Time Course of Histone Deacetylase 1 and Acetylated H3 and H4 Histones in the Brain of Rats Treated with Ladasten

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 11, pp. 533-536, November, 2010
Original article submitted December 17, 2009

We studied the effects of single intragastric administration of ladasten in a dose of 50 mg/kg on the time course of histone deacetylase 1 (HDAC1) and levels of acetylated histones H3 (Lys9) and H4 (Lys8) in the striatum, hippocampus, and hypothalamus. Ladasten reduced HDAC1 level in rat striatum and hippocampus and modified H3acK9 and H4acK8 levels in various structures of rat brain.

Key Words: *acetylated histones; histone deacylases; chromatin; HDAC inhibitors; neuroprotection*

Regulation of the expression of specific gene groups by drugs with different structures and pharmacological activities determines the immediate and long-term effects of these drugs. A vast scope of data on the primary targets of drug treatment (receptors, enzymes, *etc.*), routes of intracellular signal transmission, and transcription factors regulating the transcription activity of genes in response to drugs has been accumulated [11].

Gene expression is also regulated at the level of chromatin structure. Local chromatin decondensation in the promotor areas of actively transcribed genes is an obligatory factor providing access of transcription factors and RNA polymerase II to the DNA regulatory sites [9]. Apart from chromatin remodeling by ATP-dependent enzymatic complex, the "open" decondensed state of chromatin is maintained via modification of the histone N-terminal amino acids (mainly lysine and arginine). Histone N-terminals can be subjected to posttranslation modifications: acetylation, methylation, ubiquitinylation, sumoylation, phosphorylation, and ADP-ribosylation. Acetylation/deacetylation catalyzed by histone acetylases (HAT) and histone deacetylases (HDAC), respectively, is characterized in detail. The histone hyperacetylated state is associated with chromatin condensation and transcription activation, while deacetylation is linked with "closed" chromatin and repression of gene transcription [7,12].

Many drugs are capable of modifying chromatin function. For example, valproic acid and its salts (antiepileptic agents) inhibit HAT [13]. Long-term cocaine treatment of experimental animals reduces the levels of Lys9 H3 histones and elevation of HDAC2 level [4]. Fluoxetine (antidepressant) also elevates HGAC2 activity in the rat hippocampus and hypothalamus [15]. A single injection of neuroleptics haloperidol and risperidone (dopamine D_2 receptor antagonists) causes transitory phosphorylation of Ser10 and acetylation of Lys14 in H3 in the striatal neurons [10].

We previously carried out a comprehensive molecular biological study of ladasten ((N-(2–adamantyl)-N–parabromophenylamine, a new psychostimulant with anxiolytic and immunotropic activities [1]. Here we studied *in vivo* the effect of a single dose of ladasten on the time course of HDAC1 and acetylated histones H3 (Lys9) and H4 (Lys8) in rat striatum, hypothalamus, and hippocampus.

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0007-4888/11/1505-0603

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (200-250 g). All manipulations on animals were carried out in accordance with "Regulations on Handling Experimental Animals". Experimental animals received a single intragastric dose of ladasten (50 mg/kg in suspension with Twin-80), controls the same volume of water with Twin-80. Cytoplasmatic and histone proteins were isolated [5]. The levels of HDAC1, H3acK9, and H4acK8 were standardized by the level of β-tubulin (anti-β-tubulin, Cell Signaling). Three variants of experiments were repeated 2-9 times.

The data were statistically processed by Student's *t* test using Statistica 6.0 software. The differences were considered significant at $p<0.05$.

RESULTS

The level of HDAC1 in the striatum decreased as soon as 15 min after ladasten administration (Fig. 1, *a*), while after 1 h its level was 67% of the control ($p=0.0026$). The level of H3acK9 also decreased significantly almost by half (56% of control, $p=0.004$) after 1 h and less significantly (80% of control, $p=0.044$) 2 h after ladasten administration. The level of H4acK8 decreased only 2 h after administration by 20% in comparison with the control ($p=0.0002$; Fig. 2, *a*). The slight decrease in acetylated histone levels in this brain structure can indicate the predominating "closed" chromatin conformation under the effect of ladasten. Disagreement between HDAC1 and acetylated histone levels in the striatum is worthy of note and can be explained by the participation of HDAC of other classes, *e.g.* HDAC II into regulation of acetylation of histones H3 and H4 lysine residues. It is also possible that ladasten treatment leads to inhibition of acetyl transferase activities in the striatum.

In the hippocampus, a minor decrease in HDAC1 level (by 29%, $p=0.003$) was recorded 0.5 h after drug administration (Fig. 1, *b*), while after 1 h a trend to increase of H3acK9 and H4acK8 levels was observed. The level of HDAC1 increased by 60% in comparison with the control ($p<0.0001$) 1.5 h after ladasten administration, while after 2 h the level of H3acK9 decreased (by 20%, $p=0.032$), as did the level of H4acK8 (by 19%, $p=0.001$; Fig. 2, *b*). Relationship between the levels of HDAC1, H3acK9, and H4acK8 can indicate that ladasten-induced histone deacetylation in the hippocampus is realized mainly by HDAC1.

