BIOGERONTOLOGY

Synthesis of IL-2 mRNA in Cells of Rat Hypothalamic Structures after Injection of Short Peptides

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In situ hybridization on paraffin sections of the rat brain showed that synthetic peptides Vilon, Epithalon, and Cortagen modulated the expression of IL-2 gene *in vivo* in cells of some hypothalamic structures depending on the terms and routes of administration.

Key Words: *peptides; gene expression; IL-2 mRNA; hypothalamus*

Bioactive peptides Vilon, Epithalon, and Cortagen are characterized by immunomodulating effects: they stimulate the production of IL-2 mRNA in splenic lymphocytes and exhibit a geroprotective effect (prolong animal life span) [5,6,10]. IL-2 is expressed not only in cerebral immunocompetent cells, but also in astrocytes, glia, and neurons and regulates cell differentiation and proliferation processes and secretion of neurohormones [8].

We studied the effects of Vilon, Epithalon, and Cortagen on the expression of IL-2 mRNA in rat hypothalamic cells depending on the route and terms of administration.

MATERIALS AND METHODS

The study was carried out using peptides, synthesized at St. Petersburg Institute of Bioregulation and Gerontology on the basis of amino acid analysis of complex preparations of the thymus (Vilon, Lys-Glu), epiphysis (Epithalon, Ala-Glu-Asp-Gly), and cerebral cortex (Cortagen, Ala-Glu-Asp-Pro).

Sprague-Dawley male rats (200 g) were intramuscularly injected (into the left or right hip alternatively for 5 days) with the peptides (10 μg/kg in 200 μl saline) or received a single intranasal dose of 1.5 or 10 μl alternatively into each nostril; initial concentration 10 ng/μl). Intact animals and animals receiving normal saline served as controls. The animals were handled in accordance with the regulation of the European Convention ET/S 129, 1986, and ESC 86/609 directions.

Twenty-four hours after the last intramuscular injection or 1.5 h after intranasal treatment the rats were narcotized and intracardial perfusion with warm saline with heparin was carried out; the brain was removed, fixed, and treated as described previously [2]. Serial sections (5 μ) were prepared on a microtome (Reichardt) and mounted on slides treated with 3-aminopropyltritaxilane (6-8 sections per slide). One sections was stained with thianine for evaluating the topography and counting the total number of cells in the structure, the next section was used for detecting IL-2 mRNA by *in situ* hybridization on paraffin sections of the brain with digoxygenin-labeled IL-2 cDNA (pAA1213) [1,11]. Rat brain computer maps were used

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[12]. Cell counts on section preparations from different hypothalamic structures were evaluated using Ista Video Master software. The total count of cells per 10,000 μ^2 (evaluated by the histological method) was taken for 100%.

The results were statistically processed using Student's *t* test.

RESULTS

Involvement of the hypothalamus into the regulation of autonomic functions, including the defense functions of the body [3], prompted us to evaluate the intensity of IL-2 gene expression in this brain structure. Induction of IL-2 mRNA synthesis in the lateral hypothalamic area (LHA) was observed during stress [1,9] and intravenous injection of antigen [2]. Intramuscular injection of Epithalon to rats causes a pronounced expression of IL-2 mRNA gene in some hypothalamic structures: LHA and anterior hypothalamic fields (AHN), dorsomedial, ventromedial, and paraventricular hypothalamic nuclei (DMH, VMH, and PVH, respectively). The count of neuronal cells producing IL-2 mRNA is different in these hypothalamic structures. The count of IL-2 mRNA-positive cells is maximum in LHA, lower in DMA, VMH, PVH, and

