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Mildronate as a Regulator of Protein Expression in a Rat Model of Parkinson's Disease

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Summary. *Background. Mildronate (3-[2,2,2-trimethylhydrazinium] propionate dihydrate) traditionally is a well-known cardioprotective drug. However, our recent studies convincingly demonstrated its neuroprotective properties. The aim of the present study was to evaluate the influence of mildronate on the expression of proteins that are involved in the differentiation and survival of the nigrostriatal dopaminergic neurons in the rat model of Parkinson's disease (PD). The following biomarkers were used: heat shock protein 70 (Hsp70, a molecular chaperone), glial cell line-derived nerve growth factor (GDNF, a growth factor promoting neuronal differentiation, regeneration, and survival), and neural cell adhesion molecule (NCAM).*

Material and Methods. PD was modeled by 6-hydroxydopamine (6-OHDA) unilateral intrastriatal injection in rats. Mildronate was administered at doses of 10, 20, and 50 mg/kg for 2 weeks intraperitoneally before 6-OHDA injection. Rat brains were dissected on day 28 after discontinuation of mildronate injections. The expression of biomarkers was assessed immunohistochemically and by Western blot assay.

Results. 6-OHDA decreased the expression of Hsp70 and GDNF in the lesioned striatum and substantia nigra, whereas in mildronate-pretreated (20 and 50 mg/kg) rats, the expression of Hsp70 and GDNF was close to the control group values. NCAM expression also was decreased by 6-OHDA in the striatum and it was totally protected by mildronate at a dose of 50 mg/kg. In contrast, in the substantia nigra, 6-OHDA increased the expression of NCAM, while mildronate pretreatment (20 and 50 mg/kg) reversed the 6-OHDA-induced overexpression of NCAM close to the control values.

Conclusion. The obtained data showed that mildronate was capable to regulate the expression of proteins that play a role in the homeostasis of neuro-glial processes.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra (SN) and depletion of the neurotransmitter dopamine in the striatum (1). The conventional therapy for PD is focused mostly on the stimulation of the already degenerating dopamine cells (2). The novel pharmacotherapeutic strategies that may open new avenues in the treatment of PD are being developed in order to prevent or delay the earlier stages of neurodegenerative cascades. Therefore, the search for cell targets that could be essential to be regulated by novel drugs are on agenda of PD researchers.

Mitochondria are considered as one of essential targets. Mitochondrial damage plays a crucial role in the development of oxidative stress, which is the primary pathogenetic mechanism of nigral dopaminergic cell death in PD (3). Particularly, derangements of complex I are considered as central and early cause of PD, since deficiency in complex I activity initiates further respiratory chain dysfunction that involves mitochondria-dependent apoptotic cascades resulting in cell death (4).

In addition, the aggregation of misfolded proteins (e.g., α-synuclein) plays a crucial role in PD, because they activate proinflammatory molecules, hence switch on the neuroinflammatory and apoptotic events. Recent evidence suggests that misfolded proteins and their aggregates are in close relation with the activities of endogenous or molecular chaperons, the intracellular molecules capable of unfolding the protein conformation (5). Thus, molecular chaperones are proteins that recognize and selectively bind nonnative proteins to form stable complexes, hence preventing misfolding and aggregation of folding intermediates and keep proteins on the productive folding pathway (6). Molecu-

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lar chaperones comprise several highly conserved families of unrelated proteins, and many of them are ubiquitous and belong to the heat shock protein (Hsp) family. Hsps are molecular chaperones that assist in the proper folding of newly synthesized proteins as well as those subjected to stress-induced denaturation. Hsps also exhibit a variety of cytoprotective functions and inhibition of apoptosis (7, 8). It has been demonstrated that Hsp70 overexpression reduces α-synuclein accumulation and toxicity in both mouse and *Drosophila* models of PD (8, 9). Furthermore, it has been recently shown that 6-hydroxydopamine (6-OHDA) induces the heat shock response, leading to the increased levels of Hsp25 and Hsp70 (10). However, there are contradictory data that overexpressing Hsp70 in a mouse model of alpha-synucleinopathy did not lower the toxic load of alpha-synuclein species and had no beneficial effect on alpha-synuclein-related motor deficits (11).

Recent evidence has implicated macroglia (astrocytes) and microglia cell activation in the initiation and progression of PD (12). Moreover, activated astrocytes may promote neurogenesis by producing energy substances and growth factors. Glial cell-derived neurotrophic factor (GDNF) is a proposed substance to promote survival and differentiation of dopamine neurons (13). It has been recently demonstrated that chronic infusion of GDNF prevents 6-OHDA-induced dopaminergic cell degeneration in a rat model of Parkinson's disease (14).