In contrast to the striatum and hippocampus, the effect of ladasten on HDAC1 level in the hypothalamus was less manifest (Fig. 1, *c*). However, 1 h after drug administration the level of H4acK8 increased by 41% ($p=0.049$) and a trend to H3acK9 elevation (by 21%; $p=0.32$) was observed (Fig. 2, *b*). As was noted, high level of acetylated histones is a marker of "active" chromatin. Starting from 1.5 h after drug ad-

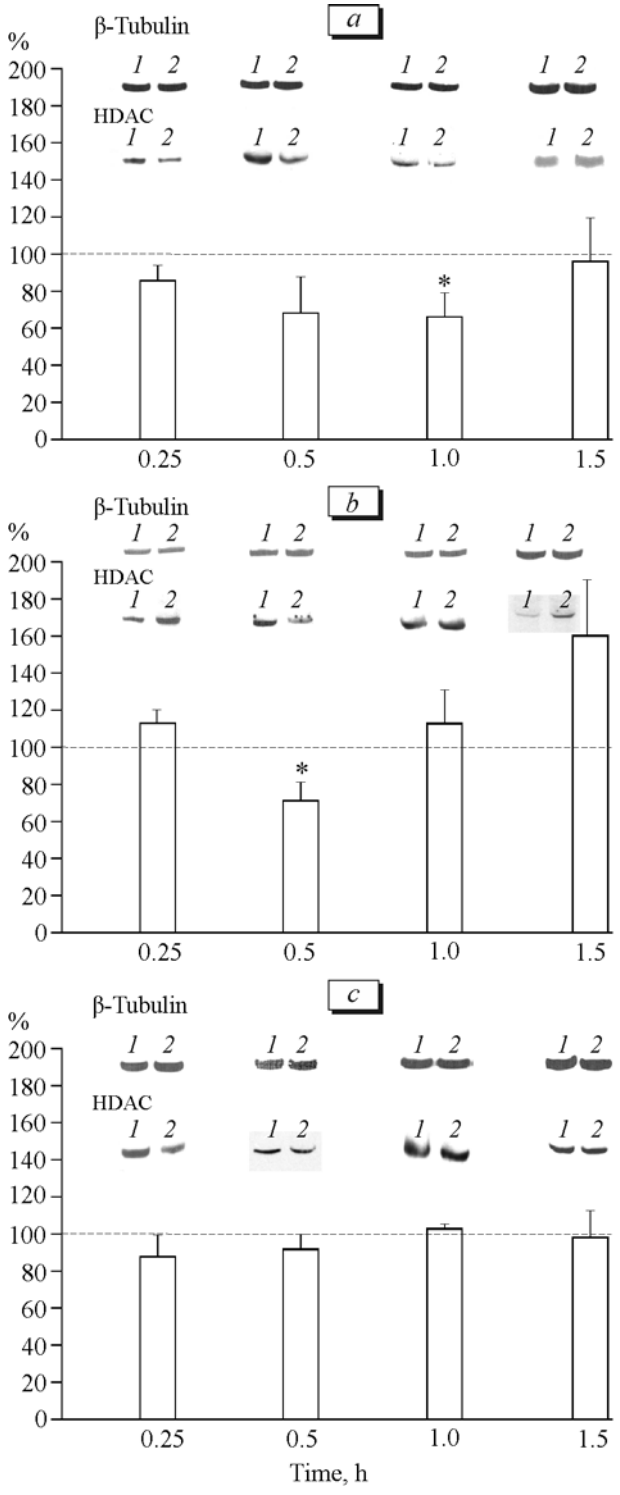

Fig. 1. Western-blot and time course of HDAC1 in cells of the striatum (*a*), hippocampus (*b*), and hypothalamus (*c*) of rats after a single intragastric dose of ladasten. All data are presented in comparison with the control (100%). *1*) control; *2*) experiment. Here and in Fig. 2: *$p<0.05$ compared to the control.

ministration the level of H3caK9 somewhat decreased (by 21%, p=0.014). Presumably, histone deacetylation in the hypothalamus (similarly as in the striatum) is maintained by other HDAC classes.

The disagreement between HDAC1 and acetylated H3 and H4 levels in the striatum and hypothalamus during ladasten treatment does not contradict published data. It is known that HAT and HDAC, belonging to different classes [13], exhibit substrate specificity and selectivity towards individual histones and sites and efficiency of lysine residues acetylation/deacetylation [8,14].

