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Antidepressant Effect of Hypoxic Preconditioning Is Associated with Modification of Expression of Transcription Factor c-Fos in Rat Brain in Response to Unavoidable Stress

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Development of post-stress depression in rats was accompanied by long-term moderate activation of the expression of transcription factor c-Fos in the neocortex, hippocampus, and paraventricular nucleus of the hypothalamus. Hypoxic preconditioning preventing depressive state in rats under conditions of unavoidable stress considerably enhanced c-Fos expression in the studied brain regions during the early stages of stress response (days 1-5) and promoted its normalization at later terms (10 days). Disturbances in the wavy dynamics of c-Fos expression can contribute to the pathogenic mechanisms of depression, in particular and induce hyperproduction of hypothalamic neurohormone corticoliberin, whereas potentiation of early expression of this factor in response to stress is obviously necessary for prevention of post-stress disorders.

Key Words: *depression; hypoxic preconditioning; transcription factors; c-Fos*

Despite high prevalence of depressive disorders, neurobiological events underlying the development of these pathologies as well as the molecular mechanisms of their correction remain insufficiently studied. Depression is characterized by sustained disorders in the expression profile of various genes and their products in brain neurons, including neurohormone corticoliberin, steroid receptors, receptors of neurotransmitters, enzymes of their metabolism and transporters, *etc.* [1,5,7]. Transcription factors are the major regulators of genome activity in cells, among them factor c-Fos plays the key role. c-Fos, an oncoprotein of the immediate early gene family, is induced rapidly and regulates activity of target genes either independently or as part of the homo-and heterodimers AP-1. Rapid but transient expression of c-Fos gene and protein in brain neurons is induced by various stimuli, therefore it was long considered as a nonspecific marker of neuronal activation [3], but now it became clear that the spatio-temporal patterns of c-Fos expression differ greatly under various physiological and pathological conditions. Sustained delayed overexpression of c-Fos is sometimes associated with the development of a pathological process [4,9]. In this connection, considerable attention is paid to spatial and temporal patterns of c-Fos expression in the brain during the development of adaptive and pathologic responses. Identification of the specific features of c-Fos expres-

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sion in the brain accompanying the development of some pathologies, *e.g*. depression, will clarify the molecular mechanisms of genome dysregulation underlying pathogenetic processes. These *in vivo* studies cannot be performed in humans, while *post-mortem* data do not provide sufficient information about the early stages of pathogenesis. Therefore, studies in animal models are of particular importance.

Here we performed a comparative analysis of c-Fos expression patterns in rat brain structures at various stages of the development of depressive disorders and during its correction with hypoxic preconditioning (HP).

MATERIALS AND METHODS

Experiments were performed on 12 Wistar rats weighing 200-250 g. Depression-like state was modeled using "learned helplessness" paradigm. The rats were subjected to unavoidable stress (US) consisting in electrocutaneous stimulation in cages 13×16×26 cm with conducting floor. Variable periods of stimulations alternated with rest so that each rat received 60 electric shocks over one hour. Control rats were places in the same cages for 1 h, but were not exposed to electric shocks. HP with mild hypobaric hypoxia (360 mm Hg, 2 h, three sessions with 24-h intervals) [1] was performed in a pressure chamber one day before US according to the method developed earlier [6].

The rats were decapitated 1, 5, and 10 days after stress exposure. The brain was promptly removed, fixed in 4% paraformaldehyde for 36-48 h, and after standard processing embedded in paraffin. Serial frontal 6 μ-thick sections of the brain were prepared. c-Fos expression on the sections was evaluated immunohistochemically using polyclonal antibodies against c-Fos (Vector; 1:100). The reaction was visualized by the avidin—biotin method using diaminobenzidine. Immunoreactivity was quantitatively assessed using image analysis system consisting of Carl Zeiss Jenaval light microscope, digital Baumer CX05c camera (Baumer Optronic), computer and software Video Test Master Morphology. The number of immunoreactive cells in hippocampal field CA1, dentate gyrus, neocortex levels, and paraventricular nucleus of the hypothalamus (PVN) was evaluated on each of 5-6 sections from each animal (each group comprised 6 animals). Mean values for the groups were calculated. The data were presented in relative units (percent of the control); control value in each group was taken as 100%.