In addition, recent data suggest that neural cell adhesion molecule (NCAM) plays an important role in neurogenesis, neural cell adaptation, and survival. Furthermore, NCAM has been identified as an alternative signaling receptor for GDNF (15).

It is increasingly recognized that the novel pharmacotherapy of PD is of great importance. It is expected that the drugs that could promote neuronal adaptation, differentiation, and survival would be beneficial. Our recent findings showed that mildronate, a representative of aza-butyrobetaine class, might regulate the expression of different proteins altered by neurotoxins 6-OHDA (PD model) and azidothymidine (AZT) (16, 17). Therefore, in PD model rats, mildronate increased the number of tyrosine hydroxylase-positive dopaminergic neurons and Notch-3-positive progenitors cells, and decreased the numbers of cells positive for glial fibrillary acidic protein (GFAP), inducible nitric oxide synthase (iNOS), ionized calcium binding adaptor molecule 1 (IBA-1), and ubiquitin (16). In AZT-induced neurotoxicity model in mice, mildronate suppressed the neuroinflammatory and apoptotic events by protecting abnormal expression of markers, such as cytochrome oxidase c, caspase 3, iNOS, cellular apoptosis susceptibility protein (CAS), and GFAP (17).

In addition, we have shown that 6-OHDA lesion caused a dramatic (5-fold) decrease in tyrosine hydroxylase (TH)-positive nerve endings and interneurons in the lesioned striatum and TH-positive neurons in substantia nigra compared with the control group. However, the administration of mildronate completely protected against a 6-OHDA-induced decrease in TH expression (16).

Furthermore, in studies on isolated rat liver mitochondria, mildronate showed mitochondria-protecting properties (17).

The aim of the present study was to investigate the effects of mildronate in a 6-OHDA rat model of PD by assessing cellular protein biomarkers that are involved in signaling cascades and that are crucial for neuronal survival, adaptation, and neural and glial integration: Hsp70, GDNF, and NCAM.

Materials and Methods

Animals. Male Wistar rats were obtained from the Laboratory of Experimental Animals, Riga Stradins University, Riga, Latvia. Each animal weighed 230–250 g at the beginning of the experiment. All of the experimental procedures were performed in accordance with the guidelines of Directive 86/609/EEC "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (1986) and were approved by the Animal Ethics Committee of the Food and Veterinary Service (Riga, Latvia).

Drugs. Mildronate (3-[2,2,2-trimethylhydrazinium] propionate dihydrate) was obtained from the Joint Stock Company "Grindex" (Riga, Latvia), dissolved in saline, and prepared as 2% stock solution. 6-OHDA was obtained from Sigma-Aldrich (St. Louis, MO, USA), dissolved in 0.2% ascorbic acid solution, and prepared as stock solution at a concentration of 20 μg/3 μL. Ketamine and imipramine were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Experimental Design. The rats were adapted to the experimental conditions and divided into 8 groups (8 animals per group). Mildronate or saline (control) in a volume of 1 mL/kg was administered intraperitoneally (IP) every day for 14 days at doses of 10, 20, and 50 mg/kg. On day 15, the neurotoxin 6-OHDA was administered into the right corpus striatum (i/str) at a concentration of 20 μg/3 μL. The control group received artificial cerebrospinal fluid (aCSF).

The groups were as follows: group 1, saline IP for 2 weeks followed by aCSF i/str; group 2, mildronate, 10 mg/kg IP for 2 weeks followed by aCSF i/str; group 3, mildronate, 20 mg/kg IP for 2 weeks followed by aCSF i/str; group 4: mildronate, 50 mg/kg IP for 2 weeks followed by aCSF i/str; group 5, saline IP for 2 weeks followed by 6-OHDA i/

str; group 6, mildronate, 10 mg/kg IP for 2 weeks followed by 6-OHDA i/str; group 7, mildronate, 20 mg/kg IP for 2 weeks followed by 6-OHDA i/str; group 8, mildronate, 50 mg/kg IP for 2 weeks followed by 6-OHDA i/str.

Surgical Procedures. After the intraperitoneal administration of mildronate or saline for 2 weeks, the rats were anesthetized (ketamine 75 mg/kg plus xylazine 10 mg/kg) and placed in a stereotaxic frame (Stoelting Inc., USA). Thirty minutes before the induction of general anesthesia, the rats received imipramine (at a dose of 20 mg/kg) to protect adrenergic neurons against the development of 6-OHDA-induced lesions. Anesthetized rats received 6-OHDA solution (20 μg/3 μL) or aCSF in the right striatum using a Stoelting microinjector. The injection rate was 1 μL/min, and the cannula was maintained in the delivery position for additional 3 minutes prior to its slow retraction. Coordinates (according to Paxinos & Watson) were as follows: AP+0.2; LM+2.5; DV-5.0 mm from the bregma.

