BRAIN-DERIVED OLIGOPEPTIDES SHOW NEUROPROTECTIVE PROPERTIES IN RATS WITH PARKINSON-LIKE SYNDROME

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OLIGOPEPTIDI IZOLOVANI IZ MOZGA IMAJU NEUROPROTEKTIVNO DEJSTVO KOD PACOVA SA SINDROMOM SLIČNOM PARKINSONIZMU

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ABSTRACT

Objective. Our research was designed to study the neuroprotective action of the PC-2 peptide complex in rats with experimental Parkinson's disease.

Methods. To evaluate the efficacy of the PC-2 peptide complex in rats with experimental Parkinson's disease, levels of 8-isoprostane, noradrenaline, dopamine acetylcholine, glutamate, GABA, aspartate, glycine, as well as the activity of isocitrate dehydrogenase and alpha-ketoglutarate dehydrogenase were determined in homogenates of the frontal lobe of the brain.

Results. Treatment with the PC-2 peptide complex normalized concentrations of noradrenaline and acetylcholine, increased concentrations of dopamine (however, they did not reach the level in rats from the control group) on the 20th day from the beginning of treatment. The levels of excitatory amino acids in rats with experimental Parkinson's disease increased, while the concentrations of inhibitory amino acids in homogenates of the frontal lobe decreased. The PC-2 protein complex normalized the content of the studied amino acids on the 20th day of treatment. In addition, the content of oxidative stress markers increased in homogenates of the frontal lobe of the brain in rats with Parkinson's disease, which resulted in a decrease in the activity of mitochondrial enzymes and energy formation against the background of high glutamate levels. The normalization of the parameters of energy metabolism was achieved on the 20th day after the surgery.

Conclusion. The PC-2 peptide complex regulates the level of biogenic amines and neurotransmitter amino acids and, as a result, affects the energy metabolism in experimental Parkinson's disease.

Key words: Parkinson disease; peptides; neurotransmitter agents.

INTRODUCTION

Parkinson's disease is a chronic progressive disease that affects the central nervous system. It is the second most common neurodegenerative disorder after Alzheimer's disease. Nowadays approximately 3.7 million people worldwide suffer from this condition. According to WHO, the number of such patients will exceed 10 million people worldwide by 2030, which can be associated with

SAŽETAK

Cilj. Naše istraživanje je dizajnirano da prouči neuroprotektivno dejstvo PC-2 peptidnog kompleksa kod pacova sa eksperimentalnom Parkinsonovom bolešću.

Metode. Da bi se procenila efikasnost kompleksa PC-2 peptida kod pacova sa eksperimentalnom Parkinsonovom bolešću, koncentracije 8-izoprostana, noradrenalina, dopamina, acetilholina, glutamata, GABA-e, aspartata, glicina, kao i aktivnost izocitratne dehidrogenaze i alfa-ketoglutaratne dehidrogenaze određeni su u homogenatima frontalnog režnja mozga.

Rezultati. Tretman sa PC-2 peptidnim kompleksom dovodi do normalizacije koncentracija noradrenalina i acetilholina, povećanja koncentracija dopamina (međutim, nisu dosegli nivo kao kod pacova iz kontrolne grupe) 20. dana od početka terapije. Koncentracije ekscitatornih aminokiselina kod pacova sa eksperimentalnom Parkinsonovom bolešću povećali su se, dok su koncentracije inhibitornih aminokiselina u homogenatima frontalnog režnja smanjene. Proteinski kompleks PC-2 normalizovao je sadržaj proučavanih aminokiselina u 20. danu lečenja. Osim toga, sadržaj markera oksidativnog stresa povećan je kod homogenata frontalnog dela mozga kod pacova sa Parkinsonovom bolešću, što je rezultiralo smanjenjem aktivnosti mitohondrijalnih enzima i stvaranjem energije pri bazalno visokim koncentracijama glutamata. Normalizacija parametara energetskeg metabolizma postignuta je 20. dana nakon operacije.

Zaključak. Kompleks PC-2 peptida reguliše nivo biogenih amina i neurotransmiterskih aminokiselina i, kao rezultat toga, utiče na energetskeg metabolizam u eksperimentalnoj Parkinsonovoj bolesti.

