NEUROPROTECTIVE EFFECTS OF ISATIN AND AFOBAZOLE IN RATS WITH ROTENONE-INDUCED PARKINSONISM ARE ACCOMPANIED BY INCREASED BRAIN LEVELS OF TRITON X-100 SOLUBLE ALPHA-SYNUCLEIN

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Effects of the endogenous neuroprotector isatin and the pharmacological drug afobazole (exhibiting neuroprotective properties) on behavioral reactions and quantitative changes in the brain proteomic profile have been investigated in rats with experimental rotenone Parkinsonism. A single dose of isatin (100 mg/kg subcutaneously on the last day of a 7-day course of rotenone administration) improved the motor activity of rats with rotenone-induced Parkinsonism in the open field test (horizontal movements) and the rotating rod test. Afobazole (10 mg/kg intraperitoneally, daily during the 7-day course of rotenone administration) reduced the manifestations of rigidity and postural instability. Proteomic analysis, performed using brain samples obtained the day after the last administration of rotenone and neuroprotectors, revealed similar quantitative changes in the brain of rats with rotenone Parkinsonism. An increase in the relative content of 65 proteins and a decrease in the relative content of 21 proteins were detected. The most pronounced changes — an almost ninety-fold increase in the alpha-synuclein content — were found in the brains of rats treated with isatin. In animals of the experimental groups treated with "Rotenone + Isatin", as well as "Rotenone + Afobazole", the increase in the relative content of this protein in the brain was almost 60 and 50 times higher than the control values. Taking into consideration the known data on the physiological role of alpha-synuclein, an increase in the content of this protein in the brain upon administration of neuroprotectors to animals with rotenone Parkinsonism may represent a compensatory reaction, at least in the early stages of this disease and the beginning of its treatment.

Key words: Parkinsonism; neurotoxin rotenone; neurodegeneration; alpha-synuclein; neuroprotectors; isatin; afobazole; brain; proteomic profiling

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INTRODUCTION

The development of *in vivo* models is an important approach actively used in studies aimed at diagnostics and treatment of Parkinson's disease (PD), a common neurodegenerative disease characterized by degeneration of the nigrostriatal dopaminergic (DA) system. In this context, one of the most adequate and widely used models of PD in rats is the so-called rotenone Parkinsonism induced by administration of the neurotoxin rotenone. By inhibiting complex I of the mitochondrial respiratory chain, this pesticide causes degeneration of cells of the DA system and the development of motor disorders and other changes that are most similar in symptoms and molecular biological characteristics to those of PD [1–4].

Previously, in a mouse model of PD, induced by the administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the endogenous neuroprotector isatin decreased manifestations of movement disorders and influenced the proteomic profile of mitochondrial proteins [5, 6].

Isatin (indole-2,3-dione) is a biological regulator found in the brain, peripheral tissues, and biological fluids of humans and animals; it has a wide range of biological and pharmacological activities [7, 8]. Isatin is an inhibitor of monoamine oxidase type B (MAO-B), an enzyme responsible for the biotransformation of MPTP and the formation of an active neurotoxin (1-methyl-4-phenylpyridinium ion) [9, 10]. In addition to MAO-B, it interacts with numerous brain proteins, including proteins pathogenetically important for the development of PD and other neurodegenerative diseases [11, 12]. Since the mechanism of the development of rotenone-induced Parkinsonism differs from MPTP-induced Parkinsonism, it is of obvious interest to assess how universal the neuroprotective effect of isatin is in different experimental models of PD. In this regard, it should be noted that the classical MAO-B inhibitor deprenyl (of which isatin is considered an endogenous functional agonist [13]) exhibits its neuroprotective activity both in the MPTP-induced Parkinsonism [9, 10] and in the rotenone-induced Parkinsonism models [14].

The aim of this work was a comparative study of the effect of isatin and afobazole on the manifestations of rotenone-induced PD in rats, including physiological reactions and quantitative changes in the brain proteomic profile.

Afobazole (5-ethoxy 2-[2-(morpholino) ethylthio]-benzimidazole dihydrochloride) is an original anxiolytic developed at the V.V. Zakusov Research Institute of Pharmacology. It acts on a number of important receptor systems in the brain [15]. In a model of MPTP-induced Parkinsonism, afobazole reduced the severity of extrapyramidal disorders, decreased rigidity and improved movement activity and coordination in mice; in a model of rotenone-induced Parkinsonism in rats, afobazole reduced the severity of postural instability and reduced the mortality rate of rats [16].

MATERIALS AND METHODS

Reagents

The following reagents were used in the study: Tris (hydroxymethyl)aminomethane, ammonium bicarbonate, dithiothreitol, urea, guanidine hydrochloride, sodium chloride, Triton X-100, 4-vinylpyridine, Coomassie brilliant blue G-250 (Merck, USA); formic acid, sodium hydroxide (Acros Organics, USA), acetonitrile (Fisher Chemical, UK); isopropanol, trifluoroacetic acid (Fluka, USA); Tris-(2-carboxyethyl)phosphine (Pierce, USA); modified trypsin (mass spectrometry grade, Promega, USA). Other reagents of the highest available purity were from local suppliers.

Experimental Animals

The study was performed on outbred albino rats obtained from the Stolbovaya nursery (the Scientific Center for Biomedical Technologies, Russia). The animals were kept under standard vivarium conditions with free access to food and water under a twelve-hour light regime.

Modeling of Experimental Parkinsonism in Rats and Administration of the Neuroprotectors Isatin and Afobazole

Modeling of PD using systemic administration of rotenone was carried out according to the guidelines described in [17]. Rotenone solution in Miglyol 840 was administered intraperitoneally (i.p.) to rats at a daily dose of 2.75 mg/kg for 7 days. Rotenone solution was prepared as described in [18]. Control animals received daily i.p. injections of the equivalent volume of saline (0.2 ml/100 g) during 7 days. Isatin was administered subcutaneously 30 min before the rotenone injection and 1 h before behavioral testing. Afobazole was administered i.p. 30 min before the rotenone injection and 1 h before testing.

The animals were randomly subdivided into the following groups:

1) animals of the passive control group ("Control") received daily saline injection (7 days; 10 rats);

2) animals of the active control group ("Rotenone") received daily i.p. injection of rotenone at a dose of 2.75 mg/kg intravenously (7 days; 21 rats);

3) animals of the experimental group ("Rotenone + Isatin") received rotenone daily (7 days) and once, on day 7, isatin at a dose of 100 mg/kg subcutaneously (9 rats);

4) animals of the experimental group ("Isatin") received saline injections daily (6 days) and once, on day 7, isatin at a dose of 100 mg/kg subcutaneously (10 rats);

5) animals of the experimental group ("Rotenone + Afobazole") receiving daily i.p. injections of (7 days) afobazole 10 mg/kg and rotenone (16 rats);

6) animals of the experimental group ("Afobazole") received daily i.p. injections of Afobazole 10 mg/kg (7 days; 10 rats).