Brain Tissue Processing. The brain tissue was cut into 10 μm-thick sections using a cryostat at –20°C (Leica CM1850, Leica Microsystems, Germany), and 24 sections each from the corpus striatum (striatum) and substantia nigra (s. nigra) were obtained.

The sections were transferred to polylysine-coated slides (3 sections on each slide). The slides were air-dried for 15 minutes, and then immersed in ice-cold acetone for 15 minutes and air-dried for 2 hours. The slides were wrapped in aluminum foil and stored at –20°C.

Immunohistochemical Examination of the Brain Tissue. Tissue sections 10 μm in thickness were stained to visualize the cells that were positive for Hsp70, GDNF, and NCAM. Endogenous peroxidase activity was blocked with 3.0% H_2O_2 for 10 minutes. Nonspecific primary antibody binding was blocked by incubating the slides with normal horse serum. These slides were incubated with rabbit polyclonal Hsp70 (AbCam, dilution at 1:100) or rabbit polyclonal GDNF (AbCam, 1:100) or rabbit polyclonal NCAM (AbCam, 1:100) for 1 hour at room temperature. The detection of bound antibody was performed using an EnVision reagent (Dako, Denmark).

The immunoperoxidase color reaction was developed by incubating the slides with diaminobenzidine for 5 minutes. A negative control without primary antibody was included in each experiment.

Imaging and Quantitation of Cells. Hsp70-, GDNF-, and NCAM-immunopositive cells were counted bilaterally in striatum and substantia nigra in 6 independent sections each from all of the experimental groups. The total number of positive cells was quantified at magnification ×400. The region of interest was captured using a Motic digital camera (Motic, China) mounted on a microscope (Motic BA400) using the Motic Image Advanced 3.2 software. Whole striatal and nigral sections were captured and analyzed. The results are expressed as cells per square millimeter.

Western Blot of the Brain Tissue. For the Western blot, frozen rat brain tissue was homogenized using RIPA lysis buffer (150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0.) and protease inhibitors (10 μM leupeptin, 1 μM pepstatin, 1 μM aprotinin, and 100 μM PMSF). The homogenate was spun at 2000 rpm for 10 minutes at 4°C. The supernatant was decanted and used for polyacrylamide gel electrophoresis (PAGE)-Western blot analysis to detect Hsp70, GDNF, and NCAM. The supernatant was spun at 12 000 rpm for 20 minutes at 4°C; the pellet was resuspended with homogenization buffer and used for PAGE-Western blot analysis. Brain homogenate proteins (20 μg of total extracts) were heated at 90°C for 5 minutes in 2× loading buffer and separated on 10% SDS-PAGE gels for 1 hour at 150 V. Proteins were transferred onto a PVDF membrane (Immobilon, Millipore) for 60 minutes at 150 mA. Membranes were blocked with 3% BSA in PBS for 1 hour and then incubated overnight at 4°C with one of the following antibodies: rabbit polyclonal anti-Hsp70 (1:10 000), anti-GDNF (1:5000), anti-NCAM (1:10 000), and rabbit polyclonal anti β-actin (1:5000). After washing with PBS, the blots were incubated for 30 minutes with peroxidase-coupled goat anti-rabbit IgG (1:10 000, Sigma-Aldrich) or rabbit anti-mouse IgG (1:10 000, Sigma Aldrich) and then washed again in PBS. The blots were developed using chemiluminescence reagents (GE Healthcare). Band densitometry analysis of the membrane was performed using scanned images of nonsaturated immunoblot films. Pixel intensities of the bands obtained in each experiment were normalized using β-actin signals.

Statistical Analysis. For the statistical analysis, the GraphPad Prism 5 software was used. The results are expressed as mean ± SEM, and the level of significance was $P<0.05$ (unpaired t test).

