GENETICS

Effect of Chronic Emotional and Pain Stress on Histone H3 Phosphorylation in the Hippocampus of Rat Strains with Different Excitability of the Nervous System

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Long-term effects of chronic emotional and pain stress on histone H3 phosphorylation by serine 10 in hippocampal CA3 neurons were examined 24 h, 2 weeks, and 2 months after termination of the stress procedure in 2 rat strains differing by excitability of the nervous system. The low excitable rats with high threshold (HT) of excitability were characterized by a high baseline level of histone H3 phosphorylation in comparison with the high excitable rats with low threshold (LT) of excitability. The long-term emotional and pain stress significantly changed the number of positive immune cells in highly excitable rats: this parameter increased in 24 h and 2 weeks after the stress, but returned to the control level in 2 months. In contrast, stress did not affect histone H3 phosphorylation in low excitable rats. Thus, long-term (up to 2 weeks) changes in histone H3 phosphorylation were reveled in rat hippocampal CA3 neurons, which depended on genetically determined functional status of the nervous system.

Key Words: *stress; hippocampus; phosphorylation; histone H3; high- and low-excitable rat strains*

Epigenetic modifications of chromatin in nerve cells are important tools to control such vital cerebral functions as learning and memory [6]. Interaction of genomic DNA and histone proteins in chromatin plays the key role in gene expression and is largely determined the post-translational modifications of histones; phosphorylation of histone H3 is of particular importance [8]. Phosphorylation of histones results from binding of negatively charged phosphate groups to these proteins (most frequently, to serine in the histone tails), which neutralizes their basic charge and diminishes their affinity to DNA triggering transcriptional activation of the genes. It was demonstrated that phosphorylation of serine 10 in histone H3 could be induced in the hippocampal neurons by the stressors [4,5], although the role of this modification in implementation of the mechanisms of trauma- or stress-related memory is still unknown. An important factor affecting these processes is genetically determined functional activity of the nervous system [1].

This work was designed to study the long-term effects of chronic emotional and pain stress (CEPS) on the degree of phosphorylation of serine 10 in histone H3 of the hippocampal neurons in the rats of two strains selected by excitability of their nervous system.

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MATERIALS AND METHODS

The experiments have been carried out on mature 5-month-old male rats of two strain selected by high (HT) and low (LT) threshold of the nervous system excitability [2]. The rats were bred under standard vivarium conditions in Department of Higher Nervous Activity Genetics with unrestricted food and water diet. All experimental procedures were carried out in accordance to European Economic Community Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. The protocols of experiments were approved by Ethics Committee of I. P. Pavlov Institute of Physiology (RAS).

The experimental rats from both strains (n=5–7) were subjected to CEPS according to K. Gekht stochastic protocol [3]. During 15 days, the experimental rats were placed one by one in a transparent case with electrified grid floor. The rats were also exposed to 10-sec light impulses, which were either reinforced with electric current (2.5 mA, 4 sec, n=6) or non-reinforced (n=6). The interstimulus time was 1 min. The control groups comprised the intact rats (n=5–7 in each group). In 24 h, 2 weeks, and 2 months after termination of CEPS, the rats of control and experimental groups were narcotized with urethane (0.3 ml/100 g body weight) and subjected to transcardial perfusion with physiological saline and 4% paraformaldehyde solution, thereafter the rats were decapitated. The brain was isolated and subjected to routine histological processing to prepare a series of paraffin-embedded thin tissue sections (7 μ). The degree of histone H3 phosphorylation was assessed with immunohistochemical method using the primary antibody against p-Histone H3 Ser10-R (1:200, Abcam) and secondary biotinylated antibody from Quick Kit (Vectastain). The reaction with primary antibodies was visualized with DAB Peroxidase Substrate Kit from Vector. For visualization of nuclear membranes, the sections were post-stained with Mayer's hematoxylin. The percentage of immunopositive nuclei was assessed relatively total number of nuclei in CA3 hippocampal field with the corresponding coordinates [7]. The specimens were examined under a Mikromed-3 light microscope equipped with CCD-camera controlled by VideoTest-FISH and ImageBase software. The data were analyzed statistically using Statgraphics PLUS 5.0 software and Student's t test.

