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Expression of the Neurotrophin BDNF in the Hippocampus and Neocortex in Rats during Formation of a Poststress Anxiety State and Its Correction by Hypoxic Postconditioning

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Quantitative immunohistochemical studies of changes in the expression of the neurotrophin BDNF (brain-derived neurotrophic factor) in the hippocampus and neocortex were performed in 24 male Wistar rats as they developed a poststress anxiety state in an experimental model of post-traumatic stress disorder and during correction of this condition by hypoxic postconditioning (PostC). The anxiety state was induced by combined psychoemotional stress (restraint stress, forced swimming, ether stress, and, after seven days, repeated restraint, i.e., restress). Correction of the anxiety state in the rats was with hypoxic postconditioning – three sessions of moderate hypobaric hypoxia (360 mmHg, 2 h). Formation of the anxiety state was accompanied by a significant reduction in the content of immunoreactive BDNF in the dorsal (CA1) and ventral (dentate gyrus) hippocampus and the neocortex, while hypoxic PostC led to partial (hippocampus) and complete (neocortex) restoration of BDNF expression. The results provided evidence that neurotrophic factors, particularly BDNF, appear to play an important role in the pathogenesis of anxious-depressive disorders and the realization of the proadaptive and neuroprotective actions of hypoxic PostC.

Keywords: hippocampus, neocortex, post-traumatic stress disorder, BDNF, hypoxic postconditioning.

The neurotrophin BDNF (brain-derived neurotrophic factor) plays an important role in the growth, development, and functioning of cerebral neurons, and in their survival, and in neuronal plasticity [8]. Furthermore, BDNF is involved in the regulation of such important processes as learning, memory, and reactions to stress [9, 11]. Data have also been obtained on the possible role of BDNF in the development and treatment of pathological anxious-depressive states [3], though this question has received insufficient study. Because of the increasing incidence of anxious-depressive disorders and the efficacy of pharmacotherapy with existing antidepressants, there is a clear need to refine our understanding of the pathogenetic mechanisms of these diseases and to develop new, including non-drug-based, means of correcting them. One such new effective means is provided by hypoxic postconditioning (PostC) [1], which has a powerful anxiolytic action, correcting formation of the anxiety state. The molecular mechanisms of hypoxic PostC have as yet received virtually no study, though there is great relevance in investigating them. Thus, the aim of the present work was to study the possible involvement of the neurotrophin BDNF both in the formation of anxious pathological states and in their correction by hypoxic PostC.

Materials and Methods

Quantitative immunohistochemical studies were performed to measure BDNF expression in the hippocampus and neocortex of rats during development of an anxiety state in a model of post-traumatic stress disorder (PTSD) and after use of PostC with hypobaric (altitude) hypoxia, which has anxiolytic actions. Experiments were performed