Results

Hsp70 Expression. The administration of 6-OHDA decreased the number of Hsp70-positive cells in the rat striatum in comparison with the control group (2±1 vs. 7±2 cells/mm^2, $P=0.03$; Fig. 1A). Mildronate per se at doses of 10, 20, and 50 mg/kg did not influence Hsp70 expression, whereas its pretreatment at doses of 20 and 50 mg/kg increased the number of Hsp70-positive cells in 6-OHDA-lesioned striatum in comparison with the 6-OHDA

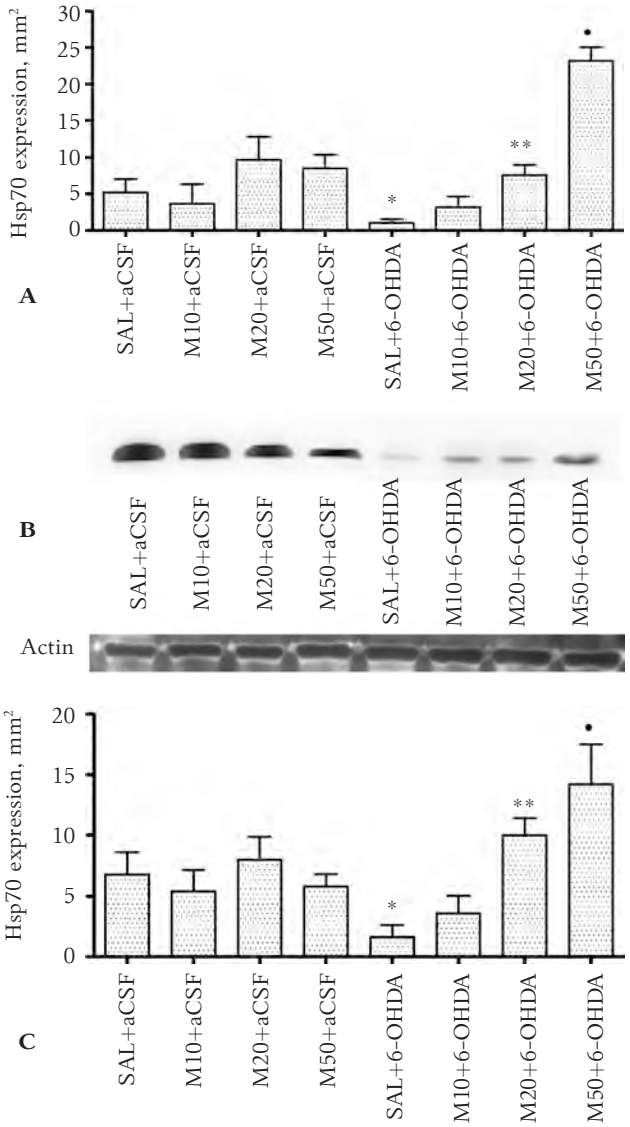

Fig. 1. The number of Hsp70-positive cells (A), Hsp70 protein expression assessed by Western blot (B) in the 6-OHDA-lesioned striatum, and the number of Hsp70-positive cells in the substantia nigra (C)
Immunohistochemical examination of rat tissue using an Hsp70 antibody. Saline (SAL, 1 mL/kg) and mildronate at doses of 10, 20, and 50 mg/kg (M10, M20, and M50) were administered intraperitoneally 2 weeks prior to the injection of 6-OHDA or artificial cerebrospinal fluid (aCSF); 6-OHDA injection in mildronate-treated rats: M10+6-OHDA, M20+6-OHDA, and M50+6-OHDA. Striatum: **P*=0.03, SAL+6-OHDA vs. SAL+aCSF; ***P*<0.0001, M20+6-OHDA vs. SAL+6-OHDA, and •*P*<0.0001, M50+6-OHDA vs. SAL+6-OHDA.
Substantia nigra: **P*=0.03, SAL+6-OHDA vs. SAL+aCSF; ***P*=0.0007, M20+6-OHDA vs. SAL+6-OHDA; and •*P*=0.003, M50+6-OHDA vs. SAL+6-OHDA; unpaired *t* test. Number of animals per group (n=8).

group (8±2 *vs.* 2±1, *P*=0.0009; and 23±2 vs. 2±1 cells/mm², *P*<0.0001; respectively). Similar results were obtained by Western blot analysis (Fig. 1B).

In the SN, the administration of 6-OHDA decreased the number of Hsp70-positive cells in comparison with the control group (2±1 *vs.* 7±2 cells/mm², *P*=0.03, Fig. 1C). Similarly, like as in the striatum, mildronate alone at doses of 10, 20, and 50 mg/kg did not influence Hsp70 expression, while mildronate pretreatment at doses of 20 and 50 mg/kg increased the number of Hsp70-positive cells in comparison with the 6-OHDA group (10±2 vs. 2±1, *P*=0.0007; and 14±4 vs. 2±1 cells/mm², *P*=0.003, respectively).

