Proteomic Analysis and Identification of Ladasten Target Proteins in Rat Brain

R. S. Yamidanov[1], M. Kh. Salimgareeva[1], S. V. Sadovnikov[1], Yu. V. Vakhitova[1,2], V. M. Govorun[3], and S. B. Seredenin[2]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 6, pp. 717-720, June, 2010
Original article submitted December 17, 2009

Two-dimensional electrophoresis and mass spectrometry detection were used to evaluate the range of proteins in rat brain after single treatment with ladasten (50 mg/kg). We identified 13 proteins with various levels of expression.

Key Words: *psychostimulant drugs; ladasten; proteomic analysis; mass spectrometry; mechanisms of action*

Modern methods of evaluation of the mechanisms for drug action include studying the profiles of genes and proteins, whose content is modified after treatment with these drugs. This approach allows us to identify potential protein targets for pharmacologically active substances. The mechanisms for action and positive or adverse effects of any drug can be evaluated [6]. The whole-genome analysis of the expression profile is a routine method at the early stages of preclinical studies with medicinal products. However, a correlation between the content of mRNA and concentration of the corresponding protein is not found in many experiments. Hence, it is difficult to interpret the results of studies with expression chips. The simultaneous measurement of a considerable number of proteins is most informative for the evaluation of drug targets [10].

We identified the target genes for a new neurotropic drug ladasten (N-(2–adamantyl)-N–*p*-bromophenyl-amine), which possesses the psychostimulant, anxiolytic, and immunomodulatory properties [2]. The range of proteins in rat brain was studied after single treatment with ladasten to evaluate the molecular targets for this drug.

MATERIALS AND METHODS

A suspension of ladasten (single dose 50 mg/kg) in Tween 80 was injected intraperitoneally to male rats. Control animals received an equivalent volume of distilled water and Tween 80. The rats were decapitated 1.5 h after drug treatment. The brain was removed. Cytosolic proteins were extracted [4], separated by means of two-dimensional gel electrophoresis [12], and identified in a mass spectrometry study. The spots of protein fragments (2 mm^3) were cut of the gel. Proteins were hydrolyzed by the standard method (http://www.expasy.org/ch2d/; Shevchenko). Mass spectrometry of gel peptides was performed on a Reflex III MALDI-TOF mass spectrometer (Bruker). Proteins were identified by a Mascot peptide fingerprint analysis with protein database (SwissProt and NCBI).

RESULTS

Scanned electrophoretograms of two-dimensional protein electrophoresis were compared with Melanie 3 software (GeneBio). The level of 27 proteins in treated samples was shown to differ from that in control samples (3 independent experiments; Fig. 1). Protein

[1]Laboratory of Molecular Immunology and Pharmacology, Institute of Biochemistry and Genetics, Ufa Research Center, Russian Academy of Sciences; [2]Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow; [3]Institute of Physicochemical Medicine, Federal Agency for Health Care and Social Development, Moscow, Russia. ***Address for correspondence:*** milyausha78@mail.ru. M. Kh. Salimgareeva

