ULTRASTRUCTURAL CHARACTERISTICS OF SOMATOSTATIN-CONTAINING HYPOTHALAMIC NEURONS IN PROTEIN-DEFICIENT RATS

I. I. Babichenko, I. Z. Eremina,
Yu. L. Grishkin, and D. I. Medvedev

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Clincial and experimental investigations have shown that during formation of the body under conditions of protein deficiency, although signs of delayed growth and development are present the serum level of pituitary somatotropic hormone (STH) is raised [5, 6, 11]. The raised STH during malnutrition can be explained by a fall in the blood level of somatomedins, which perform the function of negative feedback for this hormone [2, 15]. The possibility likewise cannot be ruled out that changes take place in the system of hypothalamic regulation of STH [5], which operates through two regulatory oligopeptides: somatotrophin releasing factor and somatostatin or somatotrophin-inhibiting factor [13]. Considering the important role of somatostatin in the mechanisms of regulation of STH, we undertook an immunohistochemical study of somatostatin-containing hypothalamic neurons of rats developing under conditions of protein deficiency.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats (CLEA Experimental Animals Laboratory, Kanagawa, Japan). From the 6th day of pregnancy and until the end of the lactation period the rats received an experimental diet containing 6% of protein or a control diet with 25% of protein, of identical calorific value (347 cal/100 g diet – CLEA, Japan). By the end of the experiment the body weight of the six experimental animals (21 days old) was 13.03 ± 0.27 g, and of the six control animals 61.1 ± 1.06 g; the weight of the brain was 1.59 ± 0.04 and 2.26 ± 0.02 g respectively. Under pentobarbital anesthesia the animals were perfused with a 4% solution of paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The immunohistochemical reaction was carried out by the indirect method on frozen sections 6 μ thick. Rabbit serum from a kit for determination of human somatostatin (Miles Scientific, Naper-

Table 1. Quantitative Characteristics of Somatostatin-Containing Axon Terminals of Hypothalamic Median Eminence of Rats with Protein Deficiency

Experimental conditions	Overall density of granular vesicles in 1 μ^2	Overall density of electron-translucent vesicles in 1 μ^2	Density of electron-translucent vesicles in 1 μ^2	Density of granular vesicles in terminals not containing electron-translucent vesicles in 1 μ^2
Control	8,56±0,39 (n=200)	3,43±0,26 (n=200)	5,55±0,30 (n=124)	10,98±0,72 (n=76)
Experiment	11,62±0,40* (n=200)	1,66±0,18* (n=200)	4,25±0,29* (n=78)	12,41±0,51 (n=122)

Legend. *$p < 0.01$ compared with control.

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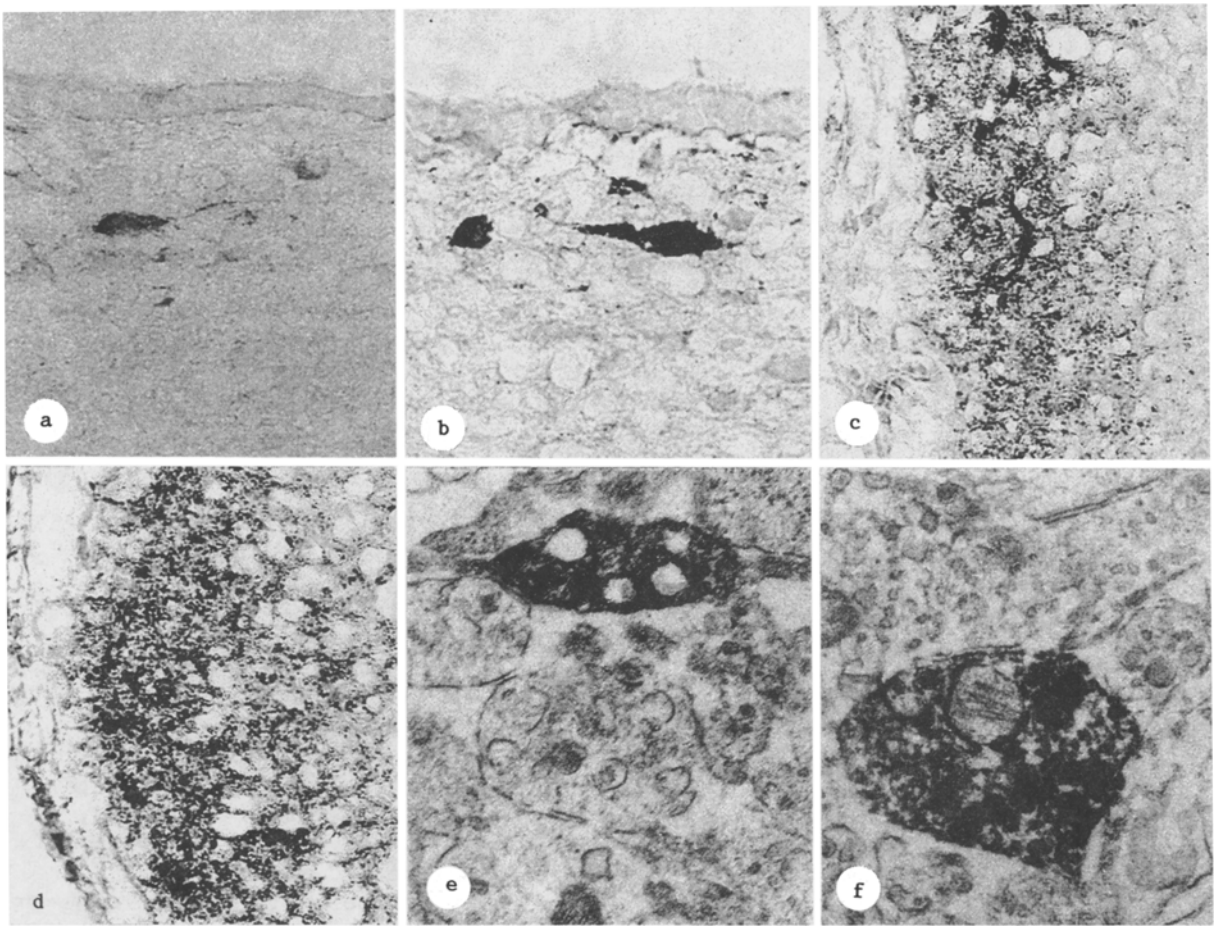