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on 24 male Wistar rats weighing 200–230 g. Experiments were performed in compliance with the requirements laid out in Decree No. 724 of the USSR Ministry of Higher Education (1984), "Regulations for Studies Using experimental Animals," as well as the directives of the Council of the European Community (86/609/EEC) on the use of animals for experimental studies. The anxiety state was induced in rats using a "stress–restress" experimental model of post-traumatic stress disorder [6]. Animals were subjected to restraint stress (120 min), forced swimming (20 min), and, after a 15-min break, were exposed to ether until they developed immobility. After seven days, the animals were subjected to restress (restraint for 30 min), which led to formation of an anxiety state. Hypoxic PostC was used to correct the anxiety state, using an original regimen previously demonstrated to be effective [1]. PostC was by three exposures to moderate hypobaric hypoxia (360 mmHg, 2 h) in a flow-type barochamber with 24-h intervals during the first three days after restress. The rats were divided into the following groups (each of six animals): PTSD – animals subjected to "stress–restress;" PTSD + PostC – animals subjected to "stress–restress" with subsequent correction of pathology with hypoxic PostC; controls – intact animals; control-R – rats subjected only to restress. As restress is a weak nonpathogenic stressor in animals which had not experienced traumatic stress, the control-R group was included in the study to control for the adaptive (not pathological, as in the PTSD group) response to restress. Animals were decapitated four days after restress. Brains were rapidly extracted and the segment including the hippocampus and adjacent parietal neocortex was excised. Specimens were fixed with the molecular fixer FineFIX (Milestone, Italy). Preparations were then subjected to standard procedures for washing, dehydration, passage through portions of xylene, and embedding in paraffin. A microtome was then used to cut series of brain sections of thickness 7 μm in the frontal plane. Sections were used for quantitative immunohistochemistry to study changes in BDNF expression in the neocortex and the dorsal (CA1) and ventral (dentate gyrus) hippocampus. After deparaffination, rehydration, and thermal antigen demasking, sections were incubated overnight at 4°C with primary rabbit polyclonal antibodies to BDNF (Santa Cruz, USA, diluted 1:100), which was followed by use of an avidin-biotin detection system (Vector Laboratories, Inc., UK) and diaminobenzidine for visualization. Quantitative analysis of neuron immunoreactivity was performed using a microimage analysis system consisting of an Olympus CX31RBSF light microscope (Optical Systems, Germany), a ProgResCT1 digital camera (Jenoptik, Germany) and a computer running the VideoTest Morphology 5.2 program (VideoTest, Russia). Numbers of immunoreactive cells were counted in hippocampal field CA1 and the dentate gyrus (on areas of length 400 μm) and in the neocortex (on areas of size 350 × 400 μm) at a level of –2.80 mm from the bregma. The statistical significance of the results was assessed using the Mann–Whitney U test (Statistica 7.0, StatSoft, Inc.). Differences between groups were regarded as significant at $p \le 0.05$. All results for experimental groups are presented as arithmetic means ± standard error of the mean (SEM).

Results

The action of pathogenic stress (the PTSD group) led to a significant decrease in immunoreactivity, which was apparent as a clear reduction in the intensity of the immunohistochemical reaction in the hippocampus and neocortex (Fig. 1, *c*, *g*) as compared with the analogous areas in control rats (see Fig. 1, *a*, *e*).

Quantitative analysis showed that the numbers of BDNF-immunopositive cells in the PTSD group decreased significantly, by 60–70%, in both the ventral (dentate gyrus – Fig. 2, *a*) and dorsal (CA1 – see Fig. 2, *b*) areas of the hippocampus, as well as in the neocortex (see Fig 2, *c*). In the mildly stressed animals (the control-R group), there were no significant differences from controls on either qualitative assessment (see Fig. 1, *b*, *f*) or quantitative analysis (see Fig. 2). The PTSD + PostC group (PostC after "stress–restress") showed an increase in the number of BDNF-immunopositive cells (see Fig. 1, *d*, *h*) to a level significantly different from that in the PTSD group in the dentate gyrus and neocortex, the number of these cells reaching control values in the neocortex (see Fig. 2).

Discussion

PTSD is one of the most widespread anxious-depressive diseases. It is characterized by a sharp increase in anxiety and a complete disruption of adaptation. As far as is currently known, impairments to the expression of a variety of genes and their products, including the neurotrophin BDNF, in cerebral neurons play an important role in the pathogenesis of this disease. A decrease in BDNF mRNA expression in the hippocampus has been described in PTSD patients [5, 11]. This is consistent with our data, reported here, showing a reduction in BDNF protein content in the hippocampus and neocortex on formation of a model analog of PTSD in rats. In contrast to other neurotrophins, BDNF has been shown to have a significant influence on synaptic transmission, with a role in the formation of long-term potentiation; there is also a relationship between its expression and secretion on the one hand and neuron activity on the other [7]. Binding of BDNF to receptors (TrB^{FL}) leads to activation of the phospholipase Cγ signal pathway, the PI-3 kinase and Akt kinase pathways, and the MEK-MAPK (mitogen-activated protein kinase) signal pathway [12]. MAPK-mediated phosphorylation of the vesicular protein synapsin is regulated by presynaptic glutamate and gamma-aminobutyric acid (GABA) release [2]. Thus, the decrease in BDNF content seen in PTSD may lead to decreased release of these two transmitters. In the case of inhibitory mediators, this can lead to decreases in $GABA_A$ receptor activity and, consequently, to the increased anxiety typical of PTSD and other disorders of this group [10]. In addition, patients with PTSD are known to develop

