PHARMACOLOGY AND TOXICOLOGY

Changes in CREB (SER133) Content and DNA-Binding Activity of Transcriptional Factors in Rat Brain Cells against the Background of Ladasten Exposure

M. Kh. Salimgareeva, Yu. V. Vakhitova, R. S. Yamidanov, S. V. Sadovnikov, S. B. Seredenin*

Translated from *Byulleten' Experimental'noi Biologii I Meditsiny*, Vol. 147, No. 5, pp. 528-531, May, 2009
Original article submitted August 14, 2008

We studied the effects of single administration of ladasten (50 mg/kg) on the level of transcriptional factor CREB (cyclic AMP response binding element protein) phosphorylated by Ser133 in rat striatum, hypothalamus, and hippocampus. Transcriptional factors with affected DNA-binding activity were identified in brain cells.

Key Words: *ladasten; hypothalamus; hippocampus; striatum; transcriptional factors.*

Ladasten (N-(2-adamantyl)-N-para-bromophenylamine) was synthesized and its pharmacology was studied in V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences. High pharmacological activity of the product was proven during II phase of clinical studies. It possesses a wide range of pharmacological properties, including psychostimulant, anxiolytic, and immunotropic activities. Ladasten appears to increase the content of neurotransmitters dopamine and serotonin predominantly in the limbic system structures (hippocampus, hypothalamus, ventral tectum area) and to induce dopamine release from the presynaptic terminals in the striatum [4].

In our previous studies the role of the main intracellular signaling systems, cAMP-sensitive protein kinase (PKA), Ca^{2+}-phospholipide dependent protein kinase (PKC), Ca^{2+}/calmodulin dependent protein kinase II (CaMKII), and mitogen-activated protein kinase cascade (MAPK), in mediation of ladasten effects was shown. Systems mediated by calcium ions are the main signaling systems, triggering changes in cell metabolism inducted by this compound [1,2,3,12]. It is known that Ca^{2+}-dependent pathways play the principal role at the early phase of target activation and readily respond to extracellular stimuli. Activation of signaling cascade proteinkinases leads to induction of the expression of certain transcriptional factors regulating transcriptional activity of the target genes.

In this study, detection of transcriptional factors activated by signaling cascades and initiated by ladasten injection was performed by hybridization using TranSignal™ Protein/DNA Arrays commercial kit (Panomics) allowing detection of changes in DNA-binding activity of 96 transcriptional factors simultaneously. The effects of ladasten on the dynamics of CREB phosphorylated by Ser 133, the most characterized and explored transcriptional factor activated in neurons in response to various stimuli, in rat striatum, hypothalamus, and hippocampus were also evaluated using Western-blot analysis.

Laboratory of Molecular Immunology and Pharmacology, Institute of Biochemistry and Genetics, Ufa Research Centre, Russian Academy of Sciences; *Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** milyausha78@mail.ru. M. Kh. Salimgareeva

MATERIALS AND METHODS

Experiments were carried out on white mongrel male rats weighing 200-250 g. The animals were kept under standard vivarium condition. All manipulations with the animals were performed in accordance with Rules for conducting studies on experimental animals (Ministry of Health of the USSR Order No. 755, December 8, 1977).

Ladasten was administered *per os* in a dose of 50 mg/kg (suspension in distilled water with Tween-80). Control animals received distilled water with Tween-80 in the corresponding volume. Nuclear extract of brain cells was obtained in accordance with Dignam protocol [4]. Protein concentration was assessed using Bradford method. Electrophoresis of proteins was carried out in polyacrylamide gel with 0.1% sodium dodecyl sulphate in a linear acrylamide gradient (6-16%). Immunoblotting and hybridization with antibodies (Cell Signaling) were performed in accordance with manufacture's protocol. Detection and identification of transcription factors were carried out using commercial biochip TranSignal™ Protein/DNA Arrays (Panomics) in accordance with manufacture's protocol. Quantitative assessment of optical density of autoradiogram bands was performed using TotalLab 2.0 software, hybridization results were analyzed using ImageQuant software 5.0 (Molecular Dynamics). Experiments were repeated 2-9 times in three biological replications. The data were statistically analyzed using Statistica 6.0 software. The differences were significant at $p<0.05$.