0007-4888/10/1496-0775

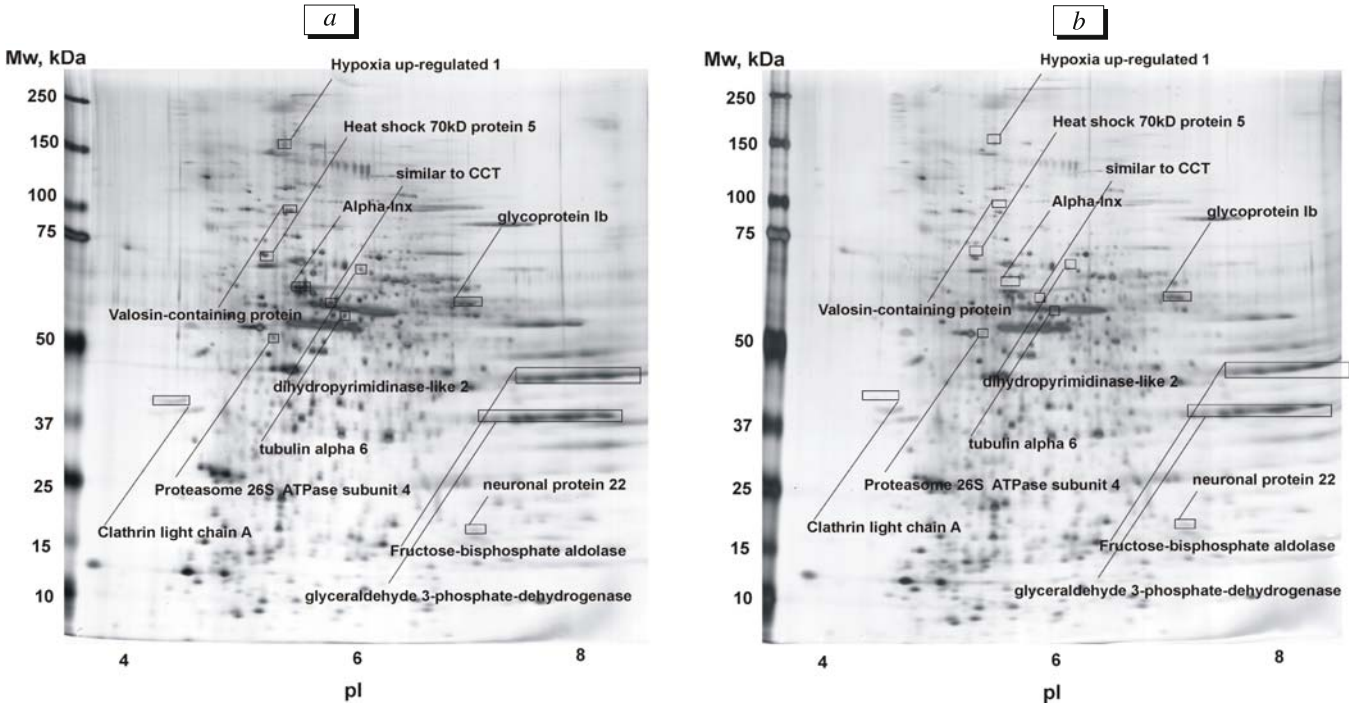

Fig. 1. Two-dimensional gel electrophoresis of rat brain proteins after intraperitoneal injection of ladasten in a single dose of 50 mg/kg. The figure illustrates differentially expressed proteins, whose intensity exceeds the threshold level for spot identification in a mass spectrometry study. Control (*a*) and treatment (*b*).

content was estimated from the normalized intensity of spots. It was calculated as the sum of pixel intensity in a certain spot divided by the sum of pixel intensity in all spots of the gel. Mass spectrometry was used to identify the proteins whose content in samples of the treatment and control groups differed by more than 1.2 times. The intensity of signals from these proteins exceeded the threshold level. This method allowed us to identify 13 proteins with various levels of expression (Table 1). The expression was shown to increase for 8 proteins, but decreased slightly for 5 proteins (as compared to the control).

Ladasten treatment is followed by a simultaneous regulation of proteins in brain cells that have a role in coupled processes. For example, the content of intracellular proteins with an impaired tertiary structure increases under adverse conditions (heat shock, oxygen deprivation, oxidative stress, *etc*.). These changes are accompanied by the increased expression of chaperone proteins for correct folding of proteins and enhanced elimination of abnormal proteins by the ubiquitin/proteosome system [9]. Ladasten activates a group of proteins involved in correct folding of proteins (hypoxia up-regulated 1, heat shock 70 kDa protein 5, and chaperone-containing TCP-1) [5,13] and directed degradation of proteins (proteosome 26S ATPase subunit 4 and valosin-containing protein) [8]. It can be hypothesized that ladasten-induced changes in the content of stress proteins is related to activation of the cytoprotective mechanisms for compensation of the possible adverse effect of this drug.

Another group of ladasten-regulated proteins includes synaptic proteins that are presented by structural proteins of the cytoskeleton in nerve cells (tubulin α6), intermediate neurofilaments (α-internexin), and endocytosis vesicles (clathrin light chain α) [7,15]. Taking into account the polyfunctionality of synaptic proteins, it is difficult to interpret the ladasten-induced changes in expression of these molecules. It is most likely that the ladasten-induced activation of neurons causes an adaptive increase in the expression of these proteins in rat brain.

Ladasten slightly inhibits the expression of two enzymes for glycolysis, fructose 1,6-diphosphate aldolase A and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Fructose 1,6-diphosphate aldolase catalyzes the degradation of fructose 1,6-diphosphate into 2 triose phosphates, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [14]. Ladasten has a role in the negative regulation of functional activity of enzymes that catalyze the consecutive reactions of glycolysis. This property of ladasten contributes to the drug-induced decrease in blood glucose level. A relationship exists between expression of the gene and protein for glyceraldehyde 3-phosphate dehydrogenase. A correlation was found between the increase in transcriptional activity of the *GAPDH* gene and content of the corresponding protein. Hence, this sys-

TABLE 1. Proteomic Analysis of Rat Brain Proteins after Intraperitoneal Injection of Ladasten in a Single Dose of 50 mg/kg