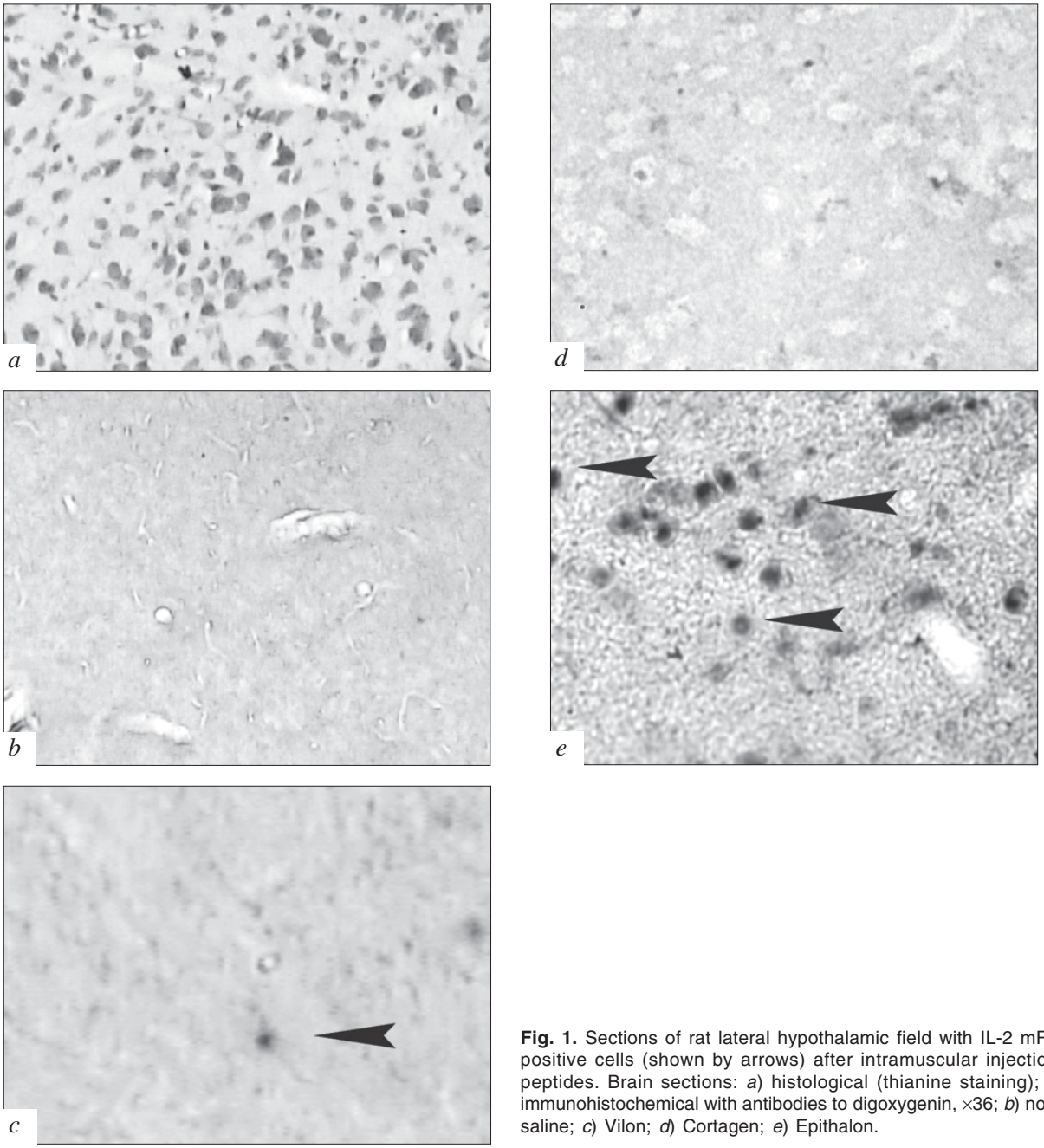

Fig. 1. Sections of rat lateral hypothalamic field with IL-2 mRNA-positive cells (shown by arrows) after intramuscular injection of peptides. Brain sections: *a*) histological (thianine staining); *b-e*) immunohistochemical with antibodies to digoxygenin, ×36; *b*) normal saline; *c*) Vilon; *d*) Cortagen; *e*) Epithalon.