RESULTS

The low excitable rats (HT strain) demonstrated greater baseline level of histone H3 phosphorylation assessed with the number of p-Histone H3 Ser10-R-immunopositive cells in comparison with the high excitable rats (LT strain). CEPS affected histone H3 phosphorylation only in LT rats in 24 h and 2 weeks after the procedure, but not in 2 months (Table 1, Fig. 1).

This observation attest to dependence of the baseline level of serine 10 phosphorylation in H3 histone of hippocampal neurons and its susceptibility to CEPS on genetically determined excitability of the nervous system.

In HT rats, CEPS increased excitability of the nervous system to electric current, suppressed long-term post-tetanic potentiation in the hippocampus for 2 months, and induced severe chronic (up to 6 months) depression-like state, which advanced the post-stressor behavioral modifications in this rat strain as a model of the post-traumatic stress disorder [1,3]. Comparing those previous data with this study negates a significant role of histone H3 phosphorylation in manifestation of the post-traumatic stress-induced disorder in HT (low excitable) rats. Our previous studies showed that this disorder could be affected by epigenetic and morphological alterations in the hippocampus such as chromatin decompaction, persistent decrease in the content of methyl-cytosine binding protein (MeCP2),

TABLE 1. Total Percentage of p-Histone H3 Ser10-R-Immunopositive Nuclei of Pyramidal Neurons in Hippocampal CA3 Field in HT and LT Rats at Various Terms after Termination of CEPS (X±m)

Strain	Group	Time after CEPS termination		
		24 h	2 weeks	2 months
HT	Control	0.49±0.04	0.45±0.04	0.44±0.04
	Experimental	0.47±0.05	0.46±0.04	0.45±0.03
LT	Control	0.14±0.04*	0.069±0.040*	0.095±0.034*
	Experimental	0.51±0.042^{+}	0.36±0.03^{+}	0.058±0.034

Note. p<0.05 compared to *corresponding value in other rat strain and $^{+}$control value in the same strain.