Fig. 2 shows the number of Hsp70-positive cells in the striatal tissue.

GDNF Expression. The administration of 6-OHDA decreased the number of GDNF-positive cells in the rat striatum in comparison with the control group (5±2 vs. 16±1 cells/mm², *P*=0.0004) (Fig. 3A). Mildronate alone at doses of 10 mg/kg did not influence GDNF expression; however, at doses of 20 and 50 mg/kg, it increased the number of GDNF-positive cells compared with the saline group (23±2 vs. 16±1, *P*=0.005; and 21±2 *vs.* 2±1 cells/mm², *P*=0.01; respectively). Similar data were obtained by Western blot analysis (Fig. 3B).

Mildronate pretreatment at doses of 20 and 50 mg/kg increased the number of striatal GDNF-positive cells in the 6-OHDA-lesioned striatum in comparison with the 6-OHDA group (18±2 vs. 5±2, *P*=0.001; and 24±3 vs. 5±2 cells/mm², *P*=0.0001). In the SN, GDNF expression did not differ significantly between the groups (Fig. 3C).

NCAM Expression. The administration of 6-OHDA decreased the number of NCAM-positive cells in the rat striatum in comparison with the control group (3±2 vs. 9±3 cells/mm², *P*=0.049) (Fig. 4A). Mildronate per se at a dose of 10 mg/kg tended to decrease NCAM expression; however, at doses of 20 and 50 mg/kg, it did not influence NCAM expression. Mildronate pretreatment at a dose of 50 mg/kg increased the number NCAM-positive cells in the 6-OHDA-lesioned striatum compared with the 6-OHDA group (17±5 vs. 3±2, *P*=0.008). Western blot analysis confirmed these data (Fig. 4B).

In the SN, the administration of 6-OHDA increased the number of NCAM-positive cells in comparison with the control group (14±2 vs. 7±2 cells/mm², *P*=0.005). Mildronate per se at doses of 10 and 50 mg/kg did not influence NCAM expression, whereas at a dose of 20 mg/kg, it increased NCAM expression compared with the control group (12±2 vs. 7±2 cells/mm², *P*=0.049). Mildronate pretreatment at doses of 10, 20, and 50 mg/kg decreased the number of NCAM-positive cells in comparison with the 6-OHDA group (Fig. 4C).

Discussion

Immunohistochemical and Western blot examination of brain tissues in our ex vivo study demonstrated that mildronate regulates the expression of biomarkers Hsp70, GDNF, and NCAM, which are

