Effect of Antiparkinsonian Drug Himantane on the Content of Dopamine Transporter DAT Protein in Rat Striatum and Cultured Pheochromocytoma PC-12 Cells

D. A. Abaimov, T. A. Zenina-Antipova, G. I. Kovalev, and S. B. Seredenin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 3, pp. 300-303, March, 2008
Original article submitted June 29, 2007.

We studied the effects of antiparkinsonian drug himantane (acute and subchronic administration) on the content of dopamine transporter protein DAT in rat striatum *ex vivo* and on the content of DTA in cultured PC-12 cells (10^{-5}-10^{-7} M, the preparation was added to the incubation medium once or 7 times). The preparation significantly reduced the content of DAT protein both *ex vivo* and *in vitro*.

Key Words: *dopamine transporter; DAT; adamantane derivatives; striatum; himntane*

Adamantane preparations (midantan, memantin, and gludantan) are widely used in the therapy of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. Himantane (N-adamant-2-yl hexamethylenimine hydrochloride), a new Russian-made drug, also exhibits antiparkinsonian and dopamine-positive properties [1,5]. Dopamine reuptake system regulating extracellular concentration of the transmitter and the efficiency of synaptic transmission is a target for antiparkinsonian drugs of the adamantane family. Midantan after acute administration inhibits catecholamine reuptake in the striatum [6]. In *in vitro* experiments we showed that himantane can block dopamine reuptake via a non-competitive mechanism [4]. Chronic treatment with midantan significantly increased the rate of dopamine reuptake in synaptosomes from the striatum by 40% [11]. Molecular and biological mechanisms of these changes are involved and difficult for interpretation. Some authors believe that these effects are related to changes in the expression of dopamine transporter protein DAT, a membrane glycoprotein responsible for dopamine elimination from the synaptic space [9]. Others attribute these properties of adamantanes to their effects on phosphorylation processes, which can induce functional modifications of DAT without changes in the expression and content of transporter protein [11].

In this context, it was interesting to evaluate the effect of single (24 h) or subchronic (7 day) exposure to himantane in a concentration range of 10^{-5}-10^{-7} M on the content of DAT protein in a culture of DAT-positive [10] PC-12 pheochromocytoma cells.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 180-220 g. The animals were maintained at 12-h high-darkness regimen with free access to food and water (6 rats per cage) for 1 week before the experiment in a vivarium of Institute of Pharmacology, Russian Academy of Medical Sciences. In experiments with single treatment with himantane, the animals were decapitated 24 h after

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** g.kovalev@relcom.ru. G. I. Kovalev

0007-4888/08/1453-0331

intraperitoneal injection of the drug. In subchronic treatment, the animals received 20 mg/kg himantane intraperitoneally once a day for 7 days. Control animals received physiological saline (0.9% NaCl, 2 ml/kg intraperitoneally). The animals were decapitated 24 h after the last injection. The striatum was isolated [8] and homogenized.

In vitro experiments were carried out on cultured PC-12 cells (Cell Culture Museum, Institute of Molecular Genetics, Russian Academy of Sciences). PC-12 cells were seeded to 6-well plates (500×10^3 cells per well) in DMEM supplemented with 5% FBS. Experiments were performed on day 3 after seeding. The cells were cultured until confluence. Himantane was added to the culture medium in final concentrations of 10^{-5}-10^{-7} M during seeding and then every 48 h for 7 days. An equivalent volume of deionized water was added to wells with control cells.

The content of dopamine-transporter protein (DAT) in the cytoplasmic fraction of PC-12 pheochromocytoma cells and cytoplasmic fraction of rat striatum homogenates were analyzed by electrophoresis followed by immunoblotting. Protein content in samples was measured by the method of Folin—Lowry. The proteins were separated in 10% PAAG and transferred to PVDF membranes by electroelution for 45 min. Western blots were incubated in the presence of primary monoclonal antibodies to DAT (Santa Cruz Biotechnology) in 1:1000 dilution for 1.5 h. After washout, the blots were incubated in the presence of goat anti-mouse IgG (Santa Cruz Biotechnology) conjugated with horseradish peroxidase in dilution of 1:1000 for 1.5 h. DAT was visualized by the reaction with ECL reagents on Kodak films, the content of DAT protein was determined densitometrically.