Cljučne reči: Parkinsonova bolest; peptidi; neurotransmiterske supstance.

the trend of increasing the proportion of "aged" people in developed countries. This condition develops as a consequence of complications of infectious diseases or exposure to toxic factors against the background of genetic predisposition. However, aging significantly contributes to the development of all neurodegenerative diseases, including Parkinson's disease (1, 2).

Its high prevalence in senile individuals can be explained by the accumulation of mutations in mitochondrial DNA, a decrease in levels of glutathione and other components of the antioxidant system, depletion of the ubiquitin-proteasome system, and inhibition of the neuronal ability to initiate a stress response (3, 4).

The first reports on the pathogenesis and etiology of Parkinson's disease appeared at the end of the 20th century, whereas researchers have been studying the biochemical features of Parkinson's disease since 1950s. It has been reported that in Parkinson's disease dopaminergic neurons of the brain structures (mainly neurons of the substantia nigra and locus coeruleus) undergo progressive degeneration, which results in a decrease in the content of dopamine and abnormal functions of dopaminergic pathways in the central nervous system (5). Long-term biochemical studies have demonstrated that Parkinson's disease is accompanied by significant metabolic changes in the brain structures. The following intracellular metabolic abnormalities observed in Parkinson's disease have been reported: the destruction of mitochondria, energy deficit in neurons (due to the reduced activity of mitochondrial complex I in dopaminergic neurons of the substantia nigra), overactivation of free radical processes and the formation of reactive oxygen species; excessive deposition of calcium ions, as well as abnormal metabolism of xenobiotics (6, 7).

Genetic factors play a pivotal role in the development of Parkinson's disease, contributing to its progressive nature (8). There are several hereditary monogenic forms of Parkinson's disease:

1) *SNCA* gene mutations cause an autosomal-dominant form of Parkinson's disease. This gene encodes α -synuclein, which is responsible for synaptic vesicular transport and storage of neurotransmitters. Gene mutations lead to conformational changes in the protein structure, resulting in α -synuclein accumulation in the neurons with the subsequent formation of aggregates (Levi bodies) (9, 10).

2) *PARK-2* gene mutations lead to the development of an autosomal-recessive form of Parkinson's disease. A product of this gene is referred to as *Parkin*, which acts as a ubiquitin-protein ligase and is responsible for delivering unfolded and misfolded proteins to proteasomes for their degradation (8, 11).

3) Mutations in the *LRRK2* gene also result in an autosomal-dominant type of the disease. The *LRRK2* gene encodes a protein called leucine-rich repeat kinase 2 or dardarin (GTP-dependent kinase) (10). Pathologically activated dardarin presumably contributes to the development of neurodegenerative changes (8, 11).

It is believed that only 5-10% of cases of Parkinson's disease are directly monogenic, while all other cases are of a multifaceted nature. The combination of genetic and environmental factors causes the development of sporadic forms (12).

A decrease in levels of dopamine and its inhibitory effects on the striatal neurons contribute to the predominance of cholinergic effects and the development of motor disorders (tremor, rigidity, and hypokinesia). It has been known that in addition to dopaminergic neurons, other groups of neurons are subject to destruction and their damage is associated with non-motor manifestations of the disease. We are talking about noradrenergic neurons of the locus coeruleus, serotonergic neurons located in the raphe nuclei, and cholinergic neurons present in the nucleus basalis of Meynert (13, 14).

Numerous research groups have demonstrated that mitochondrial dysfunction and oxidative stress play an important role in the pathogenesis of Parkinson's disease. It has been shown that patients with Parkinson's disease have structural changes and a decrease in the activity of complex I of the mitochondrial respiratory chain (15). It can be assumed that mitochondrial insufficiency develops due to damage to the mitochondrial genome. There is strong evidence that dopaminergic neurons of the substantia nigra are predisposed to the development of oxidative stress and the destruction of mitochondria, which is due to their specific biochemical characteristics: weak antioxidant defense (glutathione), aggressive nature of oxidative metabolism for catecholamines present in high amounts there, and high energy needs (6). The factors mentioned above become especially important with aging.

The development of oxidative stress in neurons of the substantia nigra is associated with the formation of peroxide radicals as a result of dopamine catabolism by monoamine oxidase B. Under normal conditions they are eliminated by glutathione peroxidase and its cofactor glutathione. It has been reported that this enzyme is downregulated in Parkinson's disease. Neurotoxic free radicals interact with lipids of the membranes of dopaminergic neurons, which leads to their destruction (16).