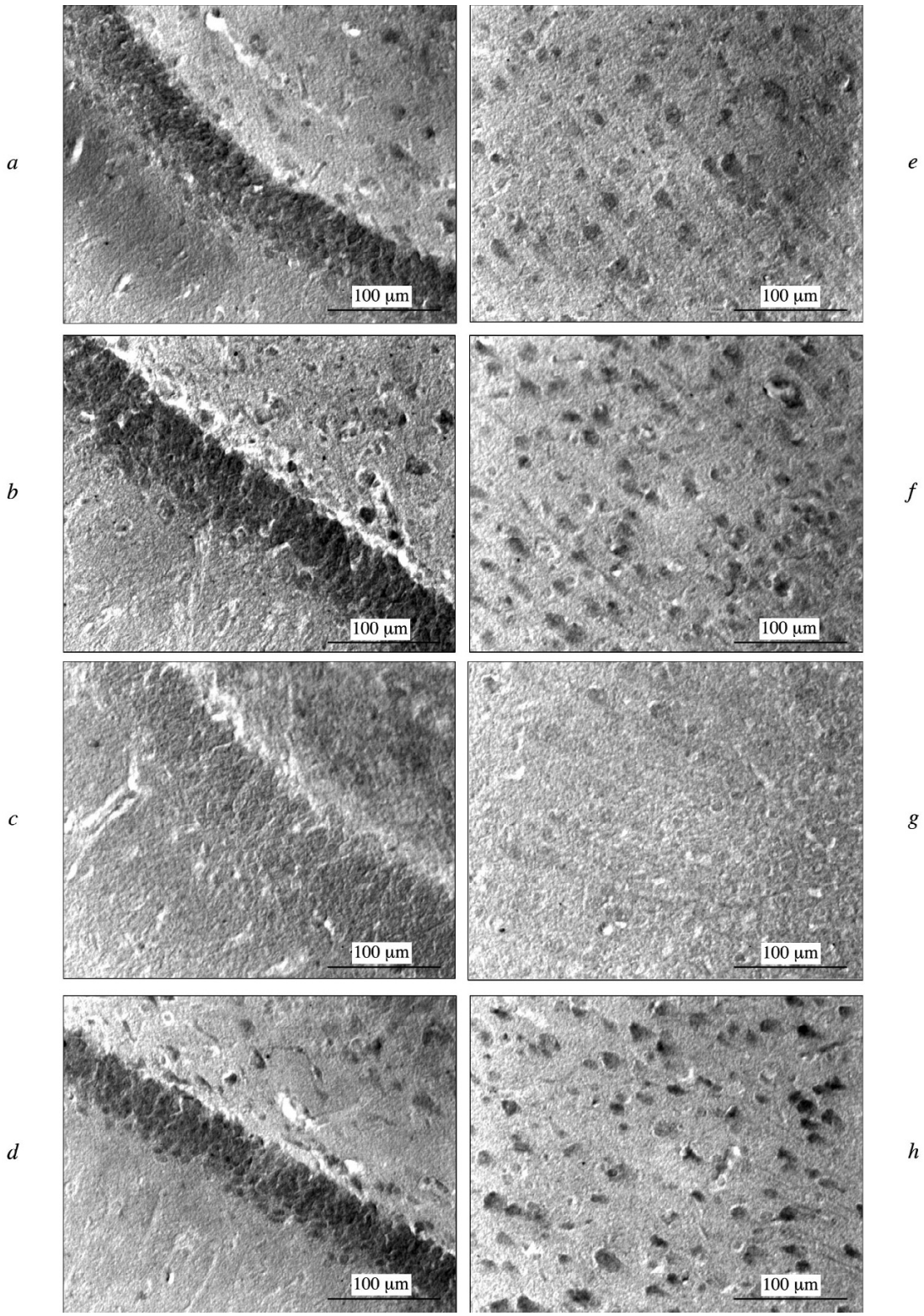

Fig. 1. The dentate gyrus (*a–d*) and the neocortex (*e–h*) in rats after modeling of post-traumatic stress disorder (PTSD) and PTSD plus use of postconditioning (PostC) with hypobaric hypoxia. *a*, *e*) Controls (intact animals); *b*, *f*) control-R (rats subjected only to re-stress); *c*, *g*) PTSD; *d*, *h*) PTSD + PostC. Immunohistochemical reaction for the neurotrophin BDNF.