Fig. 1. Somatostatin-containing neurons and their axons in the periventricular region in the median eminence of the hypothalamus of control (a, c, e) and malnourished (b, d, f) rats. a, b) Distribution of products of DAB reaction on neurons of periventricular region of hypothalamus. 480 ×. c, d) Products of DAB reaction in lateral zone of median eminence of hypothalamus. 560 ×. e, f) Electron-dense products of immunohistochemical reaction for somatostatin in axoplasm of axon terminals. 35,000 ×.

ville, Ill.) was used as the primary antibody. The secondary antibodies were Fab-fragments of donkey antirabbit immunoglobulins, conjugated with horseradish peroxidase by the periodate method [14]. Immune rabbit serum served as the negative control. After incubation in a solution of diaminobenzidine (DAB) with hydrogen peroxide in 0.05 M Tris-HCl buffer, the frozen sections were postfixed with 1% OsO_4 solution and embedded in quetol; ultrathin sections were cut from them, stained, and examined under the TESLA BS-500 electron microscope.

Quantitative analysis of axon endings containing electron-dense products of the immunohistochemical reaction was undertaken in the median eminence of the hypothalamus. Under a magnification of 6000, 200 axon terminals were analyzed in negatives obtained from the experimental and control animals. The area and number of granular and electron-transparent vesicles 120-160 nm in diameter were counted in each somatostatin-containing terminal. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

At the light-optical level, somatostatin-containing neurons were discovered in the control and malnourished animals in the periventricular region of the hypothalamus (Fig. 1a, b). Intensive distribution of products of the DAB reaction also was observed in zones of branching of the primary hypophyseo-portal vessels in the lateral part of the median eminence of the hypothalamus (Fig. 1c, d); compared with the control, an increase in the intensity of the immunohistochemical reaction was observed in the experimental animals.

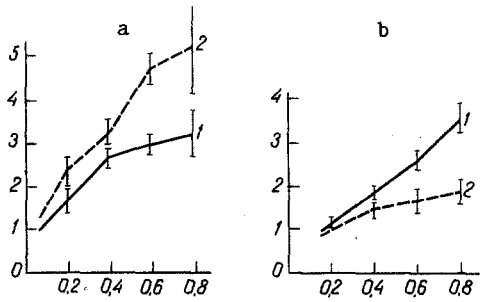

Fig. 2. Distribution of granular (a) and electron-translucent (b) vesicles depending on area of cross section of somatostatin-containing axon terminals in lateral zone of median eminence of hypothalamus of control (1) and malnourished animals (2).

In ultrathin sections of the median eminence of the hypothalamus of the malnourished and control animals somatostatin-containing axon terminals were distinguished from the other endings by the increased electron density of the axoplasm and granular vesicles, against the background of which electron-translucent vesicles measuring 120-160 nm in diameter could be clearly seen (Fig. 1e, f). Table 1 gives the results of their quantatitive analysis in experimental and control rats according to values obtained for the density of the granular and electron-translucent vesicles in the terminals. As Table 1 shows, there was a significant increase compared with the control in the density of distribution of granular vesicles and a decrease in the overall density of the electron-translucent vesicles in axon endings with an immunohistochemical reaction for somatostatin in the malnourished animals. Moreover, in the lateral zone of the median eminence of the hypothalamus in protein deficiency the number of terminals containing electron-translucent vesicles was 39%, compared with 62% in the control. Statistical comparison of these terminals revealed a significant decrease in the mean density of the electron-translucent vesicles in somatostatin-containing terminals of malnourished animals compared with those fed on a normal diet, evidence of depression of the functional activity of these terminals in the experimental animals compared with the control.