Behavioral Testing

In animals with experimental PD, the dynamics of body weight growth were recorded throughout the experiment versus the first day of the experiment before rotenone administration. Animal mortality was also recorded throughout the experiment. On day 7 of rotenone administration, behavioral reactions were tested to identify extrapyramidal disorders in the open field and rotating rod tests and assess postural instability and rigidity, as described in detail in [18].

Statistical data processing was carried out using the Statistica v. 10.0 program. The normality of distribution was checked using the Shapiro-Wilk test, followed by assessment of equality of variances using the Levene's test. Since there was no normal distribution in the experimental group, further processing was carried out using the nonparametric Mann-Whitney statistics method. The Fisher's exact test was used to evaluate categorical data in small groups. The results in the tables are presented as mean ± error of the mean (Mean ± SEM). Differences between the groups were considered significant at $p<0.05$.

Preparation of Brain Homogenate Lysates

On the next (eighth) day of the experiment the animals were decapitated under light ether anesthesia. Brain tissue (cerebral hemispheres) was homogenized using a Heidolph SilentCrusher homogenizer (Heidolph Instrument GmbH, Germany) (50,000 rpm) in 0.05 M potassium phosphate buffer (pH 7.4) to a final concentration of 30 mg/ml. To assess relative quantitative changes in the content of brain proteins in animals from different experimental groups, the same amount of total protein was used during sample preparation, which was controlled using the Bradford method [19]. After incubation in the presence of 3% Triton X-100 (4°C, 1 h),

the lysates were diluted 3 times with the same buffer and centrifuged for 30 min at 16,000 *g* to obtain a cleared supernatant.

Sample preparation for mass spectrometric analysis (protein extraction, alkylation, and trypsinolysis) was carried out as described previously [20].

The Mass Spectrometric Analysis

Mass spectrometric analysis was performed using an Ultimate 3000 RSLCnano high-performance liquid peptide separation system (Thermo Scientific, USA) in the nanoflow mode of a Q-Exactive HFX mass spectrometric detector (Thermo Scientific). The chromatographic separation of peptides was carried out on a Peaky C18 reverse phase analytical column (100 μm × 300 mm, 1.7 μm particle size, Molekta, Russia) in a linear elution gradient of mobile phase A (0.1% aqueous formic acid solution) and mobile phase B (80% acetonitrile, 0.1% formic acid) from 2% to 45% at a flow rate of 0.3 μl/min for 60 min, followed by washing the system with 99% B for 5 min and subsequent equilibration of the chromatographic system in the initial gradient conditions (A : B = 2 : 98) for 5 min.

The conditions for mass spectrometric analysis and bioinformatics data processing are detailed in [18].

Each of the proteins presented in the tables was identified in at least three independent experiments.

RESULTS AND DISCUSSION

Effects of the Neurotoxin Rotenone and the Neuroprotectors Isatin and Afobazole on the Behavioral Reactions of Rats

Under the conditions of the rotenone-induced PD model all rats treated with the toxin showed a negative dynamics of body weight throughout the experiment as compared to the group "Control". Afobazole and isatin had no effect on the body weight dynamics in animals.

Administration of rotenone caused the death of animals: on day 7 of the experiment, the mortality rate in the active control group was almost 29%, and on day 8 it reached already 43%. This significantly differed from the mortality rate in the passive control group. The percentage of dead animals during subchronic administration of afobazole on day 8 of the experiment was 5.4% less than in the "Rotenone" group. A single administration of isatin (100 mg/kg s.c.) to animals in the experimental groups "Isatin" and "Rotenone + Isatin" (see above) did not cause death of animals.

Administration of rotenone caused the development of oligokinesia in rats. On day 7 of the experiment in the "Open Field" test, a significant decrease in the horizontal (3.1-fold) and vertical (5.4-fold) locomotor activity was noted versus the group "Control", respectively, as well as a 4.4-fold decrease in search activity (the number of peeks into "holes") (Table 1). A single subcutaneous administration of isatin led to a significant decrease in the locomotor and orientation-exploratory activity of rats, which may be due to some known sedative effect of this compound. A single administration of isatin to rats treated with rotenone (the group "Rotenone + Isatin") did not reduce the activity of the animals as compared to the group "Isatin", while the number of movements was significantly higher (by 61.8%), than in the group "Rotenone". Afobazole, which was administered for 7 days before the behavioral test, had no effect on the locomotor and exploratory activity of rats in the "Open Field" test as compared to the group "Control". Afobazole did not reduce the severity of oligokinesia in rats with PD caused by 7-day administration of rotenone (Table 1).

In the rotating rod test performed on day 7 of the experiment, the rats with rotenone-induced PD demonstrated a 2.1-fold decrease in the duration of their retention on a rotating rod (10 rpm) (Table 2). A single administration of isatin 30 min before the last administration of rotenone (group "Rotenone + Isatin") reduced the locomotor deficit: the time spent on the rotating rod demonstrated a 1.7-fold increase as compared with the group "Rotenone". A seven-day administration of afobazole to rats together with rotenone caused an insignificant increase in the locomotor activity of rats by 1.2 times as compared to the group "Rotenone" (Table 2).

Systemic administration of rotenone caused the development of rigidity — the appearance of a "hump", which resulted in a significant decrease in the stride length of the animal by 1.4 times compared with this parameter in the group "Control" (Table 3). Afobazole administration decreased in the severity of rigidity, significantly increasing the step length of rats with PD (by 1.4 times) as compared to the group "Rotenone" (Table 3). Isatin administered once to rats with rotenone-induced PD did not reduce the severity of rigidity (Table 3).

The study of postural instability has shown that the development of rotenone-induced PD was accompanied by severe postural instability; on day 7 animals with rotenone-induced PD had to take a longer step to achieve body balance than animals of the group "Control". The step length of both the left and right front forelimbs in the rotenone-treated rats was significantly longer (by 1.8- and 1.5-fold, respectively) times than the corresponding stride length of the control group (Table 3). Isatin reduced the severity of postural instability in PD rats, reducing the step length of the animals by 1.3- and 1.2-fold as compared with the group "Rotenone". Afobazole also reduced the severity of postural instability in PD rats, resulted in a decrease in the step length of the front paws by 1.4 and 1.3 times as compared with the group "Rotenone" (Table 3).

