GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Interleukin-2 Concentration in Hypothalamic Structures of Rats Receiving Peptides during Mild Stress

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The number of hypothalamic IL-2-containing cells changed in rats receiving Vilon and Epithalon during mild stress (handling). The number of IL-2-positive cells in hypothalamic structures decreased 24 h after intramuscular injection of Epithalon and 2 h after intranasal administration of the test peptides. Adaptation of animals to experimental conditions prevented the decrease in the number of IL-2-positive cells in the supraoptic nucleus after intranasal administration of Epithalon.

Key Words: *peptides; Vilon; Epithalon; interleukin-2-like protein; hypothalamus*

Functional changes in physiological systems of the organism (*e.g.*, immune system) can be induced by exposure to adverse environmental factors, stress, and aging.

Peptides of the thymus, pineal gland, and cerebral cortex contribute to functional recovery of the immune and endocrine system and possess geroprotective activity [4,5,10,12]. These peptides regulate expression of several genes or transcription products. Previous studies showed that Vilon and Epithalon produce a stimulatory effect on the expression of IL-2 gene in mouse splenocytes [2,6].

IL-2 plays an important role in the regulation of the immune response to antigens [1,3]. IL-2, IL-2 mRNA, and IL-2 receptors were detected in tissues of the central nervous system (CNS) [9]. Since specific forms of IL-2 differing from IL-2 protein in lymphocytes were detected in the brain, in the analysis of IL-2 in nervous cells we use the term "IL-2-like protein".

Here we studied the effects of Vilon and Epithalon administered via different routes and using different schedules on the concentration of IL-2-like-protein in hypothalamic structures of rats during mild stress (handling).

MATERIALS AND METHODS

The experiments were performed on male Wistar rats weighing 180-200 g. The animals were maintained at room temperature and 12:12-h light/dark regimen and had free access to water and food.

Vilon (Lys-Glu) and Epithalon (Ala-Glu-Asp-Gly) in a single dose of 2 μg per 200 μl physiological saline were injected intramuscularly. Otherwise, the test peptides in a single dose of 10 ng/μl were administered intranasally into each nasal cavity. Experimental manipulations (handling; taking with the hands and placement in an immobilization cage) were considered as mild stress. Intact rats, animals adapted to the experimenter's hands for 5

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days, and rats receiving an equivalent volume of physiological saline served as the control.

The animals were narcotized 24 h after intramuscular injection or 2 h after intranasal administration of the peptides. Intracardiac perfusion was performed with phosphate buffered saline and fixative. The brain was removed after 1 h. Sections were prepared after cryoprotection.

Brain sections (20 μ) were prepared on a Reichardt microtome using a Mikonta-2 freezing device (-27°C). The sections were treated and fixed on glasses as described elsewhere [3].

IL-2-like protein was identified in an indirect immunoperoxidase test with polyclonal antibodies against recombinant IL-2 (dilution 1:20, Institute of Ultra-pure Biological Preparations). We used secondary antibodies against rabbit IgG conjugated with peroxidase (dilution 1:300, Sigma).

Cell counting and measurement of optical density were performed with the 25th section according to the rat brain atlas [13]. We used the Ista-Video-Test system. IL-2-positive cells were counted per 10,000 μ^2.

IL-2-like protein concentration in hypothalamic cells was estimated by optical density of IL-2-positive cells and expressed in percents. Baseline optical density was taken as 100%.

The results were analyzed by Student's *t* test.

RESULTS

The number of IL-2-positive cells in the anterior hypothalamic nucleus (AHN), paraventricular nucleus (PVN), and supraoptic nucleus (SO) decreased 24 h after intramuscular injection of Epithalon under conditions of mild stress (compared to intact animals and rats of the physiological saline group, Fig. 1).

Our results are consistent with published data on the transcriptome of mouse myocardium and brain. Short peptides were shown to modulate gene expression [7,8].