The results were statistically processed by ANOVA. The differences between the samples were considered significant at $p \leq 0.05$.

RESULTS

Our previous study showed that US leads to the formation of a sustained depressive-like state in rats characterized by gradual reduction in motor activity, anhedonia, and increased anxiety peaking by day 10. In addition, we observed a steady increase in the basal levels of blood corticosterone and impaired inhibition in the dexamethasone test [6]. US also steadily and moderately increased the level of c-Fos immunoreactivity in neurons of all studied rat brain regions. In pyramidal neurons of hippocampal field CA1, significantly increased expression of this protein was observed after 5 days and persisted until day 10 after stress, which clearly indicates a trend towards delayed overexpression of c-Fos in this most vulnerable hippocampal area (Fig. 1, *a*). In the dentate gyrus, more stable hippocampal area, almost 5-fold increase in the

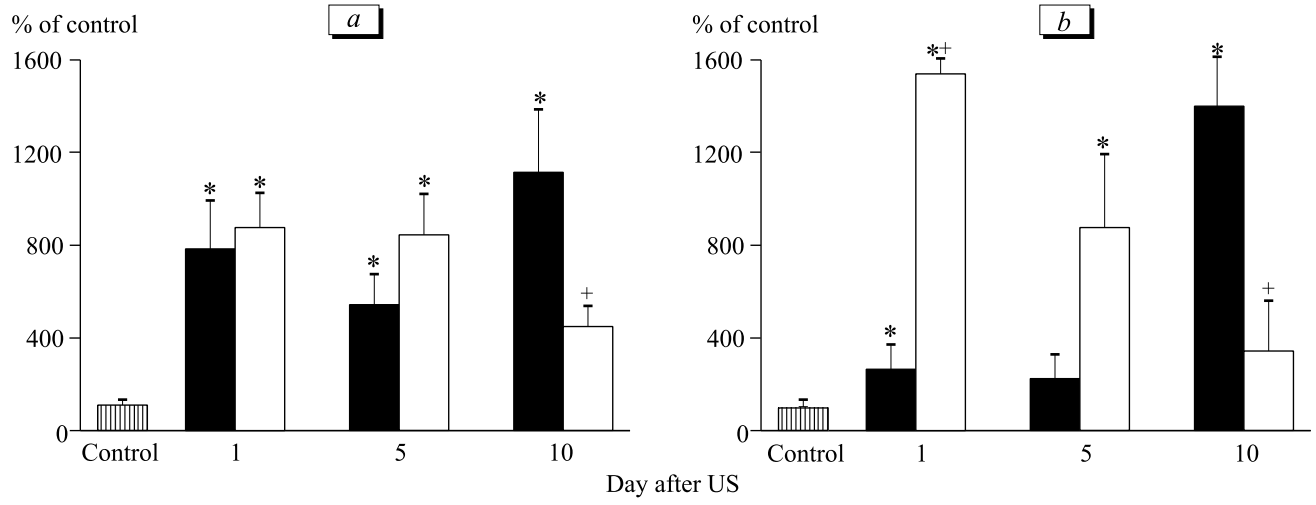

Fig. 1. Changes of c-Fos immunoreactivity in the hippocampus of rats subjected (light bars) and not subjected to HP (dark bars) followed by US. *a*) CA1; *b*) dentate gyrus. Here and in Fig. 2, 3: $p<0.05$ in comparison with: *controls; +rats without HP.