Table 1. The effects of isatin and afobazole on behavior of rats with rotenone-induced PD in the open field test

Group of animals	Number of animals	Locomotor activity on day 7	
		Horizontal (number of movements)	Vertical (number of stands)
Control	10	37.8±5.5	8.7±1.3
Rotenone	12	10.2±1.9***	1.6±0.5***
Rotenone + Isatin	9	19.9±4.7#*	1.2±0.4***
Isatin	9	18.1±5.5*	2.6±0.5***
Rotenone + Afobazole	10	12.0±4.4**	1.2±0.5***
Afobazole	10	35.9±6.2	3.6±1.1**

*, **, *** – $p<0.05$, $p<0.01$, $p<0.001$, respectively, in comparison with the group "Control"; # – $p<0.05$ as compared with the group "Rotenone".

Table 2. The effects of isatin and afobazole on coordinated locomotor activity of rats with rotenone-induced PD

Group of animals	Number of animals	Time spent on the rotating rod, s
Control	10	162.2±15.1
Rotenone	8	78.6±17.2**
Rotenone + Isatin	8	135.1±18.8#*
Isatin	9	158.7±16.1
Rotenone + Afobazole	8	94.6±27.3*
Afobazole	10	162.2±15.3

Coordinated locomotor activity of rats was evaluated on day 7 of the experiment 100 min after the last rotenone administration. *, ** – $p<0.05$, $p<0.01$, respectively, in comparison with the group "Control"; # – $p<0.05$ as compared with the group "Rotenone".

Table 3. Rigidity and postural instability in rats with rotenone-induced PD

Group of animals	Number of animals	Rigidity	Postural instability	
		Step length, cm	Left forelimb, cm	Right forelimb, cm
Control	10	10.8±0.3	2.7±0.2	2.9±0.2
Rotenone	10	7.7±0.6***	4.8±0.2***	4.4±0.3***
Rotenone + Isatin	9	8.8±0.4***	3.7±0.2##**	3.7±0.2**
Isatin	9	10.9±0.4	2.7±0.3	2.6±0.2
Rotenone + Afobazole	8	10.3±0.7#	3.4±0.2##*	3.3±0.3#*
Afobazole	10	11.1±0.3	2.4±0.2	2.7±0.2

Rigidity and postural instability of rats was evaluated on day 7 of the experiment. **, *** – $p<0.01$, $p<0.001$, respectively, in comparison with the group "Control"; # – $p<0.05$ as compared with the group "Rotenone".

Thus, systemic administration of rotenone models severe PD, characteristic of the advanced stage of PD, accompanied by a significant body weight loss in animals, their possible death, oligokinesia, rigidity, and postural instability. The corrective effect of isatin, which was administered once subcutaneously at a dose of 100 mg/kg (the "Rotenone + Isatin"), was manifested as a decrease in postural instability, as well as a decrease in oligokinesia, recorded in terms of the locomotor activity in the "Open Field" and "Rotating Rod" tests. Subchronic 7-day administration of afobazole reduced the manifestations of rigidity and postural instability in PD rats and had no effect on other parameters studied under the conditions of this PD model. These results indicate involvement of different mechanisms for the neuroprotective effects of isatin and afobazole.

Effects of the Neurotoxin Rotenone and the Neuroprotectors Isatin and Afobazole on the Relative Content of Rat Brain Proteins

Proteomic analysis has shown that the neurotoxin rotenone and the neuroprotectors afobazole and isatin have a significant impact on the relative content of eighty-six proteins belonging to various functional groups in the rat brain (Supplementary Materials, Table S1). At the same time, similar quantitative changes were found in the brain of PD treated with isatin or afobazole. An increase in the relative content of 65 proteins and a decrease in the relative content of 21 proteins were detected (76% and 24% of the total, respectively, see Fig. 1A). The proteins, demonstrating increased relative content (both in the case of separate administration

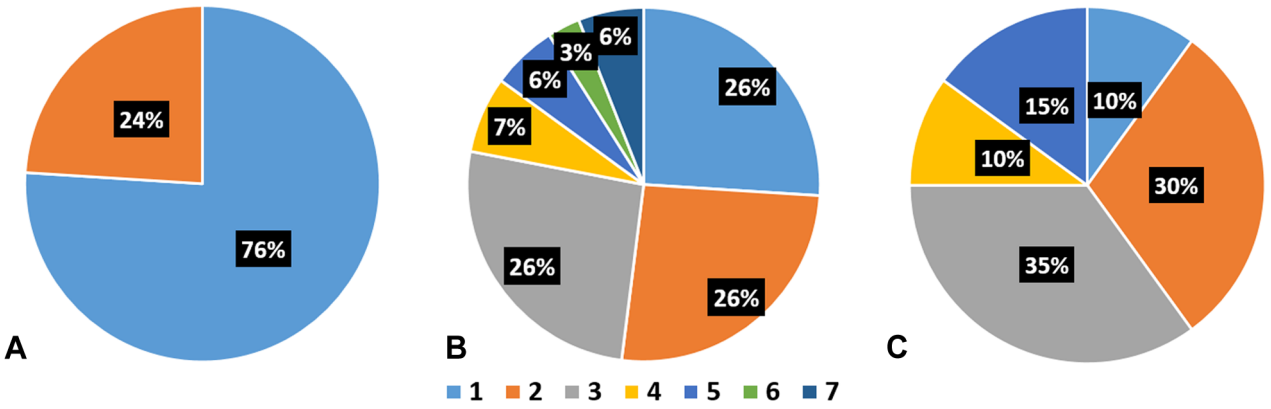

Figure 1. A – The proportion of brain proteins with the altered relative content (increased or decreased) in response to treatment of rats with neurotoxin rotenone and the neuroprotectors isatin and afobazole (percent of total proteins with altered relative content). **B**, **C** – Functional distribution of brain proteins with altered protein content in response to treatments of rats with rotenone and the neuroprotectors; **B** – increase, **C** – decrease. Functional groups of proteins: *1*. Proteins/enzymes involved in the processes of energy generation and carbohydrate metabolism. *2*. Proteins involved in the formation of the cytoskeleton and exocytosis. *3*. Proteins involved in signal transmission and regulation of enzyme activity. *4*. Antioxidant and protective proteins/enzymes. *5*. Proteins involved in regulation of gene expression, cell division and differentiation. *6*. Enzymes involved in the metabolism of proteins, amino acids and other nitrogenous compounds. *7*. Enzymes involved in lipid metabolism. A color version of the figure is available in the electronic version of the article.

of rotenone or neuroprotectors and in the case of combined administration of the neurotoxin with the neuroprotectors) belonged to several functional groups. These included groups of enzymes responsible for energy generation and carbohydrate metabolism, cytoskeletal and exocytosis proteins, and proteins involved in signal transduction and regulation of enzyme activity (each group represented 26% of the total number of proteins). The remaining 22% collectively included antioxidant and protective proteins, regulators of gene expression, cell division and differentiation, enzymes for the metabolism of proteins and other nitrogenous compounds, as well as enzymes for lipid metabolism (Fig. 1B). The highest proportions of proteins demonstrating decreased relative content in response to either separate administration of the neurotoxin and neuroprotectors or their combined injections were found in the groups of proteins involved in signal transduction and regulation of enzyme activity (35%) and cytoskeleton formation (30%). Other groups with decreased relative protein content included antioxidant and protective proteins, enzymes of energy generation and carbohydrate metabolism as well as proteins involved in regulation of gene expression, cell division and differentiation representing 10%, 10%, and 15% of the total number of proteins, respectively (Fig. 1C).

