5. H. Cantor and J. Weissman, Prog. Allergy, 20, 1 (1976).
6. W. L. Ford, Prog. Allergy, 19, 1 (1975).
7. J. B. Hay, P. J. Lachman, and Z. Trnka, Eur. J. Immunol., 3, 127 (1973).
8. R. H. Kelley and R. A. Wolstencroft, Clin. Exp. Immunol., 18, 321 (1974).
9. H. Sonozaki and S. Cohen, J. Immunol., 112, 1540 (1974).*
10. T. Yoshida and S. Cohen, J. Immunol., 112, 1540 (1974).

*As in Russian original — Publisher.

REACTION OF SOME HYPOTHALAMIC CENTERS IN RATS TO A SINGLE INJECTION OF THYROTROPIN-RELEASING HORMONE

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UDC 612.826.4.014.46:615.357.441

KEY WORDS: hypothalamus; neurosecretion; thyrotropin-releasing hormone.

The effect of exogenous thyrotropin-releasing hormone (TRH) on the level of thyroid stimulating hormone (TSH), prolactin, and thyroid gland hormones in the blood of animals and man has been investigated frequently [13, 14]. Information has been obtained on the effect of iontophoretic applications of TRH directly to neurons of various parts of the brain, including hypothalamic neurons [11, 13, 14].

It has not proved possible to show by these investigations which process in the neurosecretory cell was affected by administration of TRH: whether synthesis of the hormone, its transport along the fibers, or its liberation into the blood stream. The localization of the hypothalamic center or centers regulating thyroid gland function likewise has not yet been established. The investigation described below was devoted to the study of these problems.

EXPERIMENTAL METHOD

Male Wistar rats weighing 220-230 g were given an intramuscular injection of 8 μg synthetic TRH, dissolved in 2 ml physiological saline, 30 min before decapitation. Considering the high speed of TRH inactivation in the blood [12], its dose was chosen so that the TRH level remained high throughout the experiment, so that morphological and functional changes in the cells could be manifested. Control animals received an injection of 2 ml physiological saline.

The animals' brain was fixed in Bouin's fluid and sections 6 μ thick were stained with paraldehyde-fuchsin by the Gomori—Gabe method and counterstained with Heidenhain's azan.

The area of cross section of the nuclei and nucleoli in cells of the supraoptic, paraventricular, suprachiasmatic, ventromedial, and arcuate nuclei (SON, PVN, SCN, VMN, and AN respectively) of the hypothalamus was measured by a photographic method. Microfilming was carried out under a 60 × immersion objective, or 90 × for SCN. In each nucleus at least 35 cells were measured. The relative percentage of cells with no nucleolus or with one nucleolus and of the so-called polynucleolar cells, i.e., those containing nucleolus-like bodies, and fragments of cell nuclei also were counted in the sections among 400 cells in each animal. All measurements were made within the regions of the hypothalamic centers indicated in Fig. 1.

The significance of differences between control and experimental values was assessed by Student's t-test and the Mann—Whitney U test.

Laboratory of Neuroendocrinology, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 88-89, July, 1981. Original article submitted November 11, 1980.

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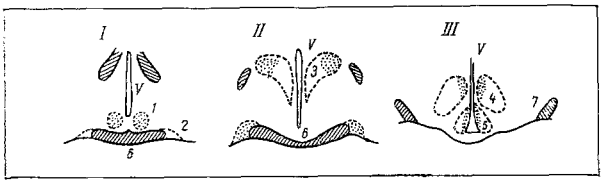

Fig. 1. Frontal section through hypothalamus in rostrocaudal direction (I, II, and III respectively). 1) SCN, 2) SON, 3) PVN, 4) VMN, 5) AN, 6) optic chiasma, 7) optic tract, V) 3rd ventricle. Dots indicate regions of hypothalamic centers in which cells were measured.

