Permanent Modification of Neurohormone Expression in the Hypothalamus of Rats on the Model of Learned Helplessness

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Depressive state of Wistar rats in the model of learned helplessness was accompanied by a persistent increase in the expression of corticotropin-releasing factor in the hypothalamus. Vasopressin content in the hypothalamus was modified only in the early poststress period and remained unchanged at the late stage of experimental depression. Our results indicate that hypothalamic corticotropin-releasing factor (but not vasopressin) plays an important role in the development of experimental mental disorders.

Key Words: *corticotropin-releasing factor; vasopressin; hypothalamus; depression; learned helplessness*

Clinical studies showed that hyperactivation and dysfunction of the pituitary-adrenocortical system are the key stages of anxiety and depressive disorders [9,14,15]. Activity of the pituitary-adrenocortical system increases in 50% patients with melancholic depression. These patients are characterized by elevated blood cortisol concentration and absence of the inhibitory response in the dexamethasone test. Hence, the negative feedback regulation (*i.e.*, suppression) of the pituitary-adrenocortical system is impaired in these patients [9,11, 14]. Basal and evoked activity of this system is primarily regulated by hypothalamic neurohormones corticotropin-releasing factor (CRF) and vasopressin (VP) acting as synergists [7]. One of the hypothesis on neuroendocrine mechanisms of depression postulates that high activity of the pituitary-adrenocortical system and disintegration of its components are associated with hypersecretion of CRF in limbic structures. This conclusion was made from the results of clinical observations. It was shown that CRF content in the cerebrospinal fluid and the number of CRF-synthesizing neurons in the hypothalamus (postmortem examination) increase in patients with various forms of anxiety and depressive disorders [10,12,15]. It was hypothesized that VP also plays an important role in the development of depression symptoms. Plasma VP concentration and number of VP-producing neurons in the hypothalamus (postmortem examination) increase in patients with melancholic depression [6]. However, these conflicting results were not confirmed by independent authors [13]. The role of VP in pathophysiology of depressive disorders (*e.g.*, dysfunction of the pituitary-adrenocortical system) remains unclear.

There are several limitations to clinical study of depression. Hence, experiments on depressive animals hold much promise in this respect.

Here we compared the role of hypothalamic neurohormones CRF and VP in the development of experimental depression in Wistar rats.

MATERIALS AND METHODS

Experiments were performed on adult male Wistar rats weighing 200-250 g. Learned helplessness ser-

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ved as an experimental model of endogenous (melancholic) depression [8]. Experimental rats (n=6) were exposed to unavoidable electrocutaneous stimulation in cages (13×16×26 cm) with conducting floor with variable periods of stimulation and rest; each rat received 60 electroshocks over 1 h. Control animals (n=6) were maintained in the same cages for 1 h, but received no electrostimulation.

For immunocytochemical study, the rats were decapitated 1, 5, and 10 days after stress. The brain was rapidly removed. The hypothalamus was isolated, fixed in 4% paraformaldehyde for 36-48 h, routinely treated, and embedded into paraffin. Serial frontal brain sections (6 μ) were prepared. To study the expression of CRF and VP in the hypothalamic paraventricular (PVN) and supraoptic nuclei, the sections were treated immunohistochemically with monoclonal antibodies against CRF (Santa Cruz Biotechnology, 1:100) and VP (Abcam, 1:500). Visualization involved the avidin-biotin method with diaminobenzidine. Immunoreactivity was studied quantitatively using an image analysis system consisting of a Jenaval light microscope (Carl Zeiss), Baumer CX05c digital camera (Baumer Optronic), computer, and Video Test Master Morphology software. By optical density, immunopositive cells were divided into groups of weakly, moderately, and highly immunoreactive cells. We estimated the total number of immunoreactive cells in the hypothalamic nuclei, as well as the count of moderately and highly immunoreactive cells in each of 5-6 sections from one animal. This quantitative approach allowed us to extend the possibility of qualitative evaluation of immunohistochemical data.

The results were analyzed by ANOVA (SPSS 12.0 software). The differences between samples were significant at $p \leq 0.05$.

RESULTS

CRF expression in rat hypothalamus increased with the progression of depressive state (exposure to unavoidable uncontrolled stress on the model of learned helplessness). After stress exposure, the total number of CRF-immunoreactive cells in the parvocellular region of hypothalamic PVN (pPVN) increased by 113% compared to the control (Fig. 1, *a*). The count of moderately and highly immunoreactive neurons increased by 243% on day 10 after stress (Fig. 1, *b*). Hence, the increase in CRF immunoreactivity was mainly related to changes in the expression of this neurohormone. CRF content in the magnocellular region of PVN (mPVN) remained unchanged under these conditions (Fig. 1).

VP expression in the hypothalamus decreased on day 1 after stress, but returned to normal by the 10th day. The total number of VP-immunoreactive cells (Fig. 2, *a*) and VP expression in pPVN decreased on day 1 after stress (Fig. 2, *b*). VP expression in pPVN returned to normal on day 5. However, the total number of immunoreactive cells remained low in this period. By the 10th day, VP expression in pPVN did not differ from the control (Fig. 2). Similar changes in VP immunoreactivity were revealed in mPVN. The decrease in VP expression on day 1 after stress was associated with changes in the expression of this neurohormone. However, VP expression returned to normal in the follow-up period (Fig. 2, *b*).