Intranasal administration of Vilon decreased the number of IL-2-positive cells in PVN. The number of IL-2-positive cells decreased in PVN and SO and tended to decrease in the lateral hypothalamic area (LHA) 2 h after Epithalon treatment (Fig. 1, *a*).

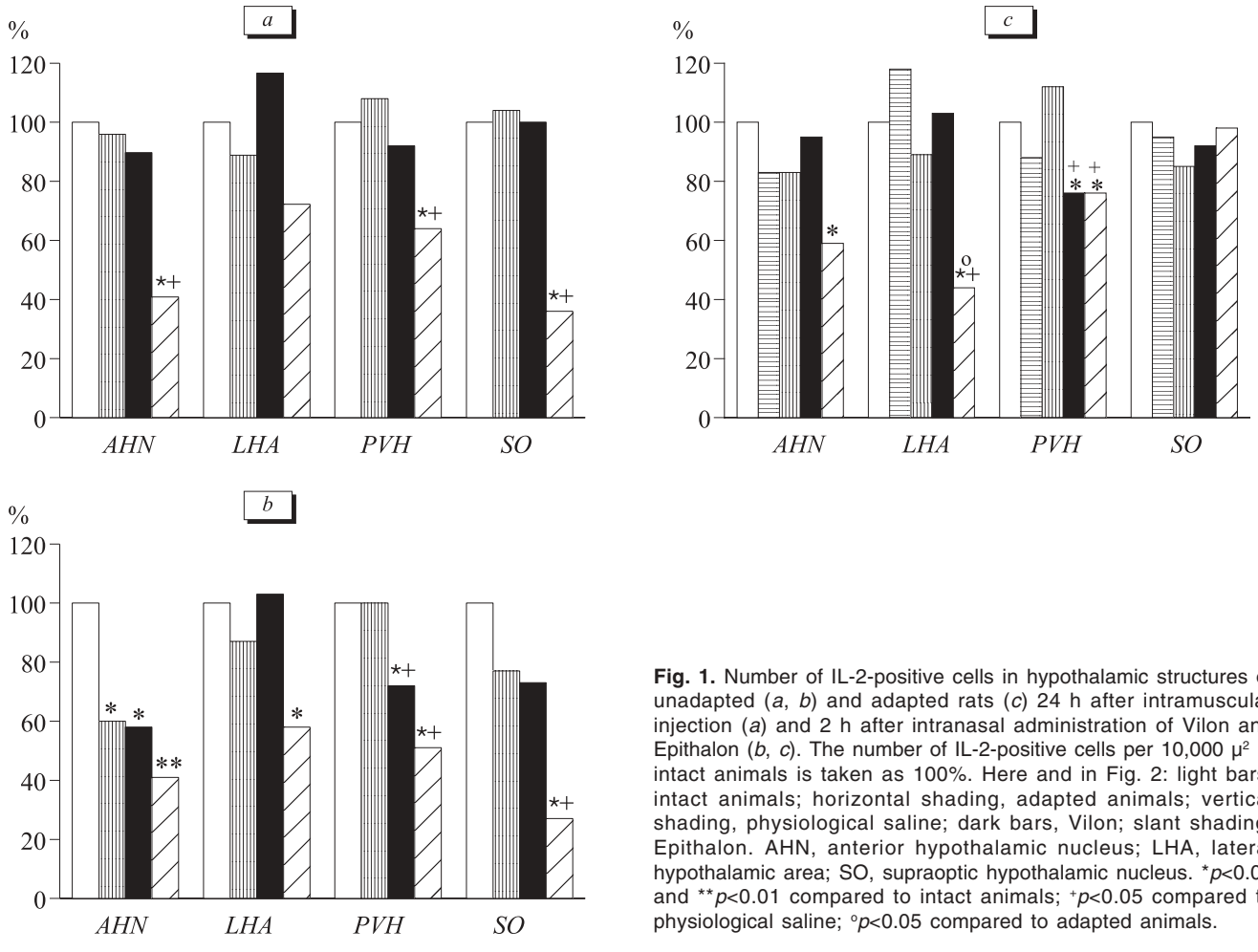

Fig. 1. Number of IL-2-positive cells in hypothalamic structures of unadapted (*a*, *b*) and adapted rats (*c*) 24 h after intramuscular injection (*a*) and 2 h after intranasal administration of Vilon and Epithalon (*b*, *c*). The number of IL-2-positive cells per 10,000 μ^2 in intact animals is taken as 100%. Here and in Fig. 2: light bars, intact animals; horizontal shading, adapted animals; vertical shading, physiological saline; dark bars, Vilon; slant shading, Epithalon. AHN, anterior hypothalamic nucleus; LHA, lateral hypothalamic area; SO, supraoptic hypothalamic nucleus. *p<0.05 and **p<0.01 compared to intact animals; ^{+}p<0.05 compared to physiological saline; $^{\circ}p$<0.05 compared to adapted animals.

