 12% formol-calcium solution for 6 days at 4°C. Pairs of serial free-floating sections, 20 μ in thickness, were cut on a freezing microtome, with a deep-freeze knife, simultaneously from two pieces of brain tissue from the experimental and control animals, respectively. To detect ACE in the hypothalamus, sections were kept for 1.5 h in

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TABLE 1. Changes in Weight of Adrenals of Rats after Administration of Hydrocortisone Solution for 10 Days

Group of animals	No. of animals	Body weight (in g)		Weight of adrenals (in mg)	
		initial	at sacrifice	absolute	relative (per 100 g body wt.)
Control (0.6 ml physiological saline daily)	12	133	175	37,7±2,3	22,1±2,0
Experimental (10 mg hydrocortisone/100 g body wt.daily)	12	134	113	14,2±0,7	12,5±0,7

Karnovsky's incubation medium [7]. The thiocholine esters acetylthiocholine and butyrylthiocholine were used as substrate. The sections were left for 1-2 days in 3% neutral formalin and mounted in glycerol-gelatin. Differentiation between specific and nonspecific cholinesterases was carried out by comparing two neighboring sections after incubation of one of them in medium with butyrylthiocholine, and the other in medium with acetylthiocholine. A 0.1% solution of neostigmine was used as an inhibitor of total cholinesterase activity. The degree of ACE activity in the nerve structures was judged from the intensity of specific precipitation in areas of enzymic activity.

EXPERIMENTAL RESULTS

The results of these investigations showed that most neurons of the ventromedial and arcuate nuclei in the animals of the control group possess acetylcholinesterase activity: the enzyme was found in the cytoplasm of the neuron body, while the nuclei of the neurons and their processes contained no enzyme, and outside the boundaries of the bodies of the nerve cells the brain substance was feebly stained. Depending on the intensity of staining of the cytoplasm, the neurons of the ventromedial and arcuate nuclei could be divided conventionally into three groups: 1) reacting strongly for ACE, with darkly stained cytoplasm; 2) with a less strong reaction for ACE – moderately stained cytoplasm; 3) neurons staining weakly, whose cytoplasm was indistinguishable in color from the surrounding brain tissue.

In the ventromedial nucleus, the "boundary" neurons of the medial part of the nucleus had highest activity, most cells in the central part of the nucleus were weakly stained, and in the lateral part neurons with varied acetylcholinesterase activity were found. In the arcuate nucleus most small neurons had intensively or moderately stained cytoplasm, while individual large neurons possessed high ACE activity. In the region of the median eminence, ACE activity was weak.

When ACTH synthesis was blocked by hydrocortisone, as shown by the significant decrease in the absolute and relative weights of the adrenals ($P < 0.001$) (Table 1), a marked decrease in ACE activity was observed in the region of the ventromedial and arcuate nuclei of the hypothalamus. In sections through the brain the basal region of the hypothalamus appeared much lighter than in the control animals. In most neurons of the ventromedial nucleus, the loss of enzyme activity was considerable: the cells were only moderately stained, including the "boundary" neurons which, under normal conditions, showed the highest ACE activity (Fig. 1a, b). In the arcuate nucleus most of the large neurons, normally strongly or moderately stained, had lost all their activity, while in some of the large neurons of the arcuate nucleus activity still remained high (Fig. 1c, d). In the region of the median eminence no changes were observed.

The results of these experiments thus show conclusively that under normal conditions the distribution of ACE activity in the neurons of the arcuate and ventromedial nuclei of rats is constant in character, and that different cell groups of the same nucleus possess unequal ACE activity. After administration of hydrocortisone for 10 days to the animals a marked decrease in ACE activity is observed in the region of the arcuate and ventromedial nuclei. The decrease in ACE activity during suppression of ACTH production occurs selectively in the system of the parvocellular hypothalamic nuclei composing the hypophysiotropic region, and this evidently reflects changes in the intensity of formation of corticotropic releasing hormone in these nuclei.

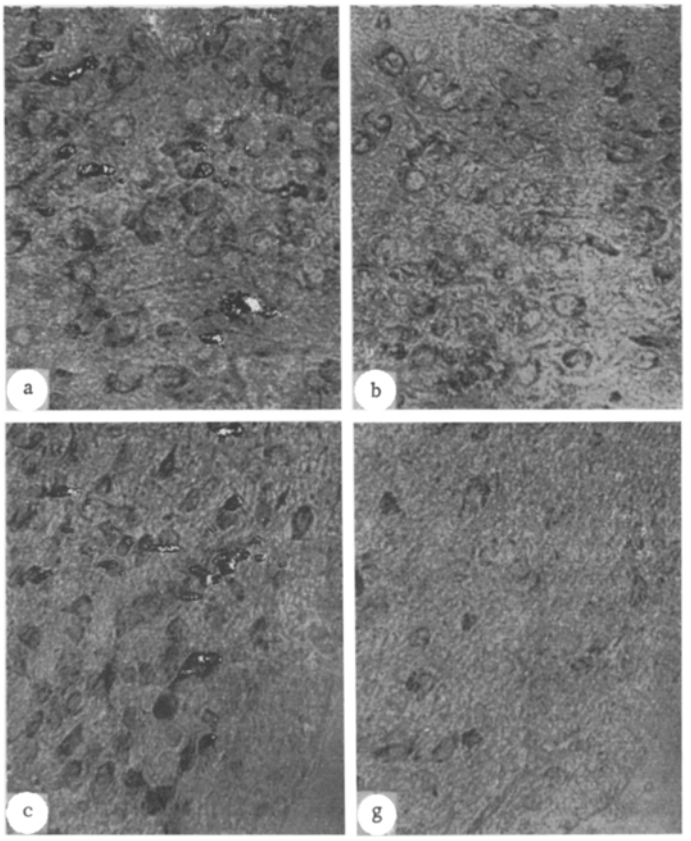

Fig. 1. ACE activity in neurons of parvocellular hypothalamic nuclei under normal conditions (a, c) and during blocking of endogenous ACTH by hydrocortisone (b, d): a, b) ventromedial nucleus; c, d) arcuate nucleus. 280 ×.

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LITERATURE CITED

1. I. G. Akmaev, Arkh. Anat., No. 8, 144 (1968).
2. A. P. Vasil'eva, in: Modern Methods of Morphological Brain Research [in Russian], Moscow (1969), p. 51.
3. M. N. Surina, Probl. Endokrinol., No. 6, 91 (1969).
4. V. C. Abrahams and M. Pickford, J. Physiol. (London), 126, 329 (1954).
5. A. Ashford and J. Jones, Brit. J. Pharmacol., 20, 95 (1963).
6. K. Iijima, T. R. Shantha, and G. H. Bourne, Z. Zellforsch., 79, 76 (1967).
7. M. Karnovsky, J. Biophys. Biochem. Cytol., 23, 217 (1964).
8. M. Motta, N. Sterescu, F. Piva, et al., Acta Neurol. Belg., 69, 501 (1969).
9. J. Szentagothai et al., Hypothalamic Control of the Anterior Pituitary, Budapest (1962).
10. J. Szentagothai et al., Hypothalamic Control of the Anterior Pituitary [Russian translation], Moscow (1965).
11. S. Watanabe and S. M. McCann, Endocrinology, 82, 664 (1968).
12. D. de Wied, P. G. Smelik, et al., in: Major Problems in Neuroendocrinology, Basel (1964), p. 156.