QUANTITATIVE CHANGES OF BRAIN ISATIN-BINDING PROTEINS OF RATS WITH THE ROTENONE-INDUCED EXPERIMENTAL PARKINSONISM

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Isatin (indoldione-2,3) is an endogenous regulator found in humans and animals. It exhibits a broad range of biological activity mediated by numerous isatin-binding proteins. Isatin produces neuroprotective effects in several experimental models of diseases, including Parkinsonism induced by the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Rotenone (a neurotoxin used to modeling Parkinson's disease in rodents) causes significant changes in the profile of isatin-binding proteins of rat brain. Comparative proteomic identification of brain proteins of control rats and the rats with the rotenone-induced Parkinsonian syndrome (PS) revealed significant quantitative changes of 86 proteins under the influence of rotenone. This neurotoxin mainly caused the increase of the quantity of proteins involved in signal transduction and regulation of enzyme activity (24), proteins involved in cytoskeleton formation and exocytosis (23), and enzymes involved in energy generation and carbohydrate metabolism (19). However, only 11 of these proteins referred to isatin-binding proteins; the content of eight of them increased while the content of three proteins decreased. This suggests that the dramatic change of the profile of isatin-binding proteins, found in the development of the rotenone-induced PS, comes from changes in the state of the pre-existing molecules of proteins, rather than altered expression of corresponding genes.

Key words: Parkinsonism; neurodegeneration; neurotoxin rotenone; brain; isatin; isatin-binding proteins; proteomic profiling

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INTRODUCTION

The neurotoxin rotenone is widely used for modeling of Parkinsonian syndrome (PS) in cell models and in rodents. The chronic administration of this substance causes the development of classic signs of Parkinson's disease: degeneration of dopaminergic neurons, accumulation of alpha-synuclein, mitochondrial dysfunction, and disruption of the ubiquitin-proteasome system [1–3].

In our experiments, the administration of rotenone to rats for 7 days led to the development of severe PS [4]. Animals showed significant body weight loss, oligokinesia, rigidity, and postural instability, as well as, in some cases, death of animals. Proteomic analysis of brain preparations from animals with rotenone-induced PS revealed changes in the profile of brain isatin-binding proteins [4].

Isatin (indoldione-2,3) is an endogenous regulator; its content in the body changes during oxidative stress and in various pathological conditions, including Parkinson's disease [5, 6]. The biological effects of isatin are mediated by altered expression of isatin-responsive genes [7] and interaction with numerous isatin-binding proteins. Administration of isatin causes neuroprotective effects in various experimental models of neurodegeneration [6, 8]. In experiments on mice with PS induced by the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), pretreatment of animals with isatin reduced the severity of locomotor impairments characteristic of this pathological condition and had a significant impact on the profile of isatin-binding proteins [9–12].

Altered profiles of proteins binding to an affinity ligand can reflect both changes (increase or decrease) in the level of these proteins in the studied object, and changes in the properties of already existing proteins. In this regard, the aim of this work was to study quantitative changes in brain isatin-binding proteins in rats with rotenone-induced experimental Parkinsonism.

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 the highest purity available were from local suppliers.

Experimental Animals

The study was performed on outbred albino rats obtained from the Stolbovaya nursery (the branch of 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

Modeling of PS using systemic administration of rotenone was carried out according to the guidelines described in [13]. Rotenone solution in Miglyol 840 was administered intraperitoneally to rats at a daily dose of 2.75 mg/kg for 7 days. Rotenone solution was prepared as described in [4]. Control animals received daily intraperitoneal injections of the equivalent volume of saline (0.2 ml/100 g) during 7 days.

Preparation of Brain Homogenate Lysates

Lysates of brain homogenate were prepared as described in [4].

For evaluation of quantitative changes of brain proteins, the same amount of total protein was used during sample preparation; it was controlled using the Bradford method [14]. Proteins were extracted with a chloroform-methanol mixture [15]. The protein sediment was dissolved in 8 M urea containing 20 mM dithiothreitol and 100 mM Tris-HCl (pH 8.5) and subjected to alkylation and subsequent trypsinolysis directly on Vivaspin 500 centrifuge membrane filters (Sartorius Stedim Biotech, Germany) with a 10,000 Da membrane, as described previously [4]. The reaction was stopped with formic acid (0.1% final concentration). The samples were evaporated using a 5301 vacuum concentrator (Eppendorf, Germany), dissolved in 0.1% formic acid, and analyzed using the equipment of the Center for collective use at the Institute of Biomedical Chemistry (Moscow).

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). Chromatographic separation of peptides was carried out on a Peaky C18 reverse phase analytical column (100 μm x 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 minutes 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 bioinformatic data processing have been described in details earlier [4].

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

RESULTS AND DISCUSSION

Comparative proteomic identification of brain proteins in control rats and rats with rotenone-induced PS showed that under the influence of rotenone, the relative content of 86 proteins significantly changed (Table 1, Table S1). Almost a quarter of them (19) belonged to mitochondrial proteins, mainly to the components of the cytochrome *c* oxidase complex and voltage-gated ion channels (Table 1, Table S1). At the same time, modeling of rotenone-induced PS was accompanied by the increase in brain proteins associated with the development of Parkinson's disease and other neurodegenerative pathologies (e.g. alpha-synuclein and others, see Table S1).

Table 1. Functional distribution of proteins with the altered relative content in the brain of rats with rotenone-induced Parkinsonian syndrome

Function	Total number in the group	Number of brain proteins after rotenone administration	
		↑	↓
Proteins involved in energy generation and carbohydrate metabolism	19	17	2
Proteins involved in cytoskeleton formation and exocytosis	23	17	6
Protein involved in signal transduction and regulation of enzyme activity	24	17	7
Antioxidant and protective proteins/enzymes	7	4	3
Protein regulators of gene expression, cell division and differentiation	7	4	3
Enzymes, involved in metabolism of proteins, amino acids and other nitrogenous compounds	2	2	0
Enzymes involved in lipid metabolism	4	4	0
Total	86	65	21

The arrows "↑" and "↓" show the increase or decrease of proteins in the functional group, respectively.