The data were processed statistically using Statistica 6.0 software. The significance of differences between the control and experimental samples was verified using Student's *t* test.

RESULTS

Himantane reduced the content of DAT protein in striatum homogenates by 59% ($p<0.05$, paired *t* test) 24 h after single administration. After subchronic treatment, the content of DAT also decreased (by 35%), but was significantly higher than after single treatment (>60%, $p<0.05$, Student *t* test), which probably reflected a tendency to recovery of the initial content of DAT reduced under the effect of the preparation (Fig. 1).

Studies on cultured PC-12 pheochromocytoma cells showed that himantane exhibits activity in a narrow micromolar concentration range (Fig. 2). For instance, himantane in a concentration of 10^{-7} M had no effect on the content of DAT in cultured PC-12 cells, while after application of 10^{-5} M himantane, a tendency ($p=0.06$, Student's *t* test) to an increase in DAT content was observed 24 h after addition of the drug into the incubation medium (by ~20% compared to the control). The most pronounced changes in the content of DAT protein in PC-12 cell culture were observed at himantane concentration of 10^{-6} M: the preparation significantly (by 51%, $p<0.05$) reduced the content of DAT 24 h after addition. Himantane applied in the subchronic regimen also significantly decreased DAT content below the control level (by 43%, $p<0.05$, Student *t* test). However, we observed a tendency to an increase in DAT content by 20-22% from the level observed 24 h after the first application of the drug ($p=0.07$, Student *t* test).

The observed activity of himantane in a concentration of 10^{-6} M *in vitro* is consistent with the data obtained in previous experiments demonstrating high activity of himantane in the micromolar concentration range. It was shown that himantane is formed in micromolar concentration in the striatum after administration of the preparation in a dose

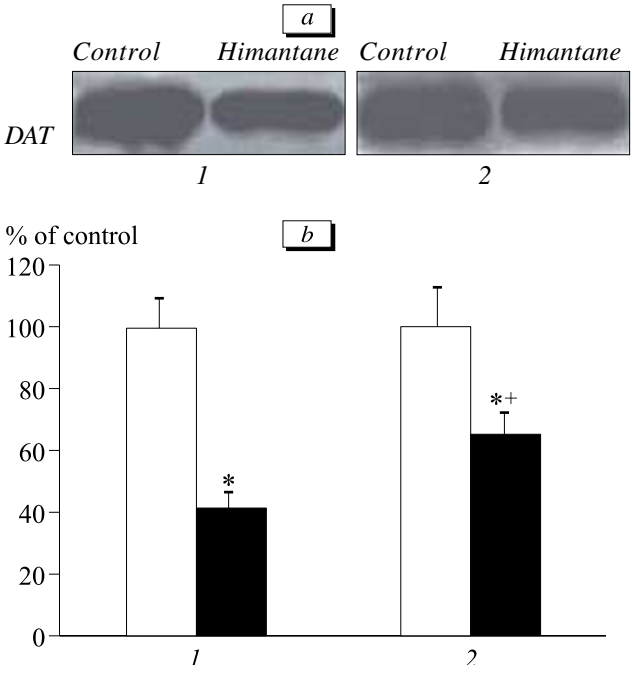

Fig. 1. Effect of single and subchronic administration of himantane on the content of dopamine transporter protein DAT in homogenates of the striatum of Wistar rats. Open bars: control; dark bars: himantane. Here and on Fig. 2: *1*) after 24 h, *2*) after 7 days. *a*) original blots; *b*) results of densitometry of original blots (3 independent experiments). Ordinate: content of DAT protein. $p<0.05$ compared to: *control (*t* test), +values 24 h after himantane administration.