RESULTS

Differential dynamics of CREB activation in different rat brain structures after exposure to ladasten was found. In the hypothalamus, an increase in pCREB level was found 1.5-2 h after ladasten administration (by 55 and 63%, respectively, Fig. 1, *a*). In the striatum, pCREB (Ser133) content slightly increased 0.5 h after ladasten administration (by 20%), but rapidly decreased after 1 h (by 75%, $p<0.001$; Fig. 1, *b*). In the hippocampus, pCREB increased 1-1.5 h after ladasten administration by 80 and 64%, respectively, and returned to control values after 2 h (98%, Fig. 1, *c*). Despite marked changes in pCREB (Ser133) content after ladasten treatment, the changes observed in the hypothalamus and hippocampus were statistically insignificant.

These findings and our previous results concerning the dynamics of PKA, PKC, CaMKII and ERK1/2 (extracellular signal-regulated kinase) suggest that early (0.5 h after ladasten dosing) increase in pCREB level is possibly associated with the fact that induction of transcriptional factor expression is realized via different signal transduction systems (PKA, PKC, CaMKII and ERK1/2) [1,2,12]. According to modern view, CREB is a central integration link for various signal pathways. For example, neurotransmitters stimulating adenylate cyclase increase CREB phosphorylation upon PKA activation, while neurotransmitters complementary to receptors negatively associated with adenylate cyclase inhibit its activity and, correspondingly, decrease pCREB phosphorylation. Various external stimuli elevating intracellular Ca^{2+} concentration (activation of inotropic receptors or some receptors coupled with G-proteins, voltage-gated Ca^{2+} channels) also initiate the chain of events resulting in increase of CREB phosphorylation by Ser133 [11]. It should be noted that the level of CREB phosphorylation by Ser133 reflects activity of various kinases and phosphatases operating synergistically or in opposite directions [8]. CREB have other phosphorylation sites apart from Ser133, for example Ser142. Phosphorylation by this site catalyzed primarily by CaMK inhibits CREB phosphorylation by Ser133 catalyzed by other protein kinases [13]. The observed rapid decrease in CREB phosphorylation by Ser133 in the striatum (after 1 h) is possibly associated with increased expression of gene (both immediate and late response genes, *e.g.* c-fos) containing CREB-binding sequences in their promoters. Regulation of CREB activity in the hypothalamus most likely depends on CaMKII and ERK1/2 kinases. This statement comes from the data about similar dynamics of protein kinase activity and pCREB level in this structure [1,12]. The same relationship was observed in the hippocampus: peaks of CaMKII and ERK1/2 activation and pCREB level are observed at the same time interval after drug administration.

Five transcriptional factors changing their DNA-binding activity 1 h after ladasten treatment were identified (Table 1).

ADR1, a PKA-phosphorylated factor, regulates expression of alcohol dehydrogenase 2 gene and some genes involved in peroxisome biogenesis and functioning [15]. Activity of this factor was increased by more than 5 times after ladasten treatment.

HFH8, a transcriptional factor from Forkhead family, is involved in the regulation of early stages of embryonic development. Sequences specific for HFH8 were found in promoters of early neurogenesis genes, D_2-dopamin receptors, neuronal acetylcholinesterase, β-retinoic acid receptor, and *oct2* gene encoding neuronal monoamine transporter in brain cells [9]. HIF-1 binding site (HBS) plays the