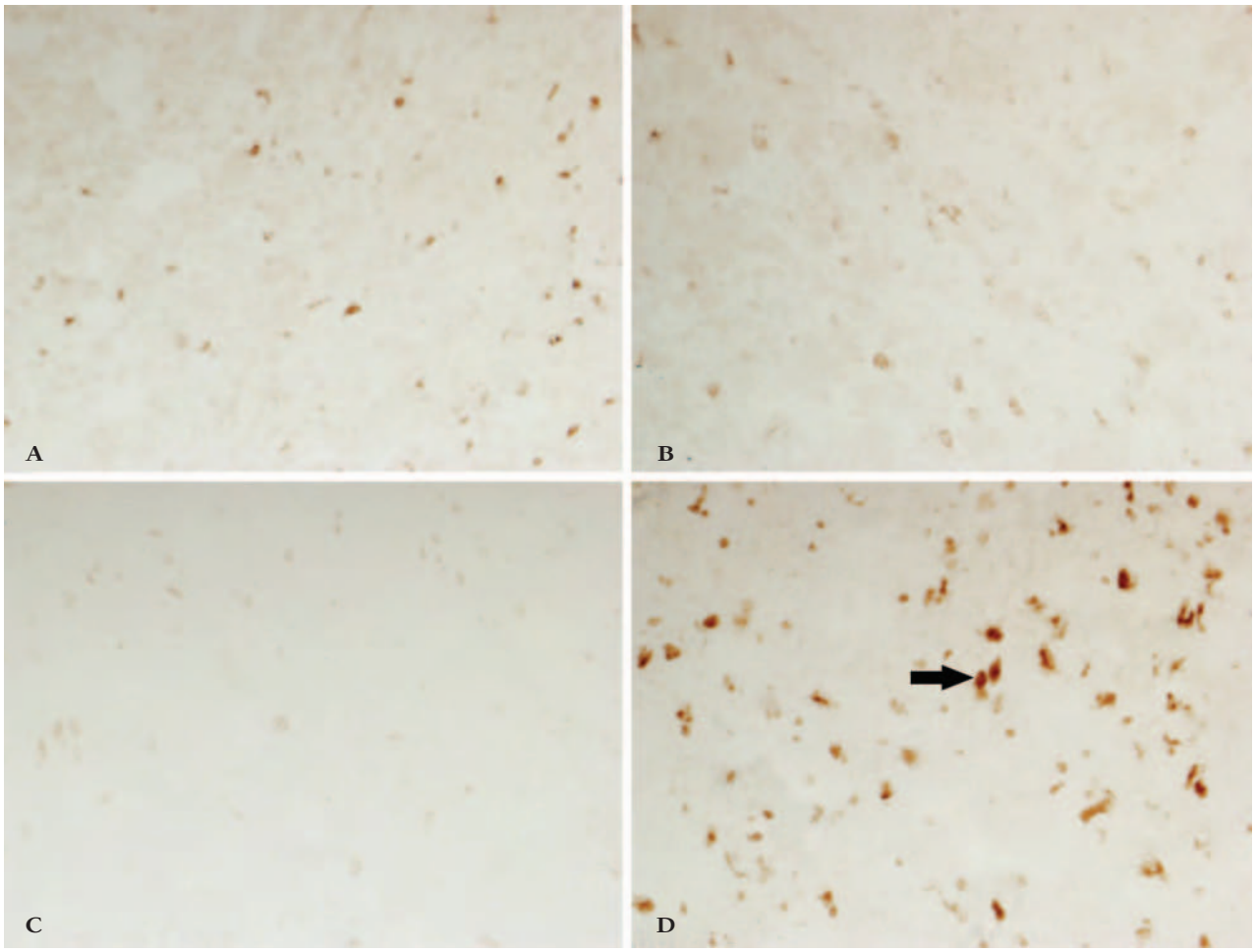

Fig. 2. Photomicrographs showing Hsp70 expression in the striatal tissue
A, SAL+aCSF; B, mildronate (M) at a dose of 50 mg/kg; C, SAL+6-OHDA; D, M50+6-OHDA.
An arrow indicates positively stained cells. Immunohistochemical staining method with an Hsp70 antibody (magnification ×200).

responsible for neuronal adaptation, survival, and neuronal glial communication.

In our study, 6-OHDA injection considerably decreased Hsp70 immunostaining in both the striatum and the SN. These findings are in agreement with literature data, which showed that the loss of Hsp70 activity is associated with neurodegeneration and the formation and induction of the pathological conversion of misfolded proteins into cytotoxic species such as amyloid deposits of alpha-synuclein, which constitute the intraneuronal inclusions in PD human patients known as Lewy bodies (8, 11). It was also demonstrated that the expression of lysosomal-associated membrane protein 2A (LAMP2A) and heat shock cognate 70 (hsc70) protein was significantly reduced in the substantia nigra of PD human brain and that was explained by chaperone-mediated autophagy activity in the PD brain (18). However, there are also contradictory data. For instance, in an in vitro study, 6-OHDA induced the heat shock response leading to the increased levels of Hsp25 and Hsp70 (10). Some other findings demonstrated that overexpression of Hsp70 in a mouse model of alpha-synucleinopathy did not lower the toxic load of alpha-synuclein species and had no beneficial effect on alpha-synuclein-related motor deficits (11).

Mildronate pretreatment (20 and 50 mg/kg) for 2 weeks increased the number of Hsp70-positive cells, which were decreased by 6-OHDA intrastriatal injections. In this context, one may suggest that mildronate in neurodegenerative conditions can act by regulating (normalizing) action on the expression of endogenous molecular chaperone molecule Hsp70. This effect may have a great importance, since Hsps recently have become a target in PD therapy (19). This action may be provided either by antioxidant activity and protecting effect of mildronate at the level of mitochondrial complex I (20) or/and by capability of mildronate to regulate ubiquitin proteasome pathway (16). However, one cannot exclude the influence of mildronate on protein conformation and its chemical chaperone-like properties. Mildronate molecule at least in part corresponds to requirements for chemical chaperons,