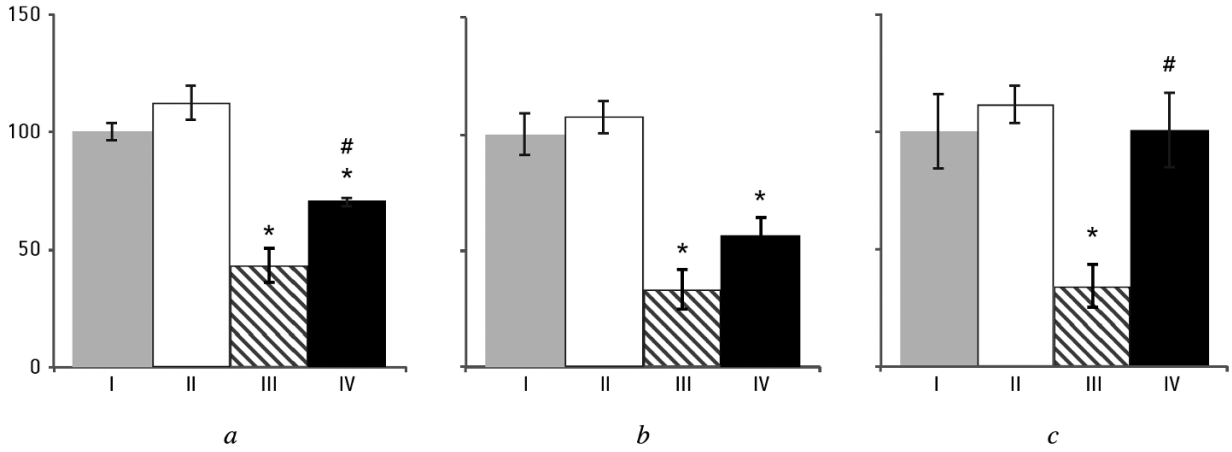

Fig. 2. Proportions of BDNF-immunopositive cells in rats in the dentate gyrus (*a*), hippocampal field CA1 (*b*), and neocortex (*c*). The abscissas show groups of rats: I) intact animals (control group); II) control-R group – animals subjected only to restress; III) after modeling of post-traumatic stress disorder (PTSD); *IV*) PTSD + use of postconditioning (PostC) with hypobaric hypoxia; the ordinates show proportions of cells (%) compared with controls, taken as 100%. *Significant differences from control group; #significant differences between PTSD + PostC and PTSD, $p \leq 0.05$.

hippocampal atrophy, presumably due to the toxic action of glucocorticoids, high contents of which are achieved in conditions of pathogenic stress [13]. This may also be due to a decreased BDNF content, resulting in a reduction in the resistance of hippocampal neurons to harmful factors and their potential for neuroplasticity [4]. The results reported here suggest that decreases in BDNF expression in the hippocampus and neocortex constitute an important pathogenetic mechanism of PTSD, as evidenced by our data on PostC animals. The protective effect of PostC in the model of PTSD was found to be accompanied by stimulation of BDNF expression, leading either to partial (hippocampus) or complete (neocortex) recovery of the content of this neurotrophin from the significantly reduced level occurring in PTSD. This probably promotes stimulation of neuroplasticity processes in these vulnerable brain formations, also normalizing the impaired release of transmitters, compensating for the negative effects of pathogenic stress. Thus, the studies reported here in an experimental rat model showed that decreases in the BDNF content in the hippocampus and neocortex were accompanied by the formation of pathological poststress anxiety, while the therapeutic and anxiolytic effects of hypoxic PostC are linked with stimulation of the expression of this neurotrophic factor in susceptible brain structures.

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