Histone acetylation/deacetylation is a dynamic process sensitive to external signals. HAT and HDAC are the objects of regulatory effects of protein kinases and protein phosphatases of different signal cascades. The site specificity of individual histones acetylation/deacetylation is presumably determined by modulation of HAT and HDAC activities [3]. Opposite effects of ladasten on the levels of acetylated histones in the striatum, hippocampus (slight reduction), and hypothalamus (elevation 1 h after the drug administration) can be due to differentiated stimulation of regulatory protein kinases by ladasten and determine the tissue-specific differences in transcription activities of genes and/or groups of genes selectively expressed in response to the drug.

The aberrant pattern of histone acetylation and disorders in HAT and HDAC regulation is a pathogenetic factor of some oncological and neurodegenerative diseases. The attention of scientists is therefore focused on the potentialities of clinical application of HDAC synthetic inhibitors [2]. Some substances with pharmacological activity, including clinically used drugs, inhibit HDAC and modify the histone function. Experiments on tumor cells showed that HDAC inhibitors block the cell growth by inducing the expression of tumor suppressor genes. Recent studies on *Drosophila melanogaster* with *htt* (huntington) gene mutation showed high survival of defective neurons in flies treated with HDAC inhibitors [6]. The efficiency of HDAC inhibitors (sodium butyrate and phenylbutyrate) improving the neuron survival was demonstrated on transgenic mice, models of Huntington's, Alzheimer's, and Parkinson's diseases, amyotrophic lateral sclerosis, and stroke [3]. Presumably, the neuroprotective effect of these compounds is due to restoration of modified histone acetylation pattern and correct gene transcription [2].

Hence, our data suggest that chromatin structure control is essential for the mechanisms of ladasten-induced changes in transcription activity of many target genes in the rat brain cells.

The study was supported by the Federal Target Program "Research and Development of Priority Trends of Scientific and Technological Complex of Russia for 2007-2012" (state contract No. 02.740.11.0290).

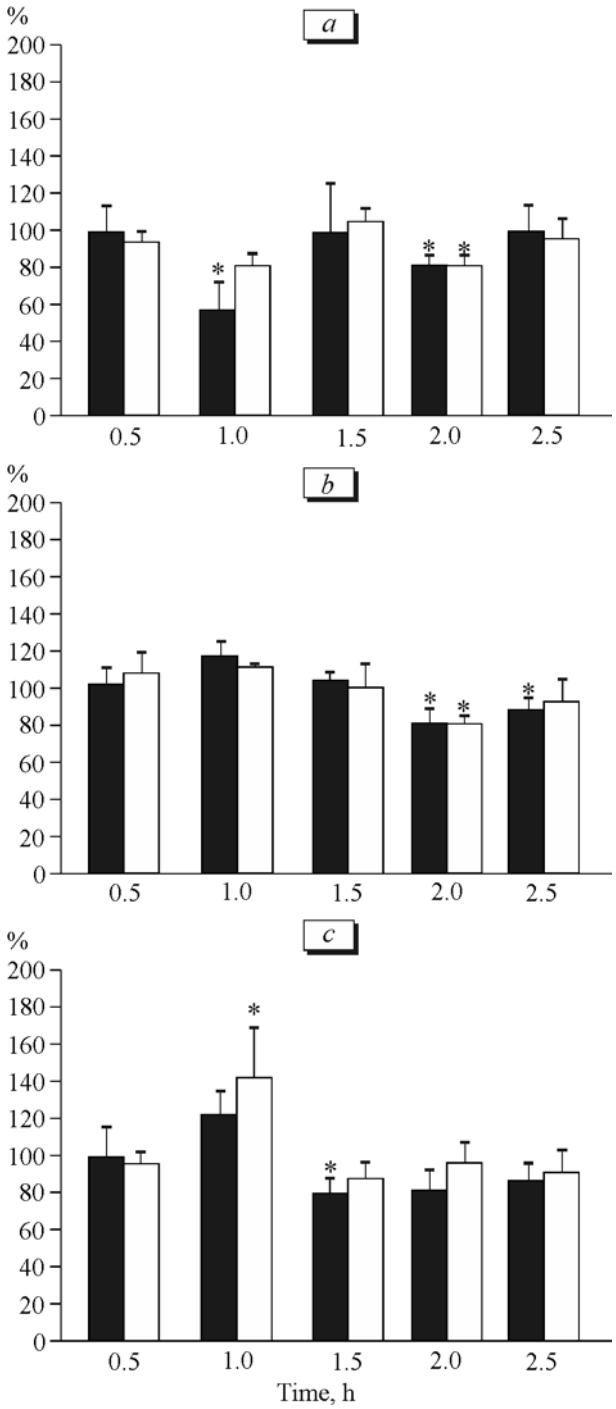

Fig. 2. Levels of H3acK9 (dark bars) and H4acK8 (light bars) in cells of the striatum (*a*), hippocampus (*b*), and hypothalamus (*c*) of rats after a single intragastric dose of ladasten. All data are presented in comparison with the controls (100%).

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