An analysis of the functional groups of proteins, characterized by changes in the relative content during PS modeling, showed that the rotenone administration mainly affected: (a) proteins involved in signal transduction and regulation of enzyme activity (24); (b) cytoskeleton and exocytosis proteins (23); (c) enzymes involved in energy generation and carbohydrate metabolism (19) (Table 1). In most cases, the relative content of these proteins as well as proteins of other functional groups increased after treatment of rats with rotenone (Table 1).

Treatment of rats with rotenone influenced the level of only 11 brain isatin-binding proteins: an increase in the level was noted in the case of 8 proteins, and the relative content of 3 proteins decreased (Table 2). This indicates that changes in the profile of isatin-binding proteins (69 isatin-binding proteins of the brain) found only in rats with rotenone-induced PS but not in control animals [4], are obviously determined by a change in the state existing protein molecules and, to a lesser extent, altered expression of the genes encoding them. This assumption can also be supported by the fact that *in vitro* modeling of oxidative stress had a significant effect on the interaction of GAPDH with the isatin ligand [16].

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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, in accordance with the Order of the Ministry of Health of the Russian Federation No. 199n dated April 1, 2016 “On Approval of the Rules for Good Laboratory Practice” and Directive 2010/63/EU of the European Parliament and the Council of the European Union dated September 22 2010 on the protection of animals used for scientific purposes.

Table 2. Brain isatin-binding proteins with the altered relative content in the brain of rats treated with rotenone (versus control)

№	Uniprot accession No.	Uniprot gene name	Uniprot protein name	Functions	Difference from control after rotenone administration	
					-Log(P-value)	Difference
1	P04797	*Gapdh*	Glyceraldehyde-3-phosphate dehydrogenase	1	2.4	0.7
2	P07943	*Akr1b1*	Aldo-keto reductase family 1 member B1	7	3.4	1.0
3	P19527	*Nefl*	Neurofilament light polypeptide	2	3.1	-0.8
4	P38983	*Rpsa*	40S ribosomal protein SA	5	4.0	1.5
5	P62744	*Ap2s1*	AP-2 complex subunit sigma	2	3.4	1.3
6	P62815	*Atp6v1b2*	V-type proton ATPase subunit B, brain isoform	2	4.4	0.8
7	Q63198	*Cntn1*	Contactin-1	3	3.0	1.9
8	Q6P0K8	*Jup*	Junction plakoglobin	2	3.0	3.4
9	A0A8I6A1Y1	*Ogdh*	Oxoglutarate dehydrogenase (succinyl-transferring)	1	3.4	-0.5
10	A0A8I6A304	*Basp1*	Brain abundant, membrane attached signal protein 1	3	5.1	4.2
11	A0A8I6A7U6	*Sfpq*	Splicing factor proline and glutamine rich	5	4.9	-1.5

Numbers in the column “Functions” designate the following groups of proteins: *1*. Proteins involved in energy generation and carbohydrate metabolism; *2*. Proteins involved in cytoskeleton formation and exocytosis; *3*. Proteins involved in signal transduction and regulation of enzyme activity; *4*. Antioxidant and protective proteins/enzymes; 5. Protein regulators of gene expression, cell division and differentiation; *6*. Enzymes, involved in metabolism of proteins, amino acids and other nitrogenous compounds; *7*. Enzymes involved in lipid metabolism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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

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КОЛИЧЕСТВЕННЫЕ ИЗМЕНЕНИЯ ИЗАТИН-СВЯЗЫВАЮЩИХ БЕЛКОВ МОЗГА У КРЫС С ИНДУЦИРОВАННЫМ РОТЕНОНОМ ЭКСПЕРИМЕНТАЛЬНЫМ ПАРКИНСОНИЗМОМ

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Изатин (2,3-диоксоиндол) — эндогенный регулятор, обнаруженный в организме человека и животных и обладающий широким спектром биологической активности, опосредованным большим количеством изатин-связывающих белков. Он проявляет нейропротекторное действие в ряде экспериментальных моделей заболеваний, включая паркинсонизм, индуцированный нейротоксином МФТП (1-метил-4-фенил-1,2,3,6-тетрагидропиридином). Ротенон — нейротоксин, используемый для моделирования у грызунов болезни Паркинсона, — вызывает существенное изменение профиля изатин-связывающих белков мозга у крыс. Сравнительная протеомная идентификация белков мозга контрольных крыс и крыс с ротенон-индуцированным паркинсоническим синдромом (ПС) выявила значимые количественные изменения 86-ти белков под воздействием ротенона. В основном нейротоксин вызывал увеличение количества белков, участвующих в передаче сигнала и регуляции активности ферментов (24), белков цитоскелета и экзоцитоза (23), а также ферментов, участвующих в процессах генерации энергии и углеводного обмена (19). Из этих белков всего 11 относилось к изатин-связывающим белкам; из них отмечено увеличение уровня у 8 белков, снижение — у 3. Это позволяет предположить, что радикальное изменение профиля изатин-связывающих белков, обнаруженное при развитии ротенон-индуцированного ПС, связано с изменением состояния существующих молекул белков и в меньшей степени с изменением экспрессии кодирующих их генов.

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

Ключевые слова: паркинсонизм; нейродегенерация; нейротоксин ротенон; мозг; изатин; изатин-связывающие белки; протеомное профилирование

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