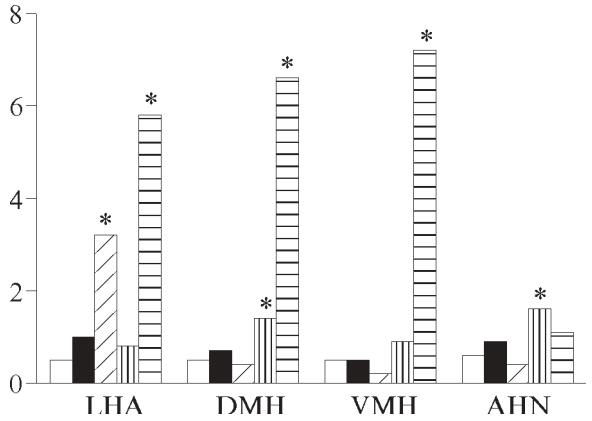

Fig. 2. Counts of IL-2 mRNA-positive cells in hypothalamic structures 2 h after intranasal administration of peptides. The content of IL-2 mRNA-positive cells is expressed in % of total number of cells on section of the structure per 10,000 μ². Ordinate: percentage of IL-2 mRNA-positive cells in the studied brain structure. Light bars: intact animals; dark bars: normal saline; cross-hatched bars: Vilon; vertical hatching: Epithalon; horizontal hatching: Cortagen. *$p<0.05$ compared to normal saline.

AHN (60, 50, 41, 34, 15%, respectively, of total number of cells in this structure).

Intramuscular injections of Vilon and Cortagen did not change the intensity of IL-2 mRNA expression in cells of the above-listed hypothalamic structures. The only exclusion was PVH, where minor (4%) stimulation of IL-2 mRNA synthesis was observed after injection of Vilon. Only solitary IL-2 mRNA-positive cells (1-4) were seen in the hypothalamic cells of intact animals and rats injected with saline (Fig. 1).

Since receptor cells of the olfactory epithelium are bipolar sensory neurons, the substances administered intranasally are transported from the nasal cavity directly into the brain (axonal transport) [7]. Cortagene produced most potent effect on IL-2 mRNA gene expression in the hypothalamic cells after intranasal administration. It was previously shown that Cortagen accelerated regeneration of damaged nerves and facilitated conduction of nerve pulse via regenerating afferent fibers [4]. Intranasal Cortagen (in contrast to intramuscular) stimulated the production of IL-2 mRNA in LHA, DMH, and VMH cells, the count of IL-2 mRNA-positive cells was maximum in VMH (7.2%; Fig. 2).

Epithalon administered intranasally produced a weaker effect compared to Cortagen; it stimulated the synthesis of IL-2 mRNA in DMH and AHN cells (1.4 and 1.6%, respectively). Hence, the effect of Epithalon administered intranasally was weaker than after intramuscular injection. Intranasal Vilon stimulated IL-2 mRNA expression only in LHA cells (3.2%).

Our experiments demonstrate the effects and selective activity of short peptides on IL-2 mRNA synthesis in hypothalamic cells.

Comparison of our findings with previous data suggests that central regulatory structures are involved into realization of immunomodulating effects of peptides.

Hence, Epithalon and Cortagen are not only immunomodulators, but exhibit central effects and modulate the expression of IL-2 gene in some neurons.

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REFERENCES

1. S. V. Barabanova, O. I. Golovko, N. S. Novikova, *et al.*, *Neirokhimiya*, **15**, No. 4, 380-387 (1998).
2. E. A. Korneva, T. B. Kazakova, and M. A. Nosov, *Allergol. Immunol.*, **1**, No. 1, 37-44 (2000).
3. E. A. Korneva and E. K. Shkhinek, *Hormones and Immune System* [in Russian], Leningrad (1988).
4. L. N. Turchaninova, L. I. Kolosova, V. V. Malinin, *et al.*, *Byull. Eksp. Biol. Med.*, **130**, No. 12, 654-656 (2000).
5. V. Kh. Khavinson, V. N. Anisimov, N. Yu. Zavarzina, *et al.*, *Ibid.*, No. 7, 88-91.
6. V. Kh. Khavinson, V. G. Morozov, V. V. Malinin, *et al.*, *Ibid.*, No. 9, 330-332.
7. W. H. Frey, J. Liu, X. Chen, *et al.*, *Drug Delivery*, **4**, 87-92 (1997).
8. U.-K. Hanish and R. Quirion, *Brain Res. Rev.*, **21**, 246-284 (1996).
9. T. B. Kazakova, S. V. Barabanova, N. S. Novikova, *et al. Int. J. Pathol.*, **7**, 39-47 (2000).
10. V. G. Morozov and V. Kh. Khavinson, *Int. J. Immunopharmacol.*, **19**, No. 9/10, 501-505 (1997).
11. *Nonradioactive In situ Hybridization. Application manual*, GmbH, Application Manual Biochemica, Mannheim (1992).
12. L. W. Swanson, *Brain Maps: Computer Graphics Files*, Amsterdam (1992).