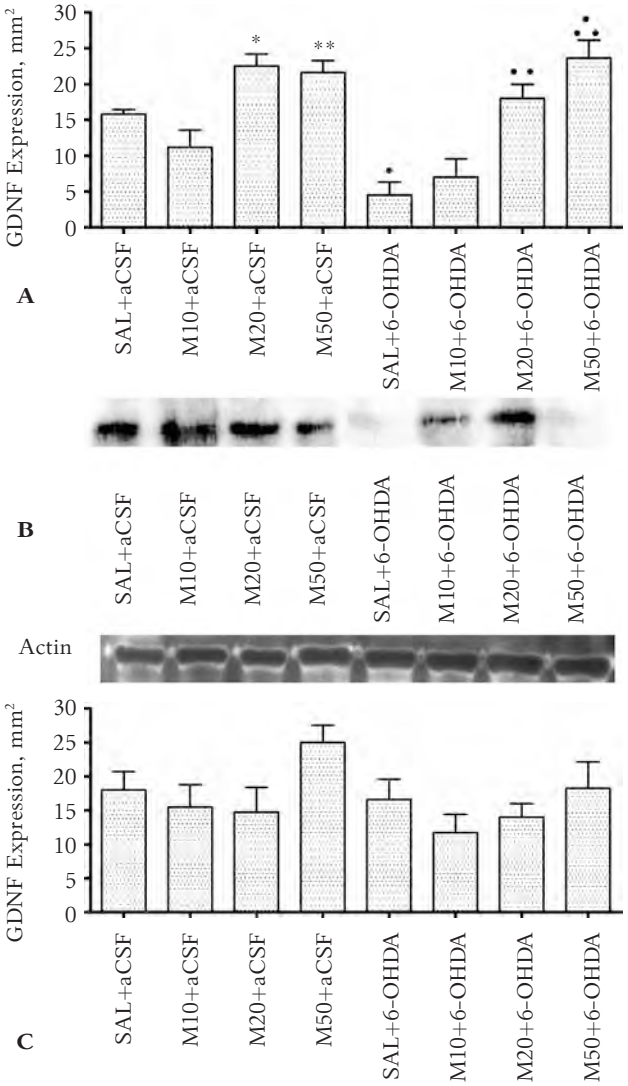

Fig. 3. The number of GDNF-positive cells in the 6-OHDA-lesioned striatum (A), GDNF protein expression assessed by Western blot (B) in the 6-OHDA-lesioned striatum, and the number of GDNF-positive cells in the substantia nigra (C)

Immunohistochemical and Western blot examination of rat tissue using a GDNF antibody. Saline (SAL, 1 mL/kg) and mildronate at doses of 10, 20, and 50 mg/kg (M10, M20, and M50) were administered intraperitoneally 2 weeks prior to the injection of 6-OHDA or artificial cerebrospinal fluid (aCSF). 6-OHDA injection in mildronate-treated rats: M10+6-OHDA, M20+6-OHDA, and M50+6-OHDA.

Striatum: **P*=0.05, M20+aCSF vs. SAL+aCSF; ***P*=0.01, M50+aCSF vs. SAL+aCSF, •*P*=0.0004, SAL+6-OHDA vs. SAL+aCSF; ••*P*=0.001, M20+6-OHDA vs. SAL+6-OHDA; and •••*P*=0.0001, M50+6-OHDA vs. SAL+6-OHDA; unpaired *t* test. Number of animals per group (n=8).