expression of c-Fos was detected in the early period (1 day) after US and persisted for 10 days (Fig. 1, *b*). A similar but less pronounced pattern was seen in layer V of neocortex (Fig. 2, *b*). In layer II of neocortex, c-Fos expression increased several times in the early period (853% of control on day 1), remained significantly elevated after 5 days, and returned to baseline levels by day 10 (Fig. 2, *a*). In hypothalamic PVN, different changes in the level of immunoreactive c-Fos were revealed in large- and small-cell parts of PVN. Specifically, persistent 5-11-fold increase in the number of c-Fos-immunopositive cells was revealed in large-cell part of PVN at all time points after US (Fig. 3, *a*), whereas in small-cell part significant increase was detected only at delayed terms (10 days), but it reached 1400% of control (Fig. 3, *b*). Thus, the development of depressive state was associated with stable overexpression c-Fos in all studied regions of rat brain except for layer II of the neocortex persisting at least until day 10, which indicated disturbances of the wavy dynamics of c-Fos expression typical of early genes products. The maximal amplitude of c-Fos overexpression was detected in delayed period in small-cell hypothalamic PVN, the main neurosecretory center regulating the pituitary-adrenocortical system. In this region, c-Fos regulates the production of neurohormone corticoliberin, the key activator of this hormonal system. Therefore c-Fos overexpression detected by us can be a molecular mechanism of pathological corticoliberin hyperproduction and, consequently, hyperfunction of the pituitary—adrenocortical system in depression.

In rats subjected to HP, US did not induce the development of post-stress depressive-like state [6].

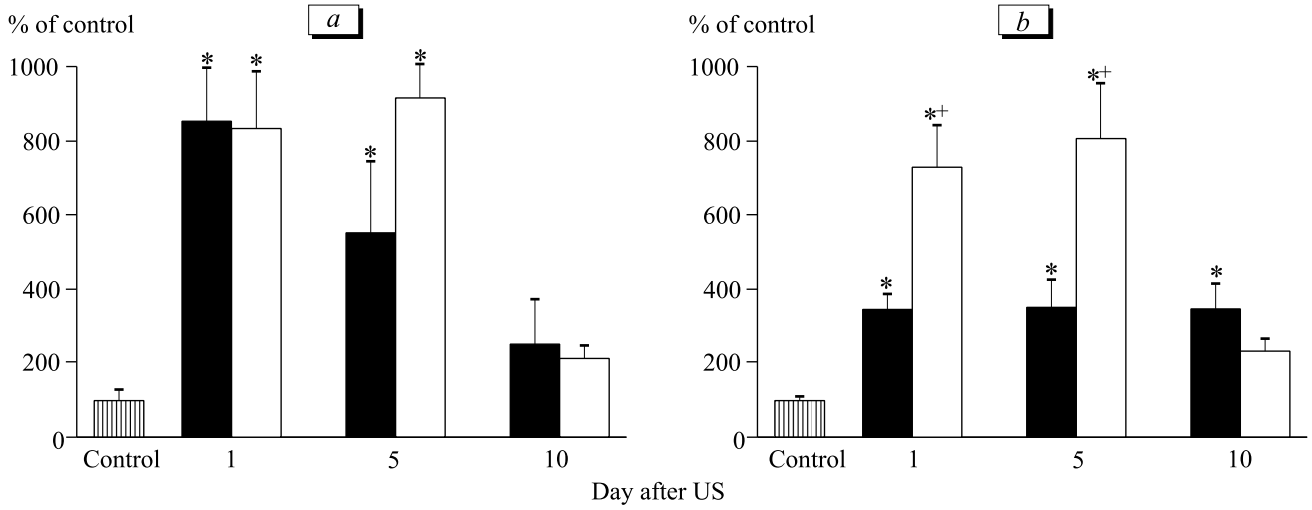

Fig. 2. Dynamics of c-Fos expression in rats subjected (light bars) and not subjected to HP (dark bars) followed by US. *a*) layer II; *b*) layer V.

