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Effect of NMDA Receptor Activity on Histone H3 Methylation and Its Asymmetry in the Hippocampal Pyramidal Neurons of Rats with Different Excitability Thresholds under Normal and Stress Conditions

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Abstract—Process of methylation of histone H3 for lysine 4 (H3K4) was studied in hippocampal pyramidal neurons of rats—intact and submitted to emotional–pain stress with active and inactivated channels of NMDA-receptors with taking into account the interhemisphere lateralization and in connection with the genetically determined level of excitability of the animals’ nervous system. There were revealed interstrain differences in the basal level of the H3K4 methylation whose direction depends on structural-functional peculiarities of hippocampal fields and lateralization. Under action of stress the direction of the observed changes in the degree of the H3K4 methylation depended on the functional states of channels of NMDA-receptors. On the background of active receptors the proportion of immunopositive cells predominantly increased. In the CA1 field this change was not connected with excitability and lateralization, whereas in the CA3 field it had a complex character and depended on these two factors. At inactivation of channels of NMDA-receptors the portion of immunopositive nuclei as a result of the stress action, on the contrary, predominantly decreased; interstrain specificity of these changes was connected with lateralization, while its direction in different hippocampal fields was different. Action of the short-time emotional–pain stress did not lead to a change of shape of interhemisphere at active state of receptors, whereas at inactivation of receptors it changes depending on the structural-functional organization of hippocampus and on excitability of the nervous system.

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INTRODUCTION

At present, the role of epigenetic mechanisms under normal and pathological conditions of the central nervous system is one of the most actual points of neurogenetics and behavior genetics [1, 2]. Studies on involvement of these mechanisms in responses to stress [3, 5] are of great interest. Among the epigenetic markers, of particular interest is the histone H3 lysine 4 methylation associated with active transcription [2, 6–10] and implicated in response to stress [4, 11], although so far its role in emotional–painful stress has not been investigated. It is noteworthy that this modification was found in c-fos and Bdnf gene promoters whose significant role in neural plasticity associat-
ed with learning, memory, and adaptive response was clearly shown [12–14].

Some kinds of stress are known to affect several epigenetic DNA and histone protein modifications [11] with participation of NMDA receptors [15, 16]. The NMDA-sensitive glutamate receptors mediating Ca\(^{2+}\) influx represent one of the key components of the stress signaling cascade in glutamatergic structures, specifically hippocampus, the brain structure extremely sensitive to stress. It seems actual to explore the effect of their activity on the level of histone H3 lysine 4 methylation in hippocampal neurons.

It is to be emphasized that there is evidence of a high heterogeneity of physiological activity parameters in different hippocampal fields as well as genetic expression gradients along the hippocampal dorso-ventral axis [17, 18]. Besides, the ever increasing number of studies demonstrate interhemispheric asymmetry of some hippocampal functional parameters in animals and humans [19–21], although its effect on manifestation of epigenetic modifications in different hippocampal fields has not been studied until now.

Thus, the goal of this study was to assess the level of histone H3 lysine 4 methylation in pyramidal neurons of three hippocampal fields (CA1–CA3) in intact and stress-exposed rats with active and inactive NMDA receptor channels as related to lateralization, separately in the left and right hemispheres, and the genetically determined excitability level of the nervous system [22, 23].

MATERIALS AND METHODS

Animals. The experiments were carried out on the HT (high threshold) and LT (low threshold) rat strains selected from the outbred Wistar population by the nervous system excitability threshold at the Laboratory of Genetics of the Higher Nervous Activity, Pavlov Institute of Physiology, Russian Academy of Sciences [22, 23]. The animals were kept in vivarium under a standard 12/12 h light/dark regime on a standard ad libitum diet. The adult 5-month old males were used. In each strain, animals were separated in four groups: (1) rats with active NMDA receptors (intact control), (2) rats injected with ketamine, the uncompetitive antagonist of NMDA receptors (ketamine), (3) rats with active NMDA receptors exposed to emotional-painful stress (stress), (4) rats with ketamine-inactivated NMDA receptors exposed to emotional-painful stress (ketamine+stress). In total, 32 rats were used in the experiments, 8 groups by 4 rats in each.

Injection of the uncompetitive NMDA receptor antagonist ketamine. Ketamine was injected intraperitoneally, 10 mg/100 g of the animal’s body weight.

Exposure to emotional-painful stress. The exposure was performed 1 h after the ketamine injection. The animal was placed into a lucent chamber with a lattice floor and an incandescent lamp. For 13 min, 12 light signals (10 s each) were fed into a chamber, 6 reinforced and 6 non-reinforced with electric current (2.5 mA, 4 s), randomly distributed according to the Hecht’s scheme [24]. A half an hour after termination of the exposure to stress, the animals were sacrificed.

Procedure of transcardiac perfusion. Rats were immobilized by urethane injection (0.3 ml/100 g of body weight), and transcardiac injection was consecutively performed with saline with heparin and with 4% paraformaldehyde.

Histological treatment of brain tissue. After sampling, the material was additionally fixed overnight in 4% paraformaldehyde at 4°C, washed in phosphate buffer (pH = 7.6) for 1 h, dehydrated in an ascending ethanol series, infiltrated with methyl benzoate for 1 day and methyl benzoate/paraffin 1:1 for 1 h at 56°C, and embedded in paraffin (twice for 1 h at 56°C) with final formation of paraffin blocks. Then the frontal brain sections (7 μm) were cut on a microtome at the level—2.80 mm from bregma [25]. The sections were mounted on gelatin-covered object slides.

Immunohistochemical staining. Anti-histone H3 (di+tri methyl K4) primary antibody (Abcam), QuickKit (Vector) and DAB-kit (Vector) were used. After a standard protocol including deparaffination in xylene (two changes for 15 min) and rehydration in a descending ethanol series, infiltrated with methyl benzoate for 1 day and methyl benzoate/paraffin 1:1 for 1 h at 56°C, and embedded in paraffin (twice for 1 h at 56°C) with final formation of paraffin blocks. Then the frontal brain sections (7 μm) were cut on a microtome at the level—2.80 mm from bregma [25]. The sections were mounted on gelatin-covered object slides.