Energy deficit is accompanied by the depolarization of neuronal membranes, which results in the removal of a NMDA glutamate receptor block with magnesium ions and the subsequent overactivation of NMDA receptors. This increases the influx of calcium ions and the intense release of them from the intracellular depot. This potentiates the cascade of calcium-dependent destructive reactions and apoptosis is triggered (17).

Nowadays Parkinson's disease has been treated with: medications (drugs that increase the level of dopamine in the brain (Levodopa), dopamine agonists) and neurosurgical methods (deep brain stimulation) (18).

However, such methods only reduce the symptoms of disorders neither replacing the dead nerve cells nor stopping the development of pathology. Prolonged pharmacotherapy (over 5 years) of the disease can cause significant side effects, mainly in the form of dyskinesia. In addition, prolonged treatment with drugs results in a decrease in sensitivity to them. Thus, fundamentally new methods of treatment that aim at restoring lost neurons should be elaborated. The method of deep brain stimulation and replacement treatment with fetal dopaminergic neurons (19) have been used for almost 15 years. Moreover, the possibility of using stem cells has been studied. It has been found that stem cells of different types serve as a valuable source of neuron-like cells and substrates for cell therapy of various diseases (20). However, the results of using such innovative techniques for treatment of Parkinson's disease are not always positive, which may be explained by the inadequacy of experimental studies revealing subtle mechanisms of tissue repair.

Regulatory peptides seem to be promising for the treatment of neurogenerative diseases. In particular, a PC-2 peptide complex (Cerluten) developed by the *Scientific-Production Center of Revitalization and Health* (Saint-Petersburg, Russia) can be used. PC-2 is a peptide complex isolated from the brain of calves (up to 12 months of age). In addition, it contains a bioantioxidant complex isolated in accordance with the unique technology from the biotechnologically cultivated ginseng. It belongs to the group of cytomaxes. PC-2 is used for the treatment of inflammatory and neurodegenerative diseases. It has been reported that neurotrophic substances present in PC-2 can significantly reduce neuroapoptosis and neurodestruction. This medication, as indicated by the manufacturers, exerts tissue-specific regulatory effects on brain cells, contributes to the improvement of cognitive functions, increases the resistance of the brain to hypoxia, and is effective in patients with neurodegenerative diseases. However, the possibility of its use for the treatment of Parkinson's disease as an independent pharmaceutical agent or in combination with other promising methods has not yet been studied.

Our research was designed to study the neuroprotective action of the PC-2 peptide complex in rats with experimental Parkinson's disease.

MATERIAL AND METHODS

1. Animals

The experiment was carried out by using 81 mature male WAG rats, which were kept in standard conditions of the vivarium. They were randomly divided into three groups. The control group consisted of intact animals.

Parkinson-like syndrome was caused in animals from group 2. Group 3 included animals treated with the PC-2 peptide complex (manufactured by the *Scientific-Production Center of Revitalization and Health*, Saint-Petersburg, Russia). PC-2 complex is commercially available (Пептидный комплекс №2) and it is recommended to be used either intranasally or rubbed into the skin. Its components are distilled water, peptide complex A-5, glycerol, bioantioxidant complex Neovitin®, phenoxyethanol with ethylhexylglycerol, propylene glycol, xanthan gum, PEG-40, castor oil, grapefruit essential oil, methylchloroisothiazolinon and methylisothiazolinone. The complex is registered and certified in the Russian Federation (№ RU 78.01.05.001.E.005601.08.11, https://npcriz.ru/products_and_services/products/peptide_complexes/pc2/certificates/). In this study we administered PC-2 at a daily dose of 0.1 mg/kg of weight, intranasally once per day during 10 days against the background of Parkinson-like syndrome. Intact rats from the control group received physiological solution in accordance with the scheme mentioned above.

Groups 1 and 2 included 30 rats each, while group 3 consisted of 21 animals. The experiment was carried out in dynamics. Ten animals from group 1 and 2 and seven rats from group 3 were taken on the 10th, 20th, 30th days from the beginning of the experiment for determining some biochemical parameters. The terms chosen by us are substantiated as follows: the most pronounced motor disorders in all rats were observed on the 10th day; the treatment was finished on the 20th day; no motor disorders in all rats were found on the 30th day.