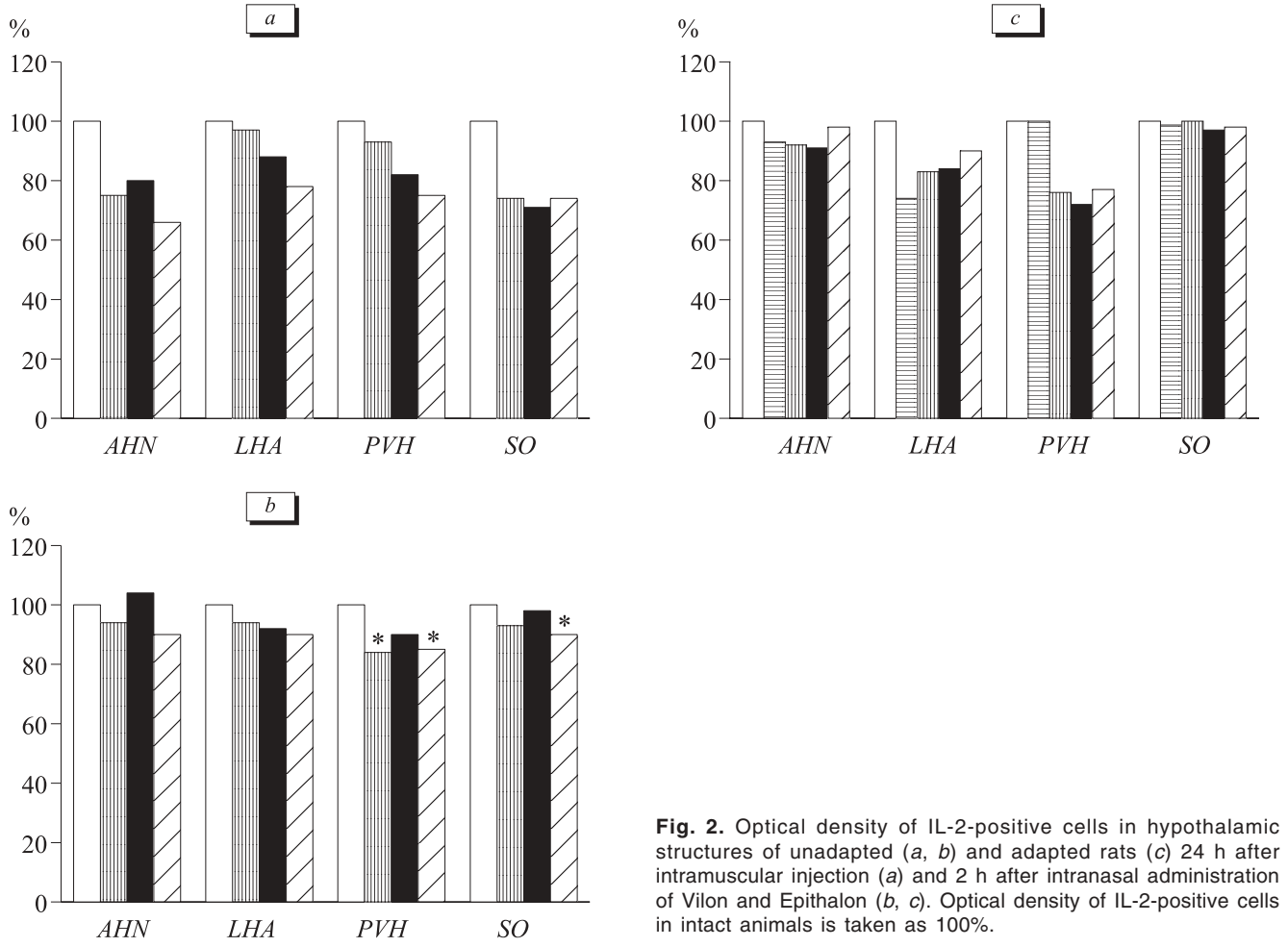

Fig. 2. Optical density of IL-2-positive cells in hypothalamic structures of unadapted (*a*, *b*) and adapted rats (*c*) 24 h after intramuscular injection (*a*) and 2 h after intranasal administration of Vilon and Epithalon (*b*, *c*). Optical density of IL-2-positive cells in intact animals is taken as 100%.

The animals were adapted to experimental manipulations for 5 days (taking with the hands and placement in an immobilization cage for 1 min) to diminish the effect of stress (handling). Further experiments were performed on adapted animals.

Similarly to rats of the handling group, the number of IL-2-positive cells in PVN of handling-adapted animals decreased after intranasal administration of Vilon. Epithalon decreased the number of IL-2-positive cells in PVN and LHA (Fig. 1, *b*), but had no effect on the count of these cells in SO of handling-adapted animals.

Optical density of IL-2-positive cells in hypothalamic structures reflects intracellular IL-2 concentration. This parameter did not differ in control and treated animals. However, optical density of IL-2-positive cells tended to decrease after treatment with the test peptides (Fig. 2). Hence, the decrease in the number of IL-2-positive cells during treatment with peptides was not accompanied by changes in intracellular IL-2 protein concentration. These results suggest that the decrease in the number of IL-2-positive cells reflects reduction of IL-2 protein concentration in hypothalamic structures.

Our findings show that Vilon and Epithalon administered via different routes and according to different schedules produce a modulatory effect on the concentration of IL-2-like protein in hypothalamic structures of rats during mild stress and adaptation to stress. It manifested in a decrease in the number of IL-2-positive