Among the proteins with altered relative content upon administration of the neurotoxin and the neuroprotectors isatin and afobazole, a group of mitochondrial proteins attracts special attention (Fig. 2); most of proteins are components of the cytochrome *c* oxidase complex and voltage-dependent ion channels. Our results are consistent with numerous literature data on the key role of mitochondrial dysfunction in the pathogenesis of PD (e.g. [21–23]).

Altered relative content influenced by rotenone, isatin, and afobazole (at their neurotoxin/neuroprotector combinations) has been also found in the brain proteins associated with neurodegeneration (Fig. 3). These included DJ-1 protein (Parkinson disease protein 7 homolog), a modulator of various signaling pathways [24–27], glyceraldehyde-3-phosphate dehydrogenase [28–32], TRIM2 (E3-ubiquitin ligase Tripartite motif-containing protein 2) [33, 34], the inner mitochondrial membrane receptor Prohibitin-2, a participant in the signaling pathway (which also includes Parkinsonism markers PINK1 and Parkin) that triggers mitophagy [35, 36], and also components of voltage-dependent ion channels (Fig. 3). The membrane protein wolframin (WFS1), responsible for the regulation of endoplasmic reticulum stress and intracellular calcium transport, also underwent quantitative changes [37]. Mutations of the *Wfs1* gene cause a rare autosomal recessive neurodegenerative disease, Wolfram syndrome [38]. At the same time, a decrease in the increased content of brain proteins associated with neurodegeneration, detected in PD rats, in response to the administration of isatin and/or afobazole (Fig. 2 and 3) was noted only for components of voltage-dependent ion channels (Vdac, Voltage-dependent anion-selective channel proteins) 1, 2 and 3. In all other cases, no antagonism was found between the effects of administration of the neurotoxin rotenone and the neuroprotectors isatin and afobazole.

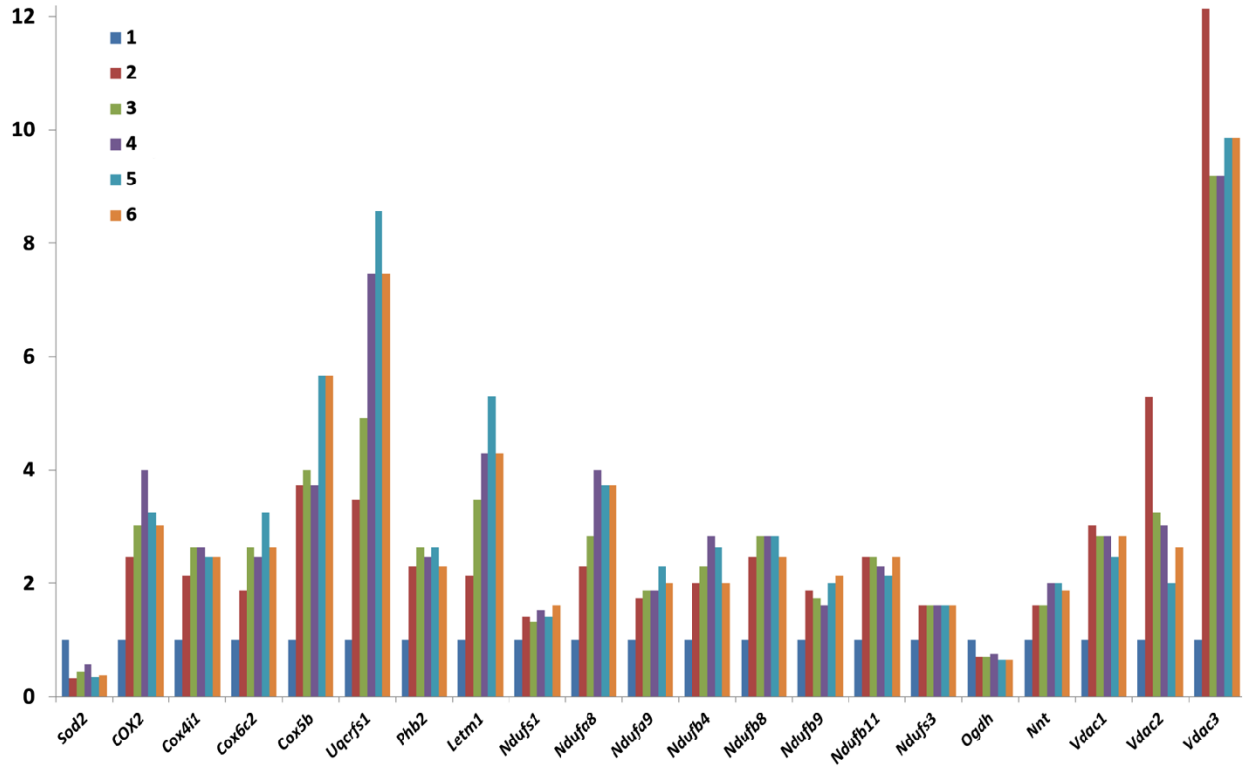

Figure 2. Fold changes in the relative content of mitochondrial proteins in the brain of rats treated with rotenone, isatin, and afobazole. The diagram shows the names of the genes. The Supplementary Materials provide accession numbers and names of proteins according to the Uniprot database (see table to Figure 2). *P*-values for each measurement are given in the overall table S1 (see Supplementary materials). Here and in the figure 3 the following experimental groups are shown: *1*. Control; *2*. Rotenone; *3*. Rotenone + Isatin; *4*. Isatin; *5*. Rotenone + Afobazole; *6*. Afobazole. A color version of the figure is available in the electronic version of the article.