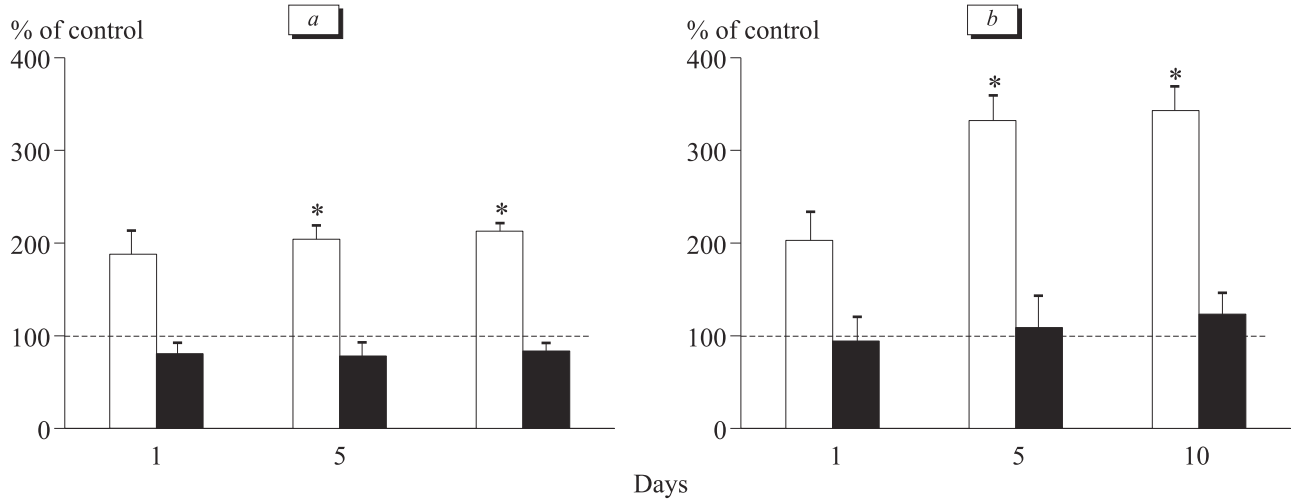

Fig. 1. CRF content in hypothalamic PVN of Wistar rats at various stages of depressive state. Here and in Fig. 2: total number of immunoreactive cells (*a*) and count of moderately and highly immunoreactive cells (*b*). Light bars, pPVN; dark bars, mPVN. *p<0.05 compared to the control (100%).

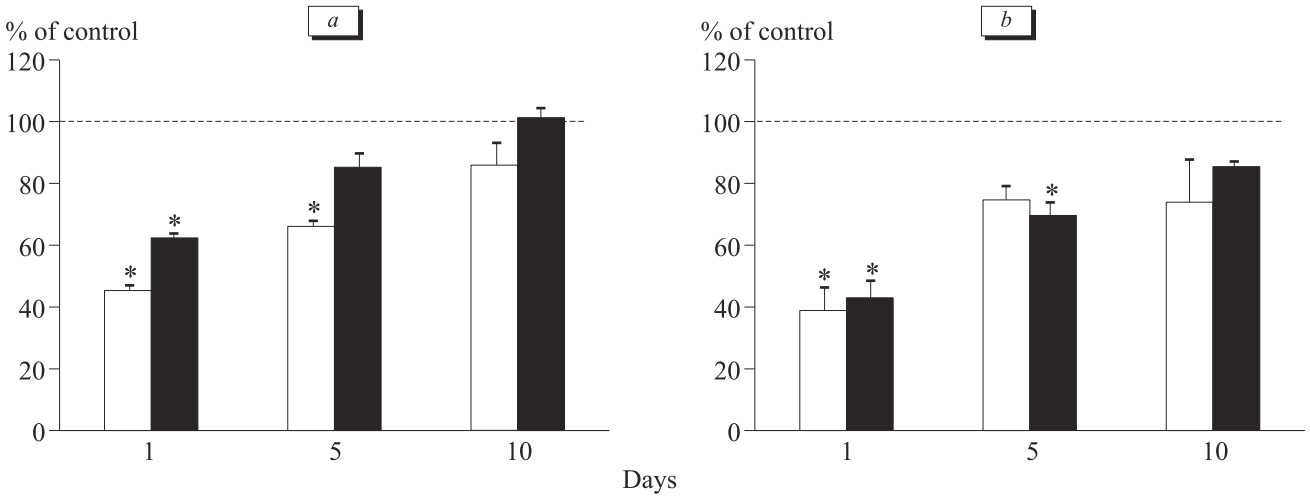

Fig. 2. VP content in hypothalamic PVN of Wistar rats at various stages of depressive state.

We conclude that the development of depressive state (model of melancholic depression) is accompanied by an increase in the number of CRF-producing neurons and neurohormone expression in hypothalamic pPVN of Wistar rats. Our results are consistent with published data and confirm the fact that the hypothalamic part of the CRF-ergic system plays the key role in the pathophysiological mechanisms of depressive state [3,9,11,14].

Other results were obtained in studying the expression of VP in the hypothalamus of Wistar rats with depressive state. VP content in the hypothalamic nuclei significantly decreased by the 24th hour after stress. These changes reflect the response of the VP-ergic system to stress and are associated with depletion of the neurohormone after its release in stress. On day 10 of depressive state, VP content in the hypothalamic nuclei did not differ from the control. These data suggest that VP is not involved in the development of behavioral depression and pituitary-adrenocortical dysfunction in Wistar rats during this period of depressive state on the model of learned helplessness [1,3-5]. Previous studies on another model of endogenous depression showed that the development of depressive sate in KLA rats (Koltushi Low Avoidance) is accompanied by similar changes in hypothalamic VP immunoreactivity. The number of VP-producing neurons in the hypothalamic nuclei and VP expression decreased on day 1, but returned to normal by the 10th day after exposure to unavoidable aversive stress [2]. The reproducibility of the data on hypothalamic VP expression in animals with various forms of endogenous depression confirms our hypothesis that the hypothalamic part of the VP-ergic system does not play a role in the development of experimental mental disorders.

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