In turn, analysis of the mean density of distribution of granular vesicles in terminals not containing electron-translucent vesicles, in the experimental and control animals, revealed no statistically significant differences (Tabel 1). This can be taken as evidence that the increase in their density in axon terminals described above in malnourished animals is associated primarily with delay of somatostatin release from terminals in the lateral zone of the hypothalamic median eminence.

In confirm this hypothesis, the number of granular and electron-translucent vesicles was counted in the experiment and control and related to the area of cross-section of the axon terminals (Fig. 2). It will be clear from Fig. 2 that, irrespective of the area of their cross sections, the axon terminals of the experimental animals contained more granular vesicles, moreover, in endings of control animals.

Since the formation of electron-translucent vesicles takes place during release of the contents of the granular vesicles from the axons [1], the results are evidence that an increase in the number of granular vesicles in somatostatin-containing axon terminals during malnutrition is associated primarily with delay of their release.

The results thus show that under conditions of protein deficiency slowing of the release of somatostatin from axon terminals of the hypothalamic median eminence is observed Such a process can be linked either with predominance of inhibitory impulses from neurotransmitters and neuropeptides modulating somatostatin relase [3, 9, 10], or with depression of the activating effect of the peptide insulin-like growth factor-1 (ILGF-1)[2]. In our view the second of these hypotheses is more likely to be true, for we know that during protein deficiency the somatomedin and ILGF-1 levels are considerably depressed [4, 8]. It is most probably this fact which leads to the decrease in functional activity of somatostatin-containing axon terminals during malnutrition observed in the present investigation, somatostatin accumulating in the granular vesicles while correspondingly less of it is supplied by the portal system of capillaries to the adenohypophysis. Since somatostatin

inhibits the secretion of pituitary STH [7], slowing of its release must be accompanied by elevation of the blood STH level in protein deficiency.

LITERATURE CITED

1. K. N. Veremeenko, Enzymes of Proteolysis and Their Inhibitors in Medical Practice [in Russian], Kiev (1971).
2. L. A. Lokshina, Mol. Biol., 13, No. 6, 1205 (1979).
3. L. A. Lokshina and É. A. Dilakyan, Mol. Biol., 20, No. 5, 1157 (1986).
   N. Back, Lancet, 2, 370 (1978).
4. D. A. Estell and M. Laskowski, Jr., Biochemistry, 19, 124 (1980).
5. H. Fritz and G. Wunderer, Drug Res., 33, 479 (1983).
6. L. Goldstein, Biochemistry, 11, 4072 (1972).
7. J. H. Griffin and C. G. Cochrane, Proc. Natl. Acad. Sci. USA, 73, 2554 (1976).
8. H. Holzer and P. C. Heinrich, Annu. Rev. Biochem., 49, 63 (1980).
9. C. Lazdunski, D. Baty, and J.-M. Pages, Eur. J. Biochem., 96, 49 (1979).
10. R. R. Porter and K. B. M. Reid, Nature, 275, 699 (1978).
11. Proteinases and their Inhibitors: Proceedings of the International Symposium, Portoroz (1980), pp. 33-34; 67-72.
12. W. Stoffel, H. Borberg, and V. Greve, Lancet, 2, 1005 (1981).
13. S. Yokoyama, R. Hayashi, M. Satani, et al., Arteriosclerosis, 5, 613 (1985).

CHOLINERGIC INNERVATION OF VASOPRESSIN-CONTAINING CELLS OF HUMAN CEREBRAL BLOOD VESSELS

A. V. Lomakin, Yu. I. Pigolkin, and P. A. Motavkin

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KEY WORDS: melanocytes; vasopressin; cerebral vessels; innervation

The role of vascular melanocytes in regulation of material mobility in the mammalian brain has been demonstrated experimentally. Stimulation of the vagus nerve by a direct current and application of acetylcholine to the pia mater cause degranulation of these cells [1]. However, the morphological substrate of the mechanism of degranulation and the vasoactive substances of melanocytes, as possible vascular effectors, are not yet known. We investigated these cells and the localization of vasopressin (VP) in them, and also their relations with cholinergic axons.

EXPERIMENTAL METHOD

The pia mater and also transverse sections through the mesencephalon and pons and blood vessels located in these regions in fetuses during the second half of intrauterine development, and in adults (aged 30-50 years 6-12 h after death) were investigated. Melanocytes were identified by presence of brown melanin granules in the cytoplasm, by Masson's reaction, and by the reaction for tyrosinase [3]. Nerve fibers were impregnaged by Campos' method and cholinergic axons were revealed by reactions for acetyl-cholinesterase (AChE) [6] and choline-acetyltransferase (ChAT) [2], and electron-microscopically by the presence of translucent synaptic vesicles in them. Material for electron-microscopic study was fixed in 1% OsO_4 and embedded in Epon-12. Sections were cut on the LKB Ultrotome and examined in the JEM-100B electron microscope. VP was detected in the MBI-15-2 luminescence microscope by an indirect immunofluorescence method [7], using monospecific hog antibodies to hog IgG, conjugated with FITC ("Sevac," Czechoslovakia). Pigmented neurons of the locus coeruleus,

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Department of Histology, Vladivostok Medical Institute.

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