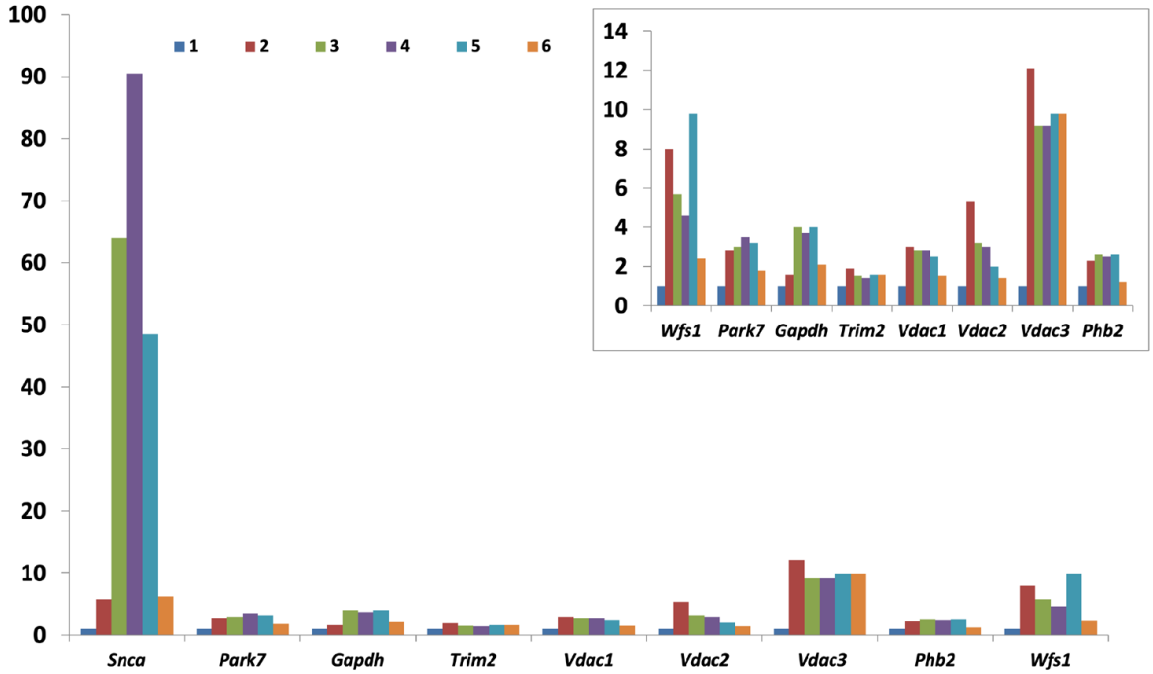

Figure 3. Fold changes in the relative content of brain proteins associated with the pathogenesis of PD, which were induced in rats by administration of rotenone, isatin and/or afobazole. In the inset, for better illustration of changes in the relative content of all other proteins, alpha-synuclein, characterized by the most pronounced changes in the relative content, was excluded. The diagrams indicate the names of the genes. The Supplementary Materials provide accession numbers and names of proteins according to the Uniprot database (see table to Figure 3). *P*-values for each measurement are given in the overall table S1 (see Supplementary materials). A color version of the figure is available in the electronic version of the article.

Among all the brain proteins characterized by relative quantitative changes identified in this study in rats treated with rotenone and/or isatin and afobazole, the most interesting results were obtained for alpha-synuclein (Fig. 3, as well as Supplementary Materials). This protein is considered to play a key role in the occurrence of PD [39–43].

Normally, alpha-synuclein is mainly localized in presynaptic terminals, where it enhances the fusion of synaptic vesicles with the presynaptic membrane. Alpha-synuclein has structural and functional homology with the 14-3-3 family proteins, chaperones, which (like alpha-synuclein) are widely abundant in the brain and are involved in the regulation of many cellular processes: apoptosis, cell cycle, division, gene expression, etc. [44]. In synucleinopathies, alpha-synuclein aggregates and can accumulate in association with the membranes of various cellular organelles. Its aggregates accumulate both in presynaptic terminals (thus inhibiting dopamine release) and in the neuron bodies in the form of Lewy bodies (which also contain lipids and membrane organelles, including autophagosomes) and their precursors, more diffuse structures known as “pale bodies” [41].

Cultivation of human neuroblastoma SK-N-MC cells for 1 week with 5 nM rotenone resulted in a 40% increase in soluble alpha-synuclein; long-term incubation of these cells for 4 weeks was accompanied by a 30% increase in synuclein, insoluble in 12% sodium dodecyl sulfate, while soluble synuclein maintained at the increased level [45]. In our experiments, a change in the relative content of synuclein was detected in the supernatant of the brain homogenate lysate with a final content of Triton X-100 of 1% (see section Materials and Methods). According to other authors, synuclein forms soluble in 1%–2% Triton are represented mainly by monomers of various lengths with various post-translational modifications [46–48]. Taking these data into account, the increase in the relative content of Triton X-100 soluble alpha-synuclein, found in this study during proteomic profiling, is apparently due to increased production and/or decreased degradation of the detergent soluble protein. It should be noted that the assessment of relative changes in brain proteins in rotenone-induced Parkinsonism was performed without taking into account the possible effects of rotenone, isatin, and afobazole on brain cell volume. It is known, for example, that under conditions of depletion of ATP resources in cells, it can increase by 200% [49] and thereby affect the relative quantitative results of proteomic analysis. It cannot be excluded that this factor has influenced the relative values of brain proteins caused by the administration of the studied neurotoxin and neuroprotectors.

Administration of rotenone increased the relative content of Triton X-100-soluble synuclein in the brain, and administration of isatin or afobazole to intact animals led to a significant increase in the relative content of this protein in the brain. This indicates that this effect is determined by administration of the studied neuroprotectors.

The neuroprotective role of alpha-synuclein has previously been shown. Overexpression of human alpha-synuclein protected rat dopaminergic neurons (N27 cells) from the toxic effects of MPP^+ (1-methyl-4-phenylpyridinium) by interacting with the proapoptotic factors BAD and PKCδ. Using the TSM1 cell line (mouse nerve cells), da Costa et al. observed the protective function of wild-type alpha-synuclein (but not alpha-synuclein with the Ala-53-Thr amino acid substitution associated with PD) in response to various apoptotic stimuli [51]. In experiments on transgenic mice, overexpression of alpha-synuclein protected against neurodegeneration caused by the toxic effects of the pesticide paraquat. These results indicate a possible correlation between toxin-induced alpha-synuclein accumulation and neurodegeneration [52].

Taking into consideration these and other known data on the physiological role of alpha-synuclein [53], an increase in the content of this protein in the brain upon administration of neuroprotectors to animals with rotenone parkinsonism may represent a compensatory reaction stimulated by neuroprotectors, at least in the early stages of this disease and the beginning of its treatment. This brings alpha-synuclein research back from the realm of pathophysiology to the realm of compensatory neurophysiology and cell biology.