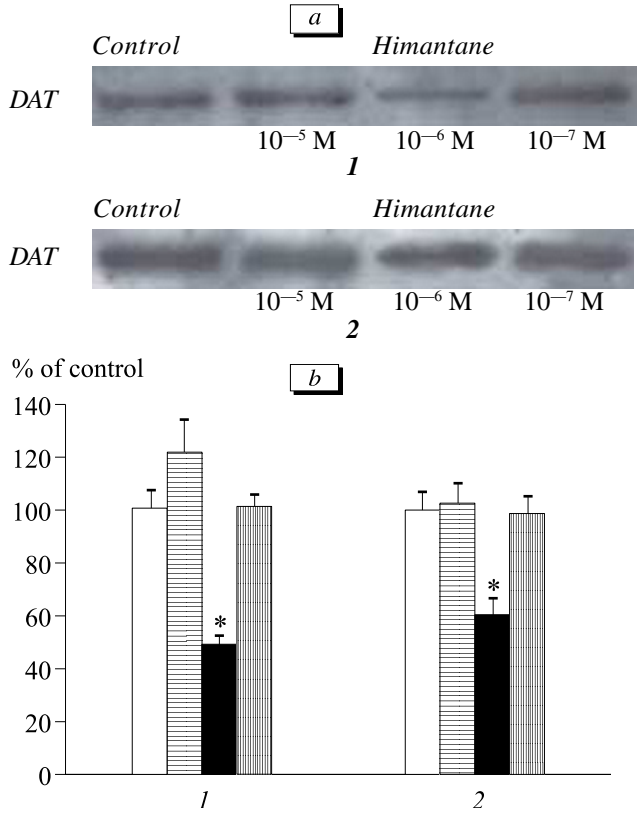

Fig. 2. Effect of himantane on the content of dopamine transporter protein DAT in culture of dopamine-positive PC-12 pheochromocytoma cells. Open bars: control; horizontal shading 10^{-5} M himantane; dark bars: 10^{-6} M himantane; vertical shading 10^{-7} M himantane.

of 20 mg/kg [3], K_i (inhibition constant) of himantane for NMDA receptor ionic channel and IC_{50} (50% inhibition constant) of [^{3}H]-dopamine reuptake lie in the micromolar himantane concentration range [2,3].

These findings attest to important contribution of DAT protein expression into the realization of dopamine-positive effects of antiparkinsonian drug himantane. It is known that elimination of DAT gene in mice leads to elevation of extracellular dopamine in the brain [7]. It can be hypothesized that himantane inhibits expression of dopamine transporter protein, which leads to a manifold increase in transmitter concentration in the synaptic space similar to that observed in DAT-knockout mice.

REFERENCES

1. E. A. Val'dman, L. N. Nerobkova, and T. A. Voronina, *Eksp. Klin. Farmakol.*, **67**, No. 1, 7-10 (2004).
2. M. V. Elshanskaya, A. I. Sobolevskii, E. A. Val'dman, and B. I. Khodorov, *Ibid.*, **64**, No. 1, 18-21 (2001).
3. G. I. Kovalev, A. P. Rodionov, E. S. Petrenko, *et al., Ibid.*, **66**, No. 3, 50-52 (2003).
4. G. I. Kovalev, D. A. Abaimov, M. V. Voronin, *et al., Neirokhimiya*, **24**, No. 2, 150-156 (2007).
5. L. N. Nerobkova, E. A. Val'dman, T. A. Voronina, *et al., Eksp. Klin. Farmakol.*, **63**, No. 3, 3-6 (2000).
6. W. Danysz, C. G. Parsons, J. Kornhuber, *et al., Neurosci. Biobehav. Rev.,* **21**, No. 4, 455-468 (1997).
7. S. R. Jones, R. R. Gainetdinov, R. M. Wightman, and M. G. Caron, *J. Neurosci.*, **18**, No. 6, 1979-1986 (1998).
8. J. Glowinski and L. Iversen, *J. Neurochem.*, **13**, No. 8, 655-669 (1966).
9. I. Gordon, R. Weizman, and M. Rehavi, *Eur. J. Pharmacol.*, **298**, No. 1, 27-30 (1996).
10. T. Kadota, T. Yamaai, Y. Saito, *et al., J. Histochem. Cytochem.*, **44**, No. 9, 989-996 (1996).
11. G. Page, M. Peeters, J. Maloteaux, *et al.*, *Eur. J. Pharmacol.*, **403**, No. 1, 75-80 (2000).