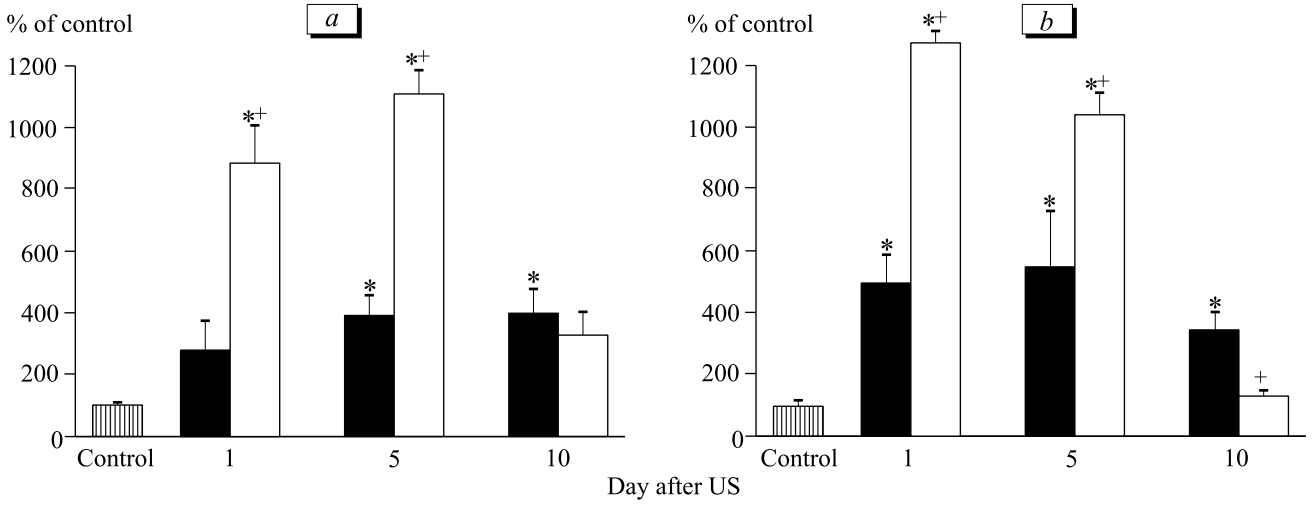

Fig. 3. Dynamics of c-Fos expression in rats subjected (light bars) and not subjected to HP (dark bars) followed by US. *a*) large-cell part; *b*) small-cell part.

However, the c-Fos expression pattern in response to stress in the studied brain regions was considerably modified. A sharp increase in the amplitude of c-Fos expression was observed at early terms (days 1-5) in the hippocampus, neocortex, and hypothalamic PVN of rats subjected to HP and its delayed expression was leveled. For instance, the early wave of c-Fos expression reached 1100% in CA1 and 1270% in the dentate gyrus of the hippocampus (Fig. 1). By day 10, the level of c-Fos immunoreactivity tended to normal. A similar pattern was observed in the neocortex (Fig. 2) and small-cell part of hypothalamic PVN (Fig. 3). In large-cell part of PVN, delayed expression of this factor after HP was leveled (Fig. 3, *a*).

Thus, the development of depressive-like state in rats leads to sustained long-term increase in c-Fos expression in the hippocampus, neocortex, and hypothalamus, this increase is most pronounced in delayed post-stress period in small-cell PVN, the neurosecretory corticoliberinergic hypothalamic center. The antidepressant effect of HP is accompanied by an increase in early c-Fos expression and leveling of its delayed overexpression in the studied brain regions. Prevention of the increase in c-Fos expression in delayed period in small-cell hypothalamic PVN promotes normalization of corticoliberin expression and prevents its abnormal hyperproduction. Enhanced expression of c-Fos in the early period can also contribute to the antidepressant effects of HP via stimulation of the transcription of many pro-adaptive target genes. This is consistent with published data that up-regulation of c-Fos plays an important role in the development of neuronal tolerance against severe hypoxia and ischemia [8,10]. The present findings extend our understanding of the functional roles of c-Fos suggesting its involvement in the mechanisms underlying induction of depressive disorders and their correction.

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