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COMPLIANCE WITH ETHICAL STANDARDS

The experiments were carried out in compliance with the generally accepted norms of humane treatment of laboratory animals.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

REFERENCES

1. *Duty S., Jenner P.* (2011) Animal models of Parkinson's disease: A source of novel treatments and clues to the cause of the disease. Br. J. Pharmacol., **164**(4), 1357-1391. DOI: 10.1111/j.1476-5381.2011.01426.x
2. *Fleming S.M., Zhu C., Fernagut P.O., Mehta A., Dicarlo C.D., Seaman R.L., Chesselet M.F.* (2004) Behavioral and immunohistochemical effects of chronic intravenous and subcutaneous infusions of varying doses of rotenone. Exp. Neurol., **187**(2), 418-429. DOI: 10.1016/j.expneurol.2004.01.023
3. *Fleming S.M., Salcedo J., Fernagut P.O., Rockenstein E., Masliah E., Levine M.S., Chesselet M.F.* (2004) Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. J. Neurosci., **24**(42), 9434-9440. DOI: 10.1523/JNEUROSCI.3080-04.2004
4. *Cannon J.R., Tapias V.M., Na H.M., Honick A.S., Drolet R.E., Greenamyre J.T.* (2009) A highly reproducible rotenone model of Parkinson's disease. Neurobiol. Dis., **34**(2), 279-290. DOI: 10.1016/j.nbd.2009.01.016
5. *Medvedev A.E., Buneeva O.A., Kopylov A.T., Tikhonova O.V., Medvedeva M.V., Nerobkova L.N., Kapitsa I.G., Zgoda V.G.* (2017) Brain mitochondrial subproteome of Rpn10-binding proteins and its changes induced by the neurotoxin MPTP and the neuroprotector isatin. Biochemistry (Moscow), **82**(3), 330-339. DOI: 10.1134/S0006297917030117
6. *Buneeva O., Kopylov A., Kapitsa I., Ivanova E., Zgoda V., Medvedev A.* (2018) The effect of neurotoxin MPTP and neuroprotector isatin on the profile of ubiquitinated brain mitochondrial proteins. Cells, **7**(8), 91. DOI: 10.3390/cells7080091
7. *Medvedev A., Buneeva O., Gnedenko O., Ershov P., Ivanov A.* (2018) Isatin, an endogenous non-peptide biofactor: A review of its molecular targets, mechanisms of actions and their biomedical implications. Biofactors, **44**, 95-108. DOI: 10.1002/biof.1408
8. *Medvedev A., Buneeva O.* (2022) Tryptophan metabolites as mediators of microbiota-gut-brain communication: Focus on isatin. Front. Behav. Neurosci., **16**, 922274. DOI: 10.3389/fnbeh.2022.922274
9. *Tetrud J.W., Langston J.W.* (1989) MPTP-induced Parkinsonism as a model for Parkinson's disease. Acta Neurol. Scand., **126**, 35-40. DOI: 10.1111/j.1600-0404.1989.tb01780.x
10. *Langston J.W.* (2017) The MPTP story. J. Parkinsons Dis., **7**(s1), S11-S19. DOI: 10.3233/JPD-179006
11. *Buneeva O., Gnedenko O., Zgoda V., Kopylov A., Glover V., Ivanov A., Medvedev A., Archakov A.* (2010) Isatin binding proteins of rat and mouse brain: Proteomic identification and optical biosensor validation. Proteomics, **10**, 23-37. DOI: 10.1002/pmic.200900492
12. *Medvedev A., Kopylov A., Buneeva O., Kurbatov L., Tikhonova O., Ivanov A., Zgoda V.A.* (2020) Neuroprotective dose of isatin causes multilevel changes involving the brain proteome: Prospects for further research. Int. J. Mol. Sci., **21**(11), 4187. DOI: 10.3390/ijms21114187
13. *Medvedev A., Buneeva O., Gnedenko O., Fedchenko V., Medvedeva M., Ivanov Y., Glover V., Sandler M.* (2006) Isatin interaction with glyceraldehyde-3-phosphate dehydrogenase, a putative target of neuroprotective drugs: Partial agonism with deprenyl. J. Neural. Transm. Suppl., **71**, 97-103. DOI: 10.1007/978-3-211-33328-0_11
14. *Saravanan K.S., Sindhu K.M., Senthilkumar K.S., Mohanakumar K.P.* (2006) L-deprenyl protects against rotenone-induced, oxidative stress-mediated dopaminergic neurodegeneration in rats. Neurochem. Int., **49**(1), 28-40. DOI: 10.1016/j.neuint.2005.12.016
15. *Seredenin S.B., Voronin M.V.* (2009) Neuroreceptor mechanisms involved in the action of afobazole. Russian Journal of Experimental and Clinical Pharmacology, **72**(1), 3-11. DOI: 10.30906/0869-2092-2009-72-1-3-11
16. *Kapitsa I.G., Ivanova E.A., Val'dman E.A., Voronina T.A.* (2017) Activity of afobazole in experimental models of Parkinson's disease. Russian Journal of Experimental and Clinical Pharmacology, **8**(6), 3-7. DOI:10.30906/0869-2092-2017-80-6-3-7
17. *Voronina T.A., Seredenin S.B., Yarkova M.A., Voronin M.V.* (2012) Rukovodstvo po provedeniyu doklinicheskih issledovanij lekarstvennyh sredstv, chast' pervaya (Mironov A.N., ed.), Grif i K, Moskva, 994 p.
18. *Kapitsa I.G., Kazieva L.S., Vavilov N.E., Zgoda V.G., Kopylov A.T., Medvedev A.E., Buneeva O.A.* (2023) Characteristics of behavioral reactions and the profile of brain isatin-binding proteins of rats with the rotenone-induced experimental parkinsonism. Biomeditsinskaya Khimiya, **69**(1), 46-54. DOI: 10.18097/PBMC20236901046
19. *Bradford M.M.* (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., **72**, 248-254. DOI: 10.1006/abio.1976.9999
20. *Buneeva O.A., Kapitsa I.G., Kazieva L.S., Vavilov N.E., Zgoda V.G., Medvedev A.E.* (2023) Quantitative changes of brain isatin-binding proteins of rats with the rotenone-induced experimental parkinsonism. Biomeditsinskaya Khimiya, **69**(3), 188-192. DOI: 10.18097/PBMC20236903188
21. *Bose A., Beal M.F.* (2016) Mitochondrial dysfunction in Parkinson's disease. J. Neurochem., **139**(Suppl 1), 216-231. DOI: 10.1111/jnc.13731
22. *Borsche M., Pereira S.L., Klein C., Grünewald A.* (2021) Mitochondria and Parkinson's disease: Clinical, molecular, and translational aspects. J. Parkinsons Dis., **11**(1), 45-60. DOI: 10.3233/JPD-201981
23. *Sohrabi T., Mirzaei-Behbahani B., Zadali R., Pirhaghi M., Morozova-Roche L.A., Meratan A.A.* (2023) Common mechanisms underlying α-synuclein-induced mitochondrial dysfunction in Parkinson's disease. J. Mol. Biol., **435**(12), 167992. DOI: 10.1016/j.jmb.2023.167992
24. *Neves M., Grãos M., Anjo S.I., Manadas B.* (2022) Modulation of signaling pathways by DJ-1: An updated overview. Redox Biol., **51**, 102283. DOI: 10.1016/j.redox.2022.102283
25. *Buneeva O.A., Medvedev A.E.* (2021) DJ-1 protein and its role in the development of Parkinson's disease: Studies on experimental models. Biochemistry (Moscow), **86**(6), 627-640. DOI: 10.1134/S000629792106002X
26. *Betarbet R., Canet-Aviles R.M., Sherer T.B., Mastroberardino P.G., McLendon C., Kim J.H., Lund S., Na H.M., Taylor G., Bence N.F., Kopito R., Seo B.B., Yagi T., Yagi A., Klinefelter G., Cookson M.R., Greenamyre J.T.* (2006) Intersecting pathways to neurodegeneration in Parkinson's disease: Effects of the pesticide rotenone on DJ-1, alpha-synuclein, and the ubiquitin-proteasome system. Neurobiol. Dis., **22**(2), 404-420. DOI: 10.1016/j.nbd.2005.12.003
27. *de Miranda B.R., Rocha E.M., Bai Q., El Ayadi A., Hinkle D., Burton E.A., Greenamyre J.T.* (2018) Astrocyte-specific DJ-1 overexpression protects against rotenone-induced