cells in hypothalamic structures. Epithalon was most potent in this respect. The effect of Epithalon was observed 24 h after intramuscular injection and 2 h after intranasal administration. The number of IL-2-positive cells in PVN decreased only 2 h after intranasal administration of Vilon. Epithalon had no effect on the number of IL-2-positive cells in SO of handling-adapted animals. However, the number of IL-2-positive cells in SO of unadapted rats decreased after Epithalon treatment (Fig. 1, *b*).

Published data provide evidence that the response of SO cells to stress correlates with IL-2 concentration in hypothalamic structures. IL-2 stimulates vasopressin secretion in SO cells. The in-

crease in IL-2 production was also observed under stress conditions [9]. Moreover, IL-2 is involved in activation of the hypothalamic-pituitary-adrenal axis.

Administration of Epithalon to rats of the handling group potentiated the reaction of SO cells in unadapted animals, but had no effect in stress-adapted rats.

The decrease in the number of IL-2-positive cells after administration of Vilon and Epithalon can be related to not only the inhibitory effect of peptides on IL-2 protein expression, but also the imbalance between its synthesis and consumption.

Several pathological processes are accompanied by changes in the concentration of IL-2 and number of IL-2 receptors in CNS and cerebrospinal fluid. Previous studies showed that IL-2 concentration in brain structures increases during cerebral injury [9,11]. The decrease in the concentration of IL-2 protein in brain structures has a positive effect under these conditions. In light of this, experiments on correction of IL-2 level in the brain under pathological conditions with Vilon and Epithalon seem to be very interesting.

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