2. Induction of Parkinson-like syndrome

Parkinson-like syndrome was caused by an injection of a neurotoxin 6-hydroxydopamine (6-OHDA) into the substantia nigra, which blocked dopamine synthesis in endogenous dopaminergic neurons resulting in the development of symptoms characteristic of Parkinson's disease. The model described above is widely used in experimental neurology and neurosurgery (21). The damaged area of the substantia nigra contained a significantly smaller number of neurons. Up to 80% of neurons were destroyed compared to the intact animals. The neurons were arranged as a narrow band along the lower surface of the brain.

The neurotoxin was injected under thiopental anesthesia (thiopental at a dose of 50 mg/kg intraperitoneally). After the administration of anesthesia the animals were fixed using a stereotaxic apparatus. Iodine solution was used for surgical-site antisepsis. The incision was made at the midline and was up to 2 cm long. Bones were skeletonized. To provide a required accuracy in reaching the substantia nigra, the following stereotaxic coordinates were used: AP = 4.0, L = 1.5, D = 8.2 mm ventrally to the surface of the cortex (22).

One-mm burr holes were placed from both sides symmetrically, and 6-OHDA was injected via a capillary at the depth of 8.2 mm. Severe motor dysfunctions, namely involuntary head movements, stuck up tails, trunk inclinations, were observed on the first day after the destruction of the substantia nigra. Motor disorders in rats with the Parkinson-like syndrome model were the most pronounced on the 10th day. Therefore, we started to treat the animals with the peptide complex on the 10th day after surgery.

3. Bioethics

All experimental procedures were performed in accordance with the provisions of the *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes* (<http://www.conventions.coe.int/treaty/en/treaties/word/123.doc>). The experiment was approved by the Bioethics Committee of Kharkiv National Medical University (Kharkiv, Ukraine).

4. Brain homogenate preparation

The brain was quickly isolated and cooled well in an isolation medium (0.1 M Tris-HCl buffer, pH 7.4). Then it was dried using filter paper. The hemispheres were separated, and shredded with scissors in mortars on ice. A sample of shredded tissue (0.2 g) was placed in a Potter glass homogenizer with a teflon pestle. Cooled samples were homogenized with 5 ml of the isolation medium in the homogenizer for 30 seconds (1000 rpm, the glass-teflon gap was 0.2 mm). The suspension obtained as a result was centrifuged for 30 minutes at 15,000 g. The supernatant was used for biochemical tests.

5. Assessment of biochemical parameters.

The content of biogenic amines was determined using column chromatography with the further quantitative analysis of fractions by spectrofluorimetric method (23). Standard samples of dopamine, noradrenaline, serotonin produced by Sigma (USA) were used.

Amino acids were separated by ascending thin layer chromatography (24). Chromatography was carried out using Silufol silica gel plates (Czech Republic). Butanol - acetic acid - water (80:20:20) were used as a solvent to carry out chromatography. The process was repeated 3 times for better results. Amino acids were identified using RF (retardation factor) values for the system of solvents mentioned above. Samples of glycine and aspartate produced by Sigma (USA) were used as standards.

The activity of isocitrate dehydrogenase and α -ketoglutarate dehydrogenase in mitochondria of cells in the cerebral cortex was determined spectrophotometrically (25).

Acetylcholine levels were also measured spectrophotometrically (26).

The concentrations of 8-isoprostane were determined by an ELISA kit manufactured by IBL Hamburg (Germany) to assess the oxidative stress rate.

6. Statistics

Data were processed using the *GraphPad Prism 5 application*. Student's t test was used to compare the means of two groups of normally distributed variables. Differences between independent groups were considered to be statistically significant at $p < 0.05$.

RESULTS

Severe motor disorders developed as a result of a bilateral dysfunction of the substantia nigra. They manifested as involuntary up-down and side-to-side movements, trunk inclinations, stuck up tail, and tremor. Motor disorders appeared in all animals on the first day after surgery. The most pronounced motor disorders were registered on the 8th-10th days after surgery. When treatment was not provided, motor disorders were present during the entire experiment.

Treatment of animals with the peptide complex led to disappearance of tremor, a decrease in the number of head movements on the 10th day. Changes in body shape became rarer. Normalization of the Parkinson-like syndrome occurred on the 20th-30th day after the beginning of treatment. Biochemical methods were used to assess the efficacy of the peptide complex more accurately and to find out possible mechanisms of its action.

Nowadays the role of dopamine in the development of Parkinson's disease has been well studied. However, the possible significance of other central neurotransmitters (noradrenaline and acetylcholine) in the pathogenesis of the disease has not yet been elucidated. It has been reported that dopaminergic inputs of the striatum are inhibited by its cholinergic neurons (27).