neurotoxicity in a rat model of Parkinson's disease. Neurobiol. Dis., **115**, 101-114. DOI: 10.1016/j.nbd.2018.04.008
28. *Vanle B.C., Florang V.R., Murry D.J., Aguirre A.L., Doorn J.A.* (2017) Inactivation of glyceraldehyde-3-phosphate dehydrogenase by the dopamine metabolite, 3,4-dihydroxyphenylacetaldehyde. Biochem. Biophys. Res. Commun., **492**(2), 275-281. DOI: 10.1016/j.bbrc.2017.08.067
29. *Nakajima H., Amano W., Kubo T., Fukuhara A., Ihara H., Azuma Y.T., Tajima H., Inui T., Sawa A., Takeuchi T.* (2009) Glyceraldehyde-3-phosphate dehydrogenase aggregate formation participates in oxidative stress-induced cell death. J. Biol. Chem., **284**(49), 34331-34341. DOI: 10.1074/jbc.M109.027698
30. *Sofronova A.A., Pozdyshev D.V., Barinova K.V., Muronetz V.I., Semenyuk P.I.* (2021) Glycation of glyceraldehyde-3-phosphate dehydrogenase inhibits the binding with α-synuclein and RNA. Arch. Biochem. Biophys., **698**, 108744. DOI: 10.1016/j.abb.2020.108744
31. *Muronetz V.I, Melnikova A.K., Seferbekova Z.N., Barinova K.V., Schmalhausen E.V.* (2017) Glycation, glycolysis, and neurodegenerative diseases: Is there any connection? Biochemistry (Moscow), **82**(8), 874-886. DOI: 10.1134/S0006297917080028.
32. *Berry M.D.* (2004) Glyceraldehyde-3-phosphate dehydrogenase as a target for small-molecule disease-modifying therapies in human neurodegenerative disorders. J. Psychiatry Neurosci., **29**(5), 337-345. PMID: 15486605; PMCID: PMC518865
33. *Ylikallio E., Pöyhönen R., Zimon M., de Vriendt E., Hilander T., Paetau A., Jordanova A., Lönnqvist T., Tyynismaa H.* (2013) Deficiency of the E3 ubiquitin ligase TRIM2 in early-onset axonal neuropathy. Hum. Mol. Genet., **22**(15), 2975-283. DOI: 10.1093/hmg/ddt149
34. *Balastik M., Ferraguti F., Pires-da Silva A., Lee T.H., Alvarez-Bolado G., Lu K.P., Gruss P.* (2008) Deficiency in ubiquitin ligase TRIM2 causes accumulation of neurofilament light chain and neurodegeneration. Proc. Natl. Acad. Sci. USA, **105**(33), 12016-1221. DOI: 10.1073/pnas.0802261105
35. *Yan C., Gong L., Chen L., Xu M., Abou-Hamdan H., Tang M., Désaubry L., Song Z.* (2020) PHB2 (prohibitin 2) promotes PINK1-PRKN/Parkin-dependent mitophagy by the PARL-PGAM5-PINK1 axis. Autophagy, **16**(3), 419-434. DOI: 10.1080/15548627.2019.1628520
36. *Dutta D., Ali N., Banerjee E., Singh R., Naskar A., Paidi R.K., Mohanakumar K.P.* (2018) Low levels of prohibitin in substantia nigra makes dopaminergic neurons vulnerable in Parkinson's disease. Mol. Neurobiol., **55**(1), 804-821. DOI: 10.1007/s12035-016-0328-y
37. *Li L., Venkataraman L., Chen S., Fu H.* (2020) Function of WFS1 and WFS2 in the central nervous system: Implications for Wolfram syndrome and Alzheimer's disease. Neurosci. Biobehav. Rev., **118**, 775-783. DOI: 10.1016/j.neubiorev.2020.09.011
38. *Mishra R., Chen B.S., Richa P., Yu-Wai-Man P.* (2021) Wolfram syndrome: New pathophysiological insights and therapeutic strategies. Ther. Adv. Rare Dis., **2**, 26330040211039518. DOI: 10.1177/26330040211039518
39. *Sulzer D., Edwards R.H.* (2019) The physiological role of α-synuclein and its relationship to Parkinson's disease. J. Neurochem., **150**, 475-486.
40. *Ottolini D., Calí T., Szabò I., Brini M.* (2017) Alpha-synuclein at the intracellular and the extracellular side: Functional and dysfunctional implications. Biol. Chem., **398**(1), 77-100. DOI: 10.1515/hsz-2016-0201
41. *Oliveira L.M.A., Gasser T., Edwards R., Zweckstetter M., Melki R., Stefanis L., Lashuel H.A., Sulzer D., Vekrellis K., Halliday G.M., Tomlinson J.J., Schlossmacher M., Jensen P.H., Schulze-Hentrich J., Riess O., Hirst W.D., El-Agnaf O., Mollenhauer B., Lansbury P., Outeiro T.F.* (2021) Alpha-synuclein research: Defining strategic moves in the battle against Parkinson's disease. NPJ Parkinsons Dis., **7**, 65. DOI: 10.1038/s41531-021-00203-9
42. *Chen R., Gu X., Wang X.* (2022) α-Synuclein in Parkinson's disease and advances in detection. Clin. Chim. Acta., **529**, 76-86. DOI: 10.1016/j.cca.2022.02.006
43. *Atik A., Stewart T., Zhang J.* (2016) Alpha-synuclein as a biomarker for Parkinson's disease. Brain Pathol., **26**(3), 410-418. DOI: 10.1111/bpa.12370
44. *Ostrerova N., Petrucelli L., Farrer M., Mehta N., Choi P., Hardy J., Wolozin B.* (1999) α-Synuclein shares physical and functional homology with 14-3-3 proteins. J. Neurosci., **19**(14), 5782-5791. DOI: 10.1523/JNEUROSCI.19-14-05782.1999
45. *Sherer T.B., Betarbet R., Stout A.K., Lund S., Baptista M., Panov A.V., Cookson M.R., Greenamyre J.T.* (2002) An *in vitro* model of Parkinson's disease: Linking mitochondrial impairment to altered alpha-synuclein metabolism and oxidative damage. J. Neurosci., **22**(16), 7006-7015. DOI: 10.1523/JNEUROSCI.22-16-07006.2002
46. *Vasili E., Dominguez-Meijide A., Flores-Leyn M., Al-Azzani M., Kanellidi A., Melki R., Stefanis L., Outeiro T.F.* (2022) Endogenous levels of alpha-synuclein modulate seeding and aggregation in cultured cells. Mol. Neurobiol., **59**(2), 1273-1284. DOI: 10.1007/s12035-021-02713-2
47. *Bhattacharjee P., Öhrfelt A., Lashley T., Blennow K., Brinkmalm A., Zetterberg H.* (2019) Mass spectrometric analysis of lewy body-enriched α-synuclein in Parkinson's disease. J. Proteome Res., **18**(5), 2109-2120. DOI: 10.1021/acs.jproteome.8b00982
48. *Klucken J., Shin Y., Masliah E., Hyman B.T., McLean P.J.* (2004) Hsp70 reduces alpha-synuclein aggregation and toxicity. J. Biol. Chem., **279**(24), 25497-25502. DOI: 10.1074/jbc.M400255200
49. *Jurkowitz-Alexander M.S., Altschuld R.A., Hohl C.M., Johnson J.D., McDonald J.S., Simmons T.D., Horrocks L.A.* (1992) Cell swelling, blebbing, and death are dependent on ATP depletion and independent of calcium during chemical hypoxia in a glial cell line (ROC-1). J. Neurochem., **59**(1), 344-352. DOI: 10.1111/j.1471-4159.1992.tb08910.x
50. *Kaul S., Anantharam V., Kanthasamy A., Kanthasamy A.G.* (2005) Wild-type alpha-synuclein interacts with pro-apoptotic proteins PKCdelta and BAD to protect dopaminergic neuronal cells against MPP+-induced apoptotic cell death. Mol. Brain Res., **139**(1), 137-152. DOI: 10.1016/j.molbrainres.2005.05.022
51. *da Costa C.A., Ancolio K., Checler F.* (2000) Wild-type but not Parkinson's disease-related ala-53 --> Thr mutant alpha-synuclein protects neuronal cells from apoptotic stimuli. J. Biol. Chem., **275**(31), 24065-24069. DOI: 10.1074/jbc.M002413200
52. *Manning-Bog A.B., McCormack A.L., Purisai M.G., Bolin L.M., di Monte D.A.* (2003) Alpha-synuclein overexpression protects against paraquat-induced neurodegeneration. J. Neurosci., **23**(8), 3095-3099. DOI: 10.1523/JNEUROSCI.23-08-03095.2003
53. *Sulzer D., Edwards R.H.* (2019) The physiological role of α-synuclein and its relationship to Parkinson's disease. J. Neurochem., **150**(5), 475-486. DOI: 10.1111/jnc.14810