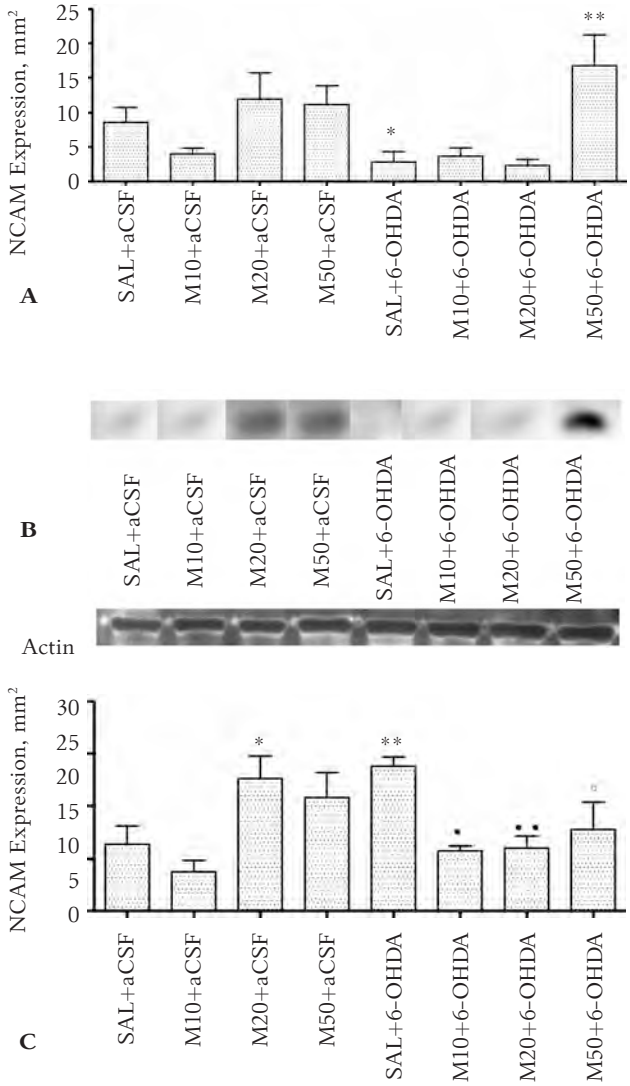

Fig. 4. The number of NCAM-positive cells (A), NCAM protein expression assessed by Western blot (B) in the 6-OHDA-lesioned striatum, and the number of NCAM-positive cells in the substantia nigra (C)

Immunohistochemical and Western blot examination of rat tissue using a NCAM antibody. Saline (SAL, 1 mL/kg) and mildronate at doses of 10, 20, and 50 mg/kg (M10, M20, and M50) were administered intraperitoneally 2 weeks prior to the injection of 6-OHDA or artificial cerebrospinal fluid (aCSF). 6-OHDA injection in mildronate-treated rats: M10+6-OHDA, M20+6-OHDA, and M50+6-OHDA.

Striatum: **P*=0.049, SAL+6-OHDA vs. SAL+aCSF; ***P*=0.008, M50+6-OHDA vs. SAL+6-OHDA.

Substantia nigra: **P*=0.04, M20+aCSF vs. SAL+aCSF; ***P*=0.049, SAL+6-OHDA vs. SAL+aCSF; •*P*=0.001, M10+6-OHDA vs. SAL+6-OHDA; ••*P*=0.001, M20+6-OHDA vs. SAL+6-OHDA; and °*P*=0.005, M50+6-OHDA vs. SAL+6-OHDA; unpaired *t* test. Number of animals per group (n=8).

since it possesses both quaternary nitrogen and negatively charged oxygen, which could easily bind to charged amino acids of cellular proteins and/or target protein-protein interfaces.

Our study demonstrated also a capability of mildronate at doses of 20 and 50 mg/kg to influence the expression of trophic protein GDNF in the striatum that was dramatically decreased by 6-OHDA. Interestingly, mildronate per se at these doses also increased GDNF expression. It is known that GDNF, which is produced in glial cells, is a substance that promotes survival and differentiation of dopamine neurons (13, 21). It has been recently demonstrated that chronic infusion of GDNF prevents 6-OHDA-

induced deficits in a rat model of Parkinson's disease (14). Furthermore, it was confirmed that GDNF has potent dopaminotrophic effects leading to a reliable recovery of phenotypic expression of tyrosine hydroxylase in surviving nigral neurons. These effects were long-lasting and sustained, remaining after the cessation of GDNF infusion (22). The capability of mildronate to increase GDNF expression is suggested to be beneficial in comparison with that of high-molecular antigenic trophic factors, because mildronate is a small molecule that lacks antigenicity. The question whether mildronate stimulates GDNF synthesis remains to be elucidated.

In addition, our data showed that mildronate at doses of 20 and 50 mg/kg normalizes the expression of NCAM close to the control values in the 6-OHDA lesioned striatum. Surprisingly, in rat SN, where 6-OHDA increased the expression of NCAM, mildronate also normalized the expression of NCAM close to the control group value. Besides, mildronate per se at a dose of 20 mg/kg increased the expression of NCAM in the SN as compared with the control group. Our results are consistent with other observations that NCAM is upregulated in the 6-OHDA-lesioned SN (23). The importance of the effect of mildronate cannot be overestimated, because NCAM has a role in cell-cell adhesion, neurite outgrowth, synaptic plasticity, and learning and memory (24).

Conclusions

Summarizing the obtained data, one may stress the capability of mildronate to normalize the 6-OHDA-altered expression of proteins involved in neuronal survival, such as molecular chaperone molecule Hsp70, trophic factor GDNF, and nerve adhesion molecule NCAM. These biomarkers may serve as the therapeutic targets in Parkinson's disease. The capability of mildronate to normalize the expression of an array of proteins involved in apoptosis, neuroinflammation, and microgliosis, reported in our previous studies, allow us to conclude that mildronate acts as a regulator of protein functioning at the levels of neuronal-glial communication. These properties of mildronate speak in favor of its beneficial effects in the treatment of Parkinson's disease.

Acknowledgments

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Statement of Conflict of Interest

The authors state no conflict of interest.

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