Therefore, dopamine deficiency may contribute to the hyperactivity of the striatal acetylcholine system leading to some characteristic motor disorders (tremor). It has been known that the ability of L-DOPA to completely eliminate akinesia in patients with Parkinson's disease can be suppressed by the preliminary administration of drugs that specifically block noradrenergic mechanisms (28). Thus, we believe that the study of the content of noradrenaline, dopamine, and acetylcholine in the tissue of the cerebral frontal lobe in rats with experimental Parkinson-like syndrome is of great importance.

It was found that on the 10th day after surgery (motor disorders were the most pronounced), the content of dopamine and noradrenaline significantly decreased, whereas the content of acetylcholine was higher (Tables 1-3). The tremor observed by us in rats during this period was rather associated with the high content of acetylcholine, but not dopamine. Acetylcholine could also contribute to the development of trunk inclinations.

Changes in the content of biogenic amines observed by us when the treatment was not provided persisted throughout the entire research (Table 1-3).

On the 10th day from the beginning of treatment with the peptide complex (the 20th day after surgery), the content of acetylcholine decreased and almost reached its level in control animals (Table 3). It is interesting to note that tremor disappeared in rats during this period, which indicated its direct connection with the level of acetylcholine. The same period of the disease was associated with the slightly increased content of noradrenaline against the background of dopamine levels that were practically similar to those of the animals untreated with the peptide complex (Table 1, 2). On the 30th day after surgery (the 20th day after the beginning of treatment with the peptide complex), the content of

noradrenaline and acetylcholine was similar to that of the intact animals, while the level of dopamine was elevated. However, it remained significantly lower compared to the intact animals (Table 1-3).

Data on the levels of GABA, glycine, aspartate and glutamate in the frontal lobe and the influence of treatment with the peptide complex are available in Tables 4-7.

The content of glutamate and aspartate was shown to increase significantly as a result of the development of Parkinson-like syndrome in rats on the 10th day after surgery when motor disorders were of the highest severity. Such changes in the content of glutamic acid and aspartic acid were observed against the background of reduced levels of GABA and glycine. It is likely that the action of excitatory amino acids is the dominant effect in the frontal lobe of the brain on the 10th day after surgery.

Table 1. Concentrations of biogenic amines in homogenates of frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	Dopamine (μmol / g tissue)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	0.54 ± 0.003	0.56 ± 0.02	0.52 ± 0.02
Group 2 (experimental Parkinson-like syndrome) n = 30	0.19 ± 0.01 **	0.21 ± 0.02 **	0.18 ± 0.01 **
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	0.19 ± 0.01 ***	0.24 ± 0.02 ***	0.37 ± 0.03 *****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****
- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 2. Concentrations of noradrenaline in homogenates of frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	Noradrenaline (μmol / g tissue)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	1.85 ± 0.12	1.79 ± 0.14	1.91 ± 0.13
Group 2 (experimental Parkinson-like syndrome) n = 30	0.18 ± 0.06 **	0.93 ± 0.05 **	1.02 ± 0.07 *
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	0.18 ± 0.06 ***	1.23 ± 0.09 ****	1.89 ± 0.12 *****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****
- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 3. Concentrations of acetylcholine in homogenates of frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	Acetylcholine ($\mu\text{mol} / \text{g tissue}$)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	2.68	2.59	2.73
	$\pm$	$\pm$	$\pm$
	0.15	0.18	0.19
Group 2 (experimental Parkinson-like syndrome) n = 30	4.11	4.22	4.30
	$\pm$	$\pm$	$\pm$
	0.28 *	0.31 *	0.37 *
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	4.1	2.67	2.79
	$\pm$	$\pm$	$\pm$
	0.28 ***	0.19 *****	0.19 ****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 4. Concentrations of glutamate in homogenates of frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	Glutamate ($\mu\text{mol} / \text{g tissue}$)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	14.65	14.09	14.78
	$\pm$	$\pm$	$\pm$
	1.02	1.19	1.31
Group 2 (experimental Parkinson-like syndrome) n = 30	26.34	27.41	27.51
	$\pm$	$\pm$	$\pm$
	1.18 **	1.38 **	1.25 **
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	26.34	20.02	15.03
	$\pm$	$\pm$	$\pm$
	1.18 ***	1.04 ****	1.28 *****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 5. Concentrations of GABA in homogenates of frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2.