TABLE 1. Area of Cross Section (in μ^2) of Nucleolus of Cells of Some Hypothalamic Centers Following a Single Injection of TRH (M ± m)

Hypothalamic center	Group of animals	Nucleus	Nucleolus	% of polynucleolar cells
SON	Control	46,5±0,5	2,28±0,03	5,5
	Experiment	44,4±0,6	1,9±0,04*	8,0
PVN	Control	41,5±0,4	2,06±0,04	15
	Experiment	44,7±0,4*	1,72±0,04*	25*
VMN	Control	39,3±0,5	1,75±0,02	3
	Experiment	45,0±0,6*	1,78±0,03	12*
SCN	Control	23,3±0,3	0,75±0,01	6,5
	Experiment	23,2±0,3	0,67±0,01*	8
AN	Control	35,3±0,8	1,30±0,03	11,7
	Experiment	34,7±0,4	1,36±0,02	11,5

*$P < 0.05$.

EXPERIMENTAL RESULTS

No visible changes were found in SON in the experimental animals, although the measurements showed a decrease in the nucleoli in the neurosecretory cells of SON, indicating depression of its functional activity (Table 1).

In PVN, darkly stained cells (according to Polenov's classification [3]), containing only a few granules of neurosecretion, were comparatively numerous. These cells were characteristic of a state of lowered intensity of neurohormone synthesis. The nuclei in the PVN cells were reduced in size and the relative percentage of cells with intranuclear nucleolus-like bodies was increased (Table 1). Sometimes two or three nucleolus-like bodies, smaller in size than the mean dimensions of the nucleoli, could be seen in the same nucleus (Fig. 2). The cell nuclei were enlarged in PVN.

Of the parvocellular hypothalamic nuclei it was VMN which reacted most clearly. The dimensions of the perikarya and nuclei of the cells were a little enlarged and the cells were rounder in shape. The dimensions of the nucleoli were not significantly changed, but the number of polynucleolar cells was signifantly increased (Fig. 3), evidence of a sharp decline in activity of RNA formation [2, 4]. VMN also contained cells with nucleolus-like bodies in their cytoplasm, but the number of these cells in the experimental animals was unchanged.

A significant decrease in size of the nucleoli was observed in the cells of SCN, although the number of polynucleolar cells was not increased under these circumstances (Table 1). These observations suggest that activity of SCN was somewhat reduced.

The most reactive nucleus under these experimental conditions was AN: No changes could be found either in the dimensions of the nuclei and nucleoli or in the number of nucleolus-like bodies in the nucleus or cytoplasm (Table 1). This reaction of AN can be regarded as evidence that these nuclei are not concerned in TRH formation. This hypothesis is not contradicted by the discovery of a high TRH concentration in the region of AN [10, 13], for the neurohormone may be contained, not in the cells of AN, but in the numerous fibers from other hypothalamic centers which pass through the AN region toward the outer zone of the median eminence [8, 9].

Depression of the functional activity of the other hypothalamic centers studied, while it may be evidence that the changes take place in the same direction, does not rule out the possibility that the mechanism of these changes may be different. Only a decrease in size of the nucleoli was observed in the cells of SON and SCN, indicating some reduction in functional activity of the cells. There was one significant difference between the reactions of PVN and VMN: In both centers of the nucleus the cells were enlarged, but the simultaneous segregation of the nucleoli suggests that the cells were not activated but, on the contrary, RNA synthesis was sharply reduced in them [2, 4]. It must be remembered that TRH was found in the smallest amounts in SON and SCN, but in the region of PVN and VMN its concen-

