GENETICS

Effect of Long-Term Mental and Pain Stress on the Dynamics of H4 Histone Acetylation in Hippocampal Neurons of Rats with Different Levels of Nervous System Excitability

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Priority data on specific effect of long-term mental and pain stress on the dynamics of H4 histone acetylation in the pyramidal neuron nuclei of the hippocampal CA3 field in rats selected by the nervous system excitability were obtained using a comparative genetic method. The congruency of long-term poststress modification of H4 histone acetylation in neurons of rats with high threshold excitability and behavioral changes intrinsic of these rats suggest that increased acetylation of H4 histone together with changes in heterochromatin conformation play a triggering role in long-term modifications of genome expression underlying the pathogenesis of posttraumatic stress disorders and other psychogenias.

Key Words: *hippocampus; acetylation; H4 histone; nervous system excitability; posttraumatic stress disorders*

Long-term mental and pain stress (LMPS) causes deep changes at the biochemical, physiological, and behavioral levels in adult rats with different levels of nervous system excitability. In animals with low excitability these changes persist for up to 6 months after the exposure and simulate the symptoms of posttraumatic stress-induced disorders in humans: mental retardation and depression [1,4]. These disorders can be caused by long-term modification of expression of genes determining the basic structural and functional peculiarities of neurons, which is confirmed by lasting differential modifications in the content of heterogeneous nuclear RNA in the hippocampal CA3 field of rats selected by nervous system excitability [3]. Acetylation of histones in nucleosomes plays an important role in the regulation of gene expression [7].

We studied the effect of LMPS on the dynamics of H4 histone acetylation in the hippocampal CA3 field neurons in rats differing by the nervous system excitability.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats aged 5 months, selected by threshold excitability of the nervous system: HT (with high threshold) and

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LT (with low threshold excitability rats). The animals were exposed to LMPS daily for 13 min for 15 days according to a stochastic scheme [5]. The animals were placed into a transparent cage with electrified grid floor; 12 light stimuli (10 sec duration, 1 min interval) were presented: 6 stimuli were not supported and 6 were supported with electric shock (2.5 mA, 4 sec) with 0.5 probability of support. According to the stochastic scheme, new current and light combinations were presented every day, which ruled out the development of a conditioned reflex. The reaction of animals consisted in jumping and vocalization. This exposure simulated a situation promoting the development of strong long-lasting behavioral stress in the animal. Control HT and LT rats were not exposed to stress.

The rats were decapitated 24 h and 2 weeks after the end of exposure. The brain was fixed in a mixture of 4% paraformaldehyde and phosphate buffer (pH 7.5) with subsequent treatment in ascending alcohols and methylbenzoate and embedding in paraffin. The sites of antibody binding to H4 histone acetyl groups in the nuclei of hippocampal CA3 pyramidal neuron were detected by ABC immunoperoxidase method using DAB-ABC-Elite Kit BA-1300 (mouse/rabbit/goat, Vector Laboratories). After antigen demasking endogenous peroxidase activity was inhibited by 30-min incubation with 0.3% H_2O_2. The sections were successively incubated with normal rabbit serum, first pan-Acetyl (C4) antibodies to H4 histone acetyl groups, biotinilated antibodies, ABC complex (rabbit-anti-goat IgG), and then stained using DAB kit (Vector Laboratories) and poststained with hematoxylin. The preparations were embedded in DPX medium and analyzed under a light microscope.

Each experimental group comprised at least 5 animals. No less than 100 cells per preparation were counted in a series of sections from one animal.

The data were statistically processed using StatgraphicsPlus 5.0 software.

RESULTS

Evaluation of H4 histone acetylation (the effect of this histone is directed to facilitation of the transcription process) showed that 24 h after LMPS the number of antibody binding sites in rats with different threshold excitability did not differ from the control (Table 1). However, 2 weeks after LMPS the level of H4 histone acetylation increased significantly, but only in HT rats. Acetylation did not change in LT rats by this term. Hence, a strain-specific relationship between the poststress dynamics of histone acetylation and genetically determined status of the nervous system and time elapsed after the end of exposure was demonstrated.

Hence, long-term poststress changes in animal behavior and content of nuclear RNA in hippocampal neurons, as well as epigenetic structural modifications of heterochromatin in HT rats [2-4] are paralleled by delayed changes in the degree of H4 histone acetylation. Acetylation of nucleosomal histones plays an important role in chromatin compactization, interactions of transcription factors with DNA, and hence, in the regulation of gene expression. DNA strand in some nucleosomes makes two turns around the histone octamer, forming a compact structure consisting of 2 histones of each type (H2A and H2B) and an H3-H4 pair. The histone molecules are characterized by irregular distribution of the main amino acids. The central sites of the molecules enriched with positively charged amino groups are responsible for histone interactions and formation of the nucleosomal core. The N-terminal amino groups of the terminal sites are reversibly acetylated by specific lysine residues and are exposed from the nucleosome. The process of histone acetylation modulates chromatin compactization and is potentially important for protein functions in the octamer. Histones are in fact minor proteins; acetylation sites in H3 and H4 are highly conservative and are unique for providing the functional essential cell growth and vital activity. Chromatin mobility is attained due to effects histone acetyltransferases and deacetylases. As a result, DNA interactions with proteins are attenuated and reactions with some transcription factors become possible. Hence, the structural role of histones is obvious: they provide not only specific packing of chromosomal DNA, but are also involved in transcription regulation. Histone acetylation is an epigenetic factor providing long-term maintenance of specific transcription activity [7]. Disturbances in

TABLE 1. Number of Binding Sites for Antibodies to H4 Histone Acetylation Groups in Nuclei of Hippocampal CA3 Pyramidal Neurons in HT and LT Rats at Different Terms after LMPS ($M \pm m$)

Strain		Group	
		24 h	2 weeks
HT	Control	2.55±0.32	2.08±0.05
	Experiment	2.76±0.21	2.72±0.11*
LT	Control	2.38±0.31	1.59±0.10
	Experiment	2.99±0.24	1.87±0.09

Note. *$p<0.05$ compared to the control.

histone acetylation process are associated with some diseases, including those characterized by mental retardation (Rubinstein-Taybi syndrome), which is also intrinsic of posttraumatic stress disorders [6].

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