Protein	NCBI number	Molecular weight, Da	Isoelectric point, Pi	Expression±standard deviation	*p*
Hypoxia up-regulated 1	gi|20302024	111220	5.11	1.633±0.040	0.102
Clathrin light chain α	gi|116503	26964	4.41	1.582±0.034	0.124
Tubulin α6	gi|34868254	63023	5.86	1.513±0.038	0.163
Proteasome 26S ATPase subunit 4	gi|38970025	47379	5.09	1.391±0.030	0.079
α-internexin	gi|1703221	56082	5.2	1.296±0.092	0.052
Dihydropyrimidinase-like 2	gi|40254595	62239	5.95	1.233±0.016	0.033
Heat shock 70 kDa protein 5	gi|38303969	72302	4.7	1.226±0.032	0.046
Valosin-containing protein	gi|38014694	89293	5.14	1.226±0.028	0.061
Fructose-bisphosphate aldolase A	gi|68186	39235	8.31	0.900±0.411	0.096
Glyceraldehyde 3-phosphate-dehydrogenase	gi|56188	35813	8.26	0.799±0.036	0.117
Glycoprotein Ib (platelet)	gi|16758814	43892	6.34	0.764±0.038	0.054
Similar to CCT (chaperonin containing TCP-1) epsilon subunit	gi|34854923	69428	6.70	0.625±0.029	0.078
Similar to Electron transfer flavoprotein alpha-subunit	gi|27720225	34929	8.62	0.490±0.050	0.114

Note. Statistically significant differences in protein expression at $p<0.05$.

tem plays a role in the effects of ladasten. These data indicate that GADPH serves as a pharmacologically important target.

Comparative study revealed the same profiles of differential proteins expression, which is induced by neurotropic drugs of various pharmacological groups (psychostimulant, antidepressant, neuroleptic, and anxiolytic drugs) and ladasten. This similarity concerns the cytoskeletal proteins, cell-cell interactions, and vesicular, transport, and membrane proteins. It is associated with common nonspecific adaptive changes of synaptic functions in response to the increase in neuronal activity. Previous studies demonstrated the existence of target proteins that are specific for 2-aminoadamantane derivative (ladasten) and associated with the pharmacological effect of this drug. The inhibitory effect of ladasten on the expression of glycolysis enzymes is consistent with published data that this drug decreases blood glucose level [3]. The cytoprotective properties of ladasten were revealed in behavioral studies with physical loads [1]. These properties are probably associated with the involvement of chaperone proteins and components of the ubiquitin/proteosome system.

We conclude that the analytical methods for differentially expressed proteins serve as a supplement for the standard psychopharmacological and biochemical studies. These methods allow us to extend the knowledge about the molecular mechanisms for pharmacological activity of ladasten.

This work was supported by the Basic Research Program "Molecular and Cellular Biology" (Presidium of the Russian Academy of Sciences, grant No. 10002-251/P-10/142-448/260503-196), Program for the Support of Leading Scientific Schools (grant No. NSh-1003.2006.4), and Federal Target Program "Research and Development in Priority Fields of S&T Complex of Russia for 2007-2012" (State Contract No. 02.740.11.02.90).

REFERENCES

1. I. S. Morozov, V. I. Petrov, and S. A. Sergeeva, *Pharmacology of Adamantanes* [in Russian], Volgograd (2001).
2. S. B. Seredenin and A. G. Miramedova, *Byull. Eksp. Biol. Med.*, **128**, No. 11, 529-531 (1999).
3. A. R. Khalimov, Kh. M. Nasyrov, and S. A. Sergeeva, *Zdravookhr. Bashkortostana*, No. 3, 7-10 (1997).
4. D. M. Bollag and S. J. Edelstein, *Protein Methods*, New York (1991), pp. 72-73.
5. I. R. Brown, *Ann. N. Y. Acad. Sci.*, **1113**, 147-158 (2007).
6. Y. D. He, *Cancer Biomark.*, **2**, Nos. 2-3, 103-133 (2006).
7. M. P. Kaplan, S. S. Chin, K. H. Fliegner, and R. K. Liem, *J. Neurosci.*, **10**, No. 8, 2735-2748 (1990).
8. J. B. Klein, M. T. Barati, R. Wu, *et al.*, *J. Biol. Chem.*, **280**, No. 36, 31,870-31,881 (2005).
9. Z. Kostova and D. H. Wolf, *EMBO J.*, **22**, No. 10, 2309-2317 (2003).

10. F. L. Moseley, K. A. Bicknell, M. S. Marber, and G. Brooks, *J. Pharm. Pharmacol.*, **59**, No. 5, 609-628 (2007).
11. A. Shevchenko, O. N. Jensen, A. V. Podtelejnikov, *et al*., *Proc. Natl. Acad. Sci. USA*, **93**, No. 25, 14,440-14,445 (1996).
12. R. J. Simpson, *Proteins and Proteomics: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (2003).
13. M. Tamatani, T. Matsuyama, A. Yamaguchi, *et al*., *Nat. Med*., **7**, No. 3, 317-323 (2001).
14. W. G. Tatton, R. M. Chalmers-Redman, M. Elstner, *et al*., *J. Neural Transm. Suppl*., **60**, 77-100 (2000).
15. K. J. Verhey and J. Gaertig, *Cell Cycle*, **6**, No. 17, 2152-2160 (2007).