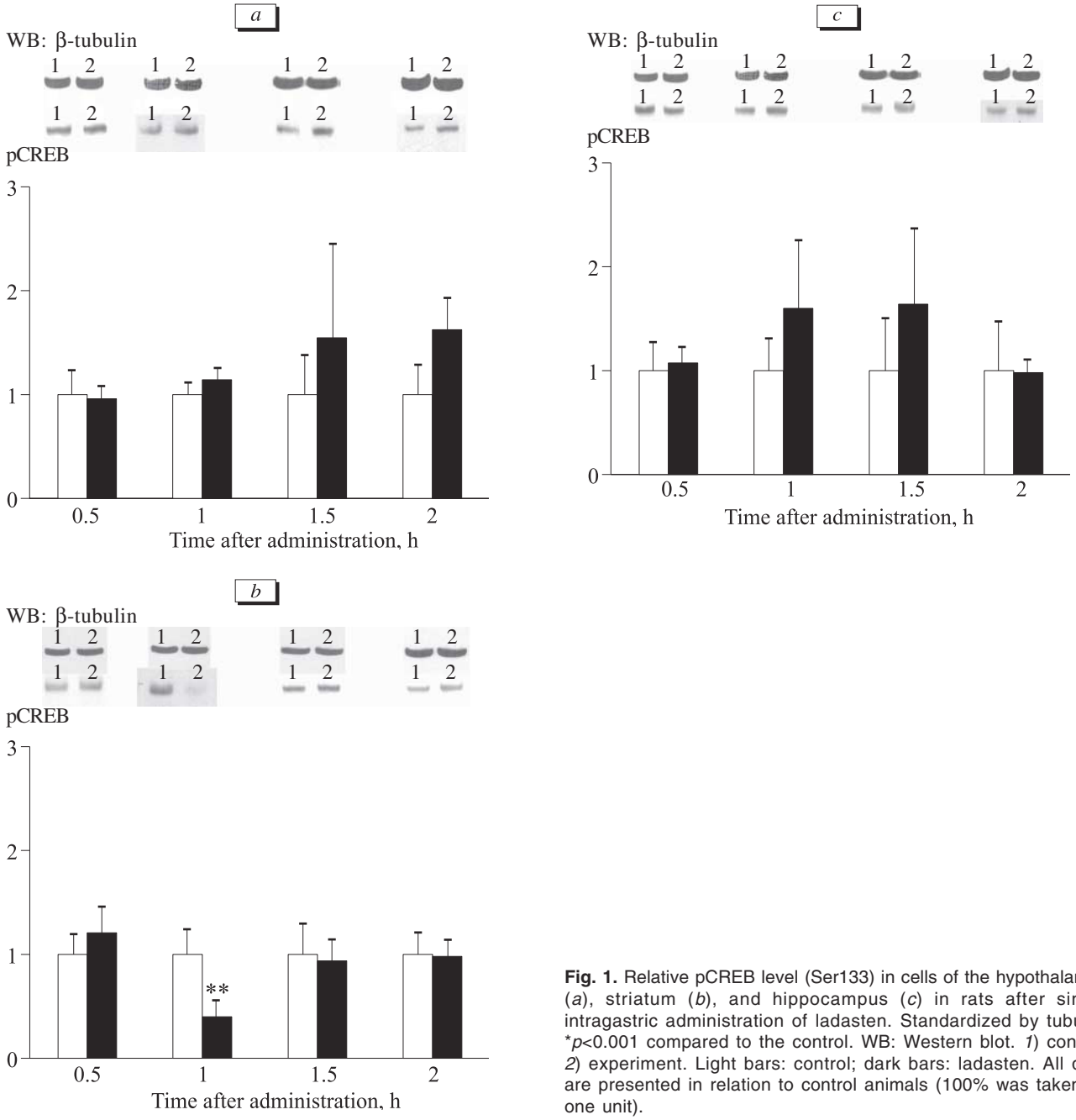

Fig. 1. Relative pCREB level (Ser133) in cells of the hypothalamus (*a*), striatum (*b*), and hippocampus (*c*) in rats after single intragastric administration of ladasten. Standardized by tubulin. **p*<0.001 compared to the control. WB: Western blot. *1*) control; *2*) experiment. Light bars: control; dark bars: ladasten. All data are presented in relation to control animals (100% was taken as one unit).

key role in hypoxia-dependent gene expression. Genes regulated by HIF-1 are identified. They include vascular endothelial growth factor, erythropoietin, glucose transporter-1, and glycolytic enzymes enolase 1 and lactate dehydrogenase. Induction of the transcription of hypoxia-sensitive genes is an essential stage of adaptation to hypoxia, since the products of these genes are involved in mechanisms responsible for increased oxygen delivery or in processes aimed at lowering of oxygen demands under conditions of hypoxia [13]. The increase in HIF-1 activity in rat brain cells after exposure to ladasten possibly reflects realization of its protective action.

