GENETICS

Effect of Prolonged Emotional and Pain Stress on the Content of Methylcytosine-Binding Protein MeCP2 in Nuclei of Hippocampal Neurons in Rats with Different Excitability of the Nervous System

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In rats with low excitability threshold of the nervous system demonstrating significant and persistent behavioral disorders under stress conditions, the content of methylcytosine-binding protein MeCP2 in neuronal nuclei of hippocampal field CA3 decreased over 2 weeks after long-term emotional and pain stress. It was hypothesized that protein MeCP2 triggers epigenetic changes in DNA that underlie "stress memory".

Key Words: *posttraumatic stress disorder; hippocampus; methylcytosine-binding protein; excitability; rats*

Study of the molecular and genetic mechanisms of delayed manifestations of posttraumatic stress disorder (PTSD) in animals with genetically determined characteristics of the nervous system allows us to evaluate individual variability in the reaction of various systems to extreme factors. This approach is of considerable importance for the diagnostics and therapy of PTSD in humans. Long-term emotional and pain stress (LEPS) is followed by a changes in instinctive behavior and learning of adult rats, which depends on genetically determined excitability of the nervous system. These reactions are accompanied by changes in hormonal and biochemical parameters that persists up to 2-6 months. Symptoms of PTSD in animals are observed under these conditions [5]. Qualitative characteristics of pericentromeric heterochromatin and heterogeneous nuclear DNA in hippocampal neurons of low-reactivity rats differ from normal over a long time after PTSD [2,4]. The epigenetic mechanisms of the delayed effect of stress are of particular interest in this respect. DNA methylation is now considered as a typical epigenetic process in eukaryotic cells playing a role in the regulation of gene expression and modulation of the state of chromatin, including mammalian CNS, not only during embryonic development, but also in adult brain [8]. DNA methylation is a mechanism underlying long-term changes in brain function and behavior after exposure to extreme factors.

Methylcytosine-binding protein (MeCP2) is a major transcription repressor protein of the methylcytosine-binding complex. This protein is also located in pericentromeric heterochromatin regions [12]. The LEPS-induced decrease in the area of

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heterochromatin can be related to conformational changes (*e.g.*, under the influence of MeCP2). There is no general agreement about the function of this protein in adult brain. However, the study of Rett syndrome in humans and animals suggests that MeCP2 plays a specific role in neurons [7] and is involved in the mechanisms of memory [6,13].

Here we studied the effect of LEPS on MeCP2 content in hippocampal neurons (CA3 field) of rats differing by excitability of the nervous system.

MATERIALS AND METHODS

Male Wistar rats aging 5 months were selected by excitability of the tibial nerve to electric current. The animals were divided into groups with high (HT, 2.5 V) and low excitability threshold (LT, 0.8 V). HT and LT animals significantly differed in excitability of the autonomic nervous system and CNS. Physiological, biochemical, morphological, and ethological characteristics of these animals were studied in details. Interlinear differences in stress reactivity at various periods after LEPS are of particular interest. Published data show that various symptoms of PTSD, including mental retardation and depression, persist over long time in LT rats (up to 2-6 months) [1,5].

LEPS was modeled daily for 15 days (13 min a day) [5]: the rats were places in a transparent chamber with an electrified grid floor and 6 non-reinforced and 6 reinforced (electric stimulation, 2.5 mA, 4 sec) light stimuli (10 sec) were presented at a 1-min interval. The probability of reinforcement was 0.5. The animals were daily exposed to a new combination of electric current and light stimulation. This situation led to the development of severe stress.

The animals were killed 24 h or 2 weeks after the end of treatment. The samples were fixed. MeCP2 content in hippocampal neurons was measured by the immunofluorescent method and DAB-ABC immunoperoxidase staining. Binding sites for specific antibodies against MeCP2 (goat polyclonal antibody MeCP2, Santa Cruz, dilution 1:200) were studied on neuronal nuclei. Experiments were performed with paraffin sections of the hippocampus (7 μ). Rhodamine-conjugated antibodies (donkey anti-goat IgG, Santa Cruz, dilution 1:200) served as secondary antibodies during immunofluorescence staining. Neuronal nuclei were identified by counterstaining with DAPI. The samples were embedded in Vectashield medium (Vector Laboratories) and examined under a MIKMED11 fluorescence microscope (Rhodamine filters, DAPI). Images of sections were obtained using a cX05 digital camera (Baumer Optronic) and analyzed on a computer (FISH Video-Test software). The DAB-ABC-immunoperoxidase method involved DAB-ABC-Elite Kit (mouse/rabbit/goat, Vector). Staining and counterstaining were performed with DAB kit (Vector) and hematoxylin, respectively. The samples were embedded in DePeX medium and examined under a light microscope.

Each experimental group consisted of at least 5 animals. Examination of serial sections from each animal included calculation of at least 25 cells in each section (not less than 100 cells per sample). The number of labels per nucleus was estimated in MeCP2-positive cells.

The results were statistically analyzed using StatgraphicsPlus 5.0 software.

RESULTS

Immunohistochemical studies showed that the number of binding sites for anti-MeCP2 antibodies in HT animals decreases 1 day after LEPS and remains low over 2 weeks after exposure (Table 1). LT animals did not differ from the control 1 day and 2 weeks after LEPS.

LEPS was followed by a decrease in MeCP2 content in neuronal nuclei of hippocampal field

TABLE 1. Number of Binding Sites for Antibodies against MeCP2 in Neuronal Nuclei of the Hippocampus in HT and LT Rats at Various Periods after LEPS ($M \pm m$)

Line	Group	Immunofluorescence method		DAB-ABC-immunoperoxidase method	
		24 h	2 weeks	24 h	2 weeks
HT	Control	2.94±0.10	3.15±0.09	4.93±0.12	4.25±0.07
	Treatment	2.43±0.11*	2.25±0.10*	3.21±0.11*	3.79±0.09*
LT	Control	2.17±0.08*	1.69±0.14*	2.36±0.13*	3.44±0.08*
	Treatment	2.35±0.08	1.98±0.12	1.96±0.16	3.39±0.09

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

CA3, which depended on linear characteristics of the excitability of the nervous system. MeCP2 expression in the hippocampus of adult animals also depends on kindling and cerebral ischemia induced by cerebral artery blockade. It should be emphasized that the decrease in methyltransferase concentration contributes to reparation of the postischemic brain [10,11].

Persistence of these changes was not evaluated. The effect of various factors involved in DNA methylation (*e.g.*, MeCP2) on CNS function is determined by its regulatory role in the expression of neuronal genes, including BDNF gene (brain-derived neurotrophic factor) [9]. This gene improves viability of neurons and modulates neuronal synaptic plasticity and long-tern posttetanic potentiation. Published data show that generation of long-tern posttetanic potentiation in the hippocampus of HT rats decreases 2 months after LEPS [1].

Our previous studies showed that LEPS is followed by decondensation of chromatin and increase in the overall genome expression in hippocampal neurons of HT rats [3,4]. These changes are probably associated with the decrease in MeCP2 content over 2 weeks after exposure. A negative correlation exists between DNA methylation and chromatin condensation. Stress exposure affects the state of heterochromatin and MeCP2 content in HT rats, which suggests that protein MeCP2 plays a role in epigenetic regulation of adult brain function and formation of memory traces.

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