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НЕЙРОПРОТЕКТОРНЫЕ ЭФФЕКТЫ ИЗАТИНА И АФОБАЗОЛА СОПРОВОЖДАЮТСЯ УВЕЛИЧЕНИЕМ УРОВНЯ РАСТВОРИМОГО В ТРИТОНЕ Х-100 АЛЬФА-СИНУКЛЕИНА В МОЗГЕ КРЫС С ЭКСПЕРИМЕНТАЛЬНЫМ РОТЕНОНОВЫМ ПАРКИНСОНИЗМОМ

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Исследовали влияние эндогенного нейропротектора изатина и фармакологического препарата афобазола, проявляющего нейропротекторные свойства, на поведенческие реакции крыс с экспериментальным ротеноновым паркинсонизмом, а также на количественные изменения протеомного профиля мозга этих животных. Изатин (100 мг/кг подкожно однократно, на 7 день курсового введения ротенона) улучшал двигательную активность крыс с ротеноновым паркинсонизмом в тесте открытого поля (горизонтальные перемещения) и вращающегося стержня. Афобазол (10 мг/кг внутрибрюшинно, ежедневно в течение 7-дневного курса введения ротенона) снижал проявления ригидности и постуральной неустойчивости. Протеомный анализ, выполненный на следующий день после окончания введения нейропротекторов, выявил сходные количественные изменения в мозге крыс с ротеноновым паркисонизмом. Обнаружено увеличение относительного содержания 65 белков и снижение относительного содержания 21 белка. Наиболее выраженные изменения — почти девяностократное увеличение содержания альфа-синуклеина — обнаружены в мозге крыс, получавших изатин. У животных экспериментальных групп “Ротенон+Изатин”, “Ротенон+Афобазол” увеличение относительного содержания этого белка в мозге почти в 60 и 50 раз, соответственно, превышало контрольные значения. С учётом известных данных о физиологической роли альфа-синуклеина, увеличение содержания этого белка в мозге при введении нейропротекторов животным с ротеноновым паркинсонизмом может представлять компенсаторную реакцию, во всяком случае, на ранних стадиях этого заболевания и начала его лечения.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: паркинсонизм; нейротоксин ротенон; нейродегенерация; альфа-синуклеин; нейропротекторы; изатин; афобазол; мозг; протеомное профилирование

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