Marked (10-fold) increase in DNA-binding activity of SAA element of β-amyloid precursor protein (*β-APP*) gene promoter, which is normally involved in the organization of interneuron contacts and plays a key role in the pathogenesis of Alzheimer disease is worth noting. Sequence of SAA gene contains binding sites for transcriptional factors Sp-1, AP-4, USF, AP-1 [10].

NPAS2 is involved in the regulation of transcription of genes controlling circadian rhythms in soma-

TABLE 1. Effects of Ladasten (50 mg/kg) on DNA-Binding Activity of Transcriptional Factors in Rat Brain Cells (*M*±*m*)

Transcriptional factor	Changes*
ADRI — alcohol dehydrogenase regulatory gene binding element	↑5.54±2.04
HFH-8 — forkhead box Fla (HNF-3/Fkh Homolog-8)	↑2.22±0.24
HBS/xbpl — HIF binding sequence (rat, as human xbp-1)	↑2.05±0.26
SAA — amyloid precursor protein (APP) regulatory element	↑10.16±1.44
NPA2 — neuronal PAS domain protein 2	↓0.33±0.03

Note. *The values were standardized by the control level taken as 1.

tosensory and visual cortex and olfactory bulbs of the mesencephalon [6]. *NPAS2* knock-out mice show disturbances in emotional long-term memory formation and impaired perception of sensory stimuli [7].

Thus, our findings attest to CREB involvement in the regulation of gene transcription in rat brain cells after single exposure to ladasten. The identified transcriptional factors assume presumptive set of genes regulated by them and, consequently, the direction of changes in cell metabolism. This allows more precise definition and widening of the spectrum of action at the stage of preclinical studies.

This work is supported by Program of fundamental research Presidium of Russian Academy of Sciences "Molecular and Cellular Biology" (10002-251/P-10/142-448/260503-196), Support Program for leading scientific schools (ScSc-1003.2006.4).

REFERENCES

1. Yu. V. Vakhitova, M. Kh. Salimgareeva, and S. B. Seredenin, *Eksper. Klin. Farmakol*., **67**, No. 2, 12-15 (2004).
2. Yu. V. Vakhitova, M. Kh. Salimgareeva, and S. B. Seredenin, *Eksper. Klin. Farmakol*., **67**, No. 3, 7-9 (2004).
3. Yu. V. Vakhitova, R. S. Yamidanov, V. A. Vakhitov, and S. B. Seredenin, *Mol. Biol.*, **39**, No. 2, 279-285 (2005).
4. V. S. Kudrin, S. A. Sergeeva, L. M. Krasnykh, *et al.*, *Eksper. Klin. Farmakol*., **58**, No.4, 8-11 (1995).
5. N. N. Dewji and C. Do, *Brain Res. Mol. Brain Res.*, **35**, Nos. 1-2, 325-328 (1996).
6. C. A. Dudley, C. Erbel-Sieler, S. J. Estill, *et al.*, *Science*, **301**, 379-383 (2003).
7. J. A. Garcia, D. Zhang, S. J. Estill, *et al.*, *Ibid*, **288**, 2226-2230 (2000).
8. G. A. Gonzalez, P. Menzel, J. Leonard, *et al.*, *Mol. Cell. Biol.*, **11**, No. 3, 1306-1312 (1991).
9. B. C. Granadino, C. Perez-Sanchez, and J. Rey-Campos, *Current Genomics*, **1**, 353-382 (2000).
10. P. W. Hoffman and J. M. Chernak, *Nucleic Acids Res.*, **23**, No. 12, 2229-2235 (1995).
11. B. E. Lonze and D. D. Ginty, *Neuron*, 2002, **35**, No. 4, 605-623 (2002).
12. M. G. Mikhaylova, J. V. Vakhitova, R. S. Yamidanov, *et al.*, *Neuropharmacology*, **53**, No. 5, 601-608 (2007).
13. G. L. Semenza, L. A. Shimoda, and N. R. Prabhakar, *Novartis Found Symp.*, **272**, 2-8 (2006).
14. T. R. Soderling, *Trends Biochem. Sci.*, **24**, No. 6, 232-236 (1999).
15. W. E. Taylor and E. T. Young, *Proc. Natl. Acad. Sci. USA*, **87**, No. 11, 4098-4102 (1990).