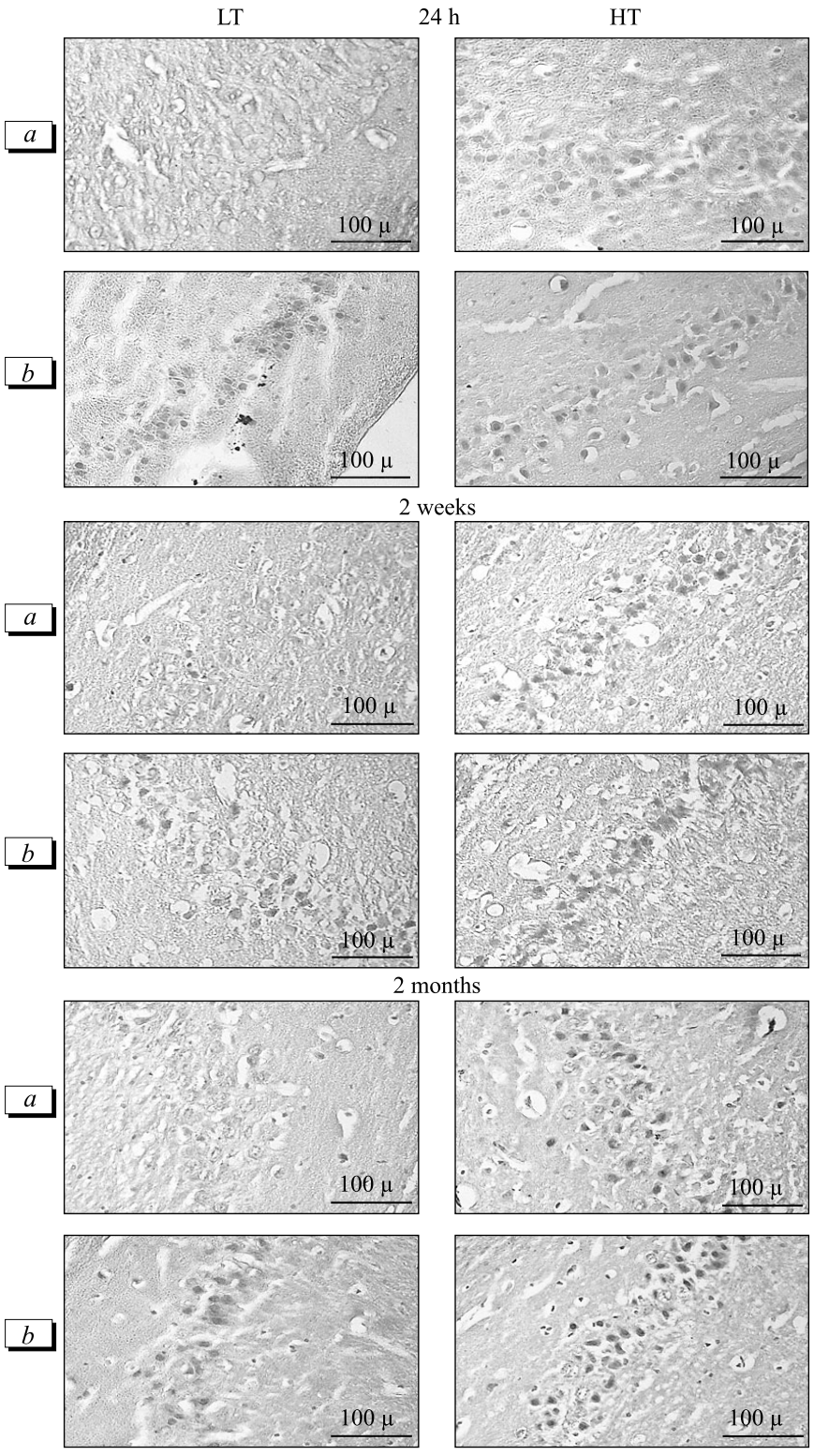

Fig. 1. Immunopositive nuclei of hippocampal CA3 pyramidal neurons of HT (right) and LT (left) rats subjected to CEPS. Shown are the control (*a*) and experimental (*b*) histological sections. Immunohistochemical staining was carried out with primary antibodies against phosphorylated serine 10 in Histone H3 (1:200, Abcam) and with secondary universal biotinylated antibody (Quick Kit, Vectastain).

up-regulation of acetylation of H3 and H4 histones accompanied by significant increase in the amount of nuclear RNA and possible death of hippocampal CA3 neurons observed for 2 months [1].

It has also been shown that in LT (high excitable) rats, CEPS triggered the obsessive-compulsive stereotypic movements (jactacio capitis, the rhythmic nodding convulsions) retained for up to 6 months [3]. These rats can be used for modeling obsessive-compulsive disorder (OCD) in humans (F42.1 according to ICD-10 classification) previously referred to as obsessive-compulsive neurosis. In the preset study,

we have revealed long-term changes in histone H3 phosphorylation in hippocampal CA3 neurons determined by enhanced genetically-dependent activity of the nervous system. The data obtained suggest correlation between histone phosphorylation in hippocampal neurons with CEPS-induced behavioral modifications. Moreover, they showed that elevated excitability of CNS is a risk factor leading to OCD in response to chronic stress. In addition to histone H3 phosphorylation, other epigenetic and cellular and molecular mechanisms provoking OCD could be the persistent morphological alterations in the hippocampus (significant decrease in the number of neurons), which appear in two months after termination of stress exposure concurrently with the epigenetic modifications of chromatin in hippocampal CA3 neurons manifested by a decrease in the content of methyl-cytosine binding protein (MeCP2) and upregulation of H3 histone acetylation [1].

Thus, genetically determined level of CNS excitability can shape peculiarities of the long-term behavioral modifications and specificity of associated cell, molecular, and epigenetic alterations in hippocampal neurons.

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