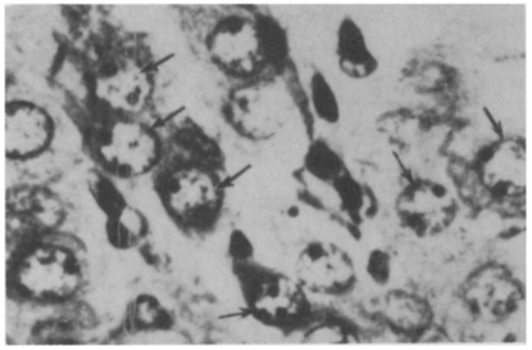

Fig. 2

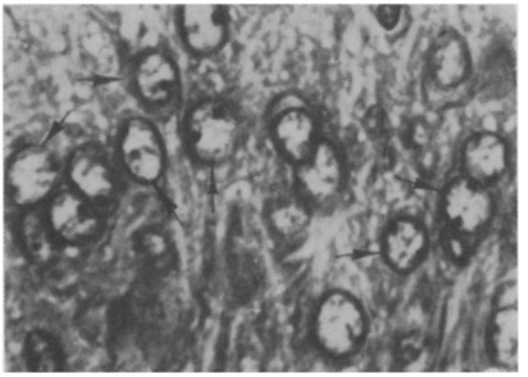

Fig. 3

Fig. 2. PVN of experimental rat. Arrows indicate polynucleolar cells. Fixation in Bouin's fluid, staining with paraldehyde-fuchsin by Gomori—Gabe method with counterstaining by Heidenhain's azan. Objective 40×, ocular 10×.

Fig. 3. VMN of experimental rat. Legend as to Fig. 2.

tration was high [10, 13]. It can accordingly be postulated that the reaction of SON and SCN reflects the modulating inhibitory action of TRH as a neurotransmitter. The sharp decline in RNA synthesis and, consequently, in protein synthetic activity in PVN and VMN may be due to an excess of TRH as neurohormone [8, 12, 13], which leads to a decrease in activity of the cells which regulate the TRH level in the body. On the basis of these results it is possible to put forward a hypothesis which contradicts the conclusion drawn by Hefco et al. [6, 7], who studied regulation of TSH by the hypothalamic nuclei by a technique of deafferentation of different parts of the hypothalamus. These workers claimed that AN plays the principal role in the regulation of TSH. However, this conclusion must be questioned, for their experiments were carried out 4 weeks after deafferentation, when contact between fibers of neurosecretory cells staining positively by Gomori's method with vessels of the portal plexus in the region of the "island" [1], is already restored. It is therefore most probable that neurohormones reached the capillaries of the portal plexus of the "island" not only from AN, but also from the magnocellular neurosecretory centers and, most probably of all, from PVN [15]. The results of the present experiments, and also the possible existence of an efferent connection of VMN with PVN [5], suggest that it is this combined activity of PVN and VMN that is of great importance for regulation of the TRH level in the body.

LITERATURE CITED

1. O. A. Danilova and A. L. Polenov, Cell Tissue Res., 176, 121 (1977).
2. A. A. Zavarzin, Principles of Special Cytology of Multicellular Animals [in Russian], Leningrad (1976).
3. A. L. Polenov, Hypothalamic Neurosecretion [in Russian], Leningrad (1968).
4. S. Ghosh, Int. Rev. Cytol., 44, 1 (1976).
5. M. C. Harris and M. Sanghera, Brain Res., 81, 401 (1974).
6. E. Hefco, L. Krulich, and J. E. Aschenbrenner, Endocrinology, 97, 1226 (1975).
7. E. Hefco, L. Krulich, and J. E. Aschenbrenner, Endocrinology, 97, 1234 (1975).
8. T. Hökfelt, K. Fuxe, O. Johansson, et al., Eur. J. Pharmacol., 34, 389 (1975).
9. M. S. Krieger, L. C. A. Conrad, and D. W. Pfaff, J. Comp. Neurol., 183, 785 (1979).
10. M. Kubek, J. F. Wilber, and J. M. George, Endocrinology, 105, 537 (1979).
11. L. P. Renaud and J. B. Martin, Brain Res., 86, 150 (1975).
12. K. Sterling and J. H. Lazarus, Annu. Rev. Physiol., 39, 349 (1977).
13. W. Vale, C. Rivier, and M. Brown, Annu. Rev. Physiol., 39, 473 (1977).
14. A. Winokur and A. L. Beckmann, Brain Res., 150, 205 (1978).
15. E. A. Zimmerman, M. A. Stillman, L. D. Recht, et al., Colloq. Int. CNRS, 280, 375 (1978).