Group of animals	GABA ($\mu\text{mol} / \text{g tissue}$)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	8.79	8.35	8.54
	$\pm$	$\pm$	$\pm$
	0.66	0.59	0.47
Group 2 (experimental Parkinson-like syndrome) n = 30	4.55	4.21	4.37
	$\pm$	$\pm$	$\pm$
	0.31 **	0.22 **	0.18 **
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	4.55	6.63	8.45
	$\pm$	$\pm$	$\pm$
	0.31 ***	0.29 ****	0.41 *****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 6. Concentrations of aspartate in homogenates of frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	Aspartate ($\mu\text{mol} / \text{g tissue}$)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	3.82	3.94	3.79
	$\pm$	$\pm$	$\pm$
	0.24	0.29	0.25
Group 2 (experimental Parkinson-like syndrome) n = 30	6.19	6.41	6.37
	$\pm$	$\pm$	$\pm$
	0.45 **	0.29 **	0.31 **
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	6.19	5.08	4.02
	$\pm$	$\pm$	$\pm$
	0.48 ***	0.22 ****	0.27 ****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 7. Concentrations of glycine in homogenates of frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	Glycine ($\mu\text{mol} / \text{g tissue}$)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	1.25	1.23	1.28
	$\pm$	$\pm$	$\pm$
	0.05	0.09	0.08
Group 2 (experimental Parkinson-like syndrome) n = 30	0.83	0.79	0.85
	$\pm$	$\pm$	$\pm$
	0.07 *	0.06 *	0.04 *
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	0.83	1.34	1.36
	$\pm$	$\pm$	$\pm$
	0.07 ***	0.08 *****	0.09 *****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 8. The activity of isocitrate dehydrogenase in frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	Isocitrate dehydrogenase ($\mu\text{mol NADH}_2 / \text{s mg tissue}$)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	51.28	52.85	50.87
	$\pm$	$\pm$	$\pm$
	2.79	1.79	2.25
Group 2 (experimental Parkinson-like syndrome) n = 30	35.62	33.53	36.12
	$\pm$	$\pm$	$\pm$
	2.17 **	3.00 **	2.79 **
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	35.62	43.11	51.64
	$\pm$	$\pm$	$\pm$
	2.17 ***	2.71 ****	1.77 *****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Table 9. The activity of α -ketoglutarate dehydrogenase in frontal lobes of the brain in rats with experimental Parkinson's syndrome and with experimental Parkinson's syndrome treated with PC-2

Group of animals	α -Ketoglutarate dehydrogenase ($\mu\text{mol NADH}_2$ / s mg tissue)		
	Day 10	Day 20	Day 30
Group 1 (control group) n = 30	59.23 $\pm$ 3.24	60.00 $\pm$ 3.75	58.89 $\pm$ 2.11
Group 2 (experimental Parkinson-like syndrome) n = 30	28.74 $\pm$ 1.75 **	27.98 $\pm$ 1.34 **	29.07 $\pm$ 2.11 **
Group 3 (experimental Parkinson-like syndrome + treatment with PC-2) n = 21	28.74 $\pm$ 1.75 ***	52.17 $\pm$ 1.24 *****	60.31 $\pm$ 2.15 *****

Notes:

* - $p < 0.01$ compared to the control group; ** - $p < 0.001$ compared to the control group; *** - $p > 0.05$ compared to Group 2; **** - $p < 0.05$ compared to Group 2; *****
- $p < 0.01$ compared to Group 2; ***** - $p < 0.001$ compared to Group 2.

Against the background of the treatment with the peptide complex, the content of glycine in the frontal lobe of rats reached its level in the control group on the 10th day from the beginning of treatment, whereas concentrations of the other amino acids become normal only on the 30th day of the experiment.

According to the oxidative theory of the pathogenesis of Parkinson's disease, one of the main factors in its development and progression is oxidative stress. To assess the state of free radical processes in the experimental Parkinson-like disease and the impact of treatment on it, we determined the level of 8-isoprostane (a marker of oxidative stress) in homogenates of the frontal lobe. The content of 8-isoprostane in intact animals was revealed to be 20.58 ± 1.39 pg / g protein, whereas in rats with Parkinson-like syndrome it reached 64.17 ± 2.19 pg / g protein, $p < 0.001$. The treatment with the peptide complex led to the normalization of 8-isoprostane levels, which decreased to 21.92 ± 2.16 pg / g protein on the 10th day.

Oxidative stress is known to promote destabilization of membranes, including the mitochondrial ones. Many authors have reported that one of the leading metabolic disorders resulting in neuronal death is mitochondrial dysfunction, which can be caused by oxidative stress and glutamate excitotoxicity (17, 29). To assess the functions of mitochondria, we studied the activity of isocitrate dehydrogenase (the key enzyme of TCA cycle) and the α -ketoglutarate dehydrogenase complex in rats with experimental Parkinson-like disease treated and untreated with the peptide complex. Our results are available in Table 8 and Table 9.

The activity of both enzymes mentioned above is significantly reduced in rats with Parkinson-like syndrome compared to the control group. Administration of the

peptide complex led to normalization of the activity of both enzymes on the 20th day from the beginning of the treatment, i.e. on the 30th day of the experiment.

DISCUSSION

There is increasing evidence that Parkinson's disease is characterized by degeneration of nigrostriatal neurons, low production of dopamine, and abnormal functions of basal ganglia. Our experimental model is based on the administration of the 6-hydroxydopamine (6-OHDA) neurotoxin that inhibits the synthesis of dopamine in dopaminergic neurons imitating the central links of the mechanism of Parkinson's disease development and can be used to evaluate the effectiveness of a treatment. Thus, bilateral destruction of the substantia nigra caused the reduction of dopamine and noradrenaline levels, elevation of acetylcholine concentrations, low levels of GABA and glycine, and high concentrations of glutamate and aspartate in the frontal lobe of the brain. In this study, changes in the system of monoamines and amino acid neurotransmitters are accompanied by the development of oxidative stress, which contributes to mitochondrial dysfunction and neuronal death. PC-2 peptide complex is found to be effective in reducing metabolic abnormalities in the experimental Parkinson-like syndrome.

It has been known that there is a functional interaction between dopamine, noradrenaline and amino acid neurotransmitters in the basal ganglia and limbic regions of the brain. In addition, it has been reported that inadequate production of dopamine in Parkinson's disease results in the activating influence of basal ganglia on the cerebral cortex. At the same time, the dynamics of the

content of amino acid neurotransmitters in the experimental Parkinson-like syndrome has to be elucidated, and some new approaches to its treatment should be elaborated. It has been reported that the GABA (gamma-aminobutyric acid)-glutamate system participates in the pathogenesis of Parkinson's disease. However, there are no reliable data about the role of aspartic acid and glycine whose receptors are found in many structures of the brain.

The results of this study strongly suggest that the development of motor disorders in rats with experimental Parkinson-like disease is associated with a decrease in the concentration of not only dopamine but also noradrenaline, as well as an increase in the content of acetylcholine in the frontal lobe. Our results are consistent with and confirmed by the information that stimulation of both dopamine and noradrenaline receptors is required in order to completely restore the motor activity in animals with pharmacologically induced akinesia (30), as well as by the fact that dopaminergic disorders in Parkinson's disease cause hyperactivity of the striatic acetylcholine system (31). It has been reported that acetylcholinergic drugs can cause hyperactivity and potentiate the behavioral effects of dopamine agonists.

Analysis of the level of the neurotransmitter amino acids in rats with experimental Parkinson-like syndrome revealed a significant increase in the content of glutamate and aspartate against the background of GABA and glycine deficiency. There is strong evidence to suggest that glutamic acid plays a pivotal role in the pathogenesis of Parkinson's disease. According to some authors, its excitotoxic properties explain the development of mitochondrial dysfunction (17), which is consistent with our observations - an increase in glutamic acid levels and a decrease in the activity of mitochondrial enzymes in rats with Parkinson-like syndrome. The search for papers dedicated to the role of aspartic acid and glycine in the pathogenesis of Parkinson's disease has revealed very little published data. However, there is evidence that aspartate is capable of forming peptide complexes with lysine and other amino acids. Such complexes can regulate the permeability of cell membranes and accelerate the degradation of biogenic amines. Taking into account the information mentioned above, it can be assumed that high levels of aspartate found by us contributes to a decrease in the content of dopamine and noradrenaline, as well as to the development of mitochondrial dysfunction. Focal GABA bilateral injection in the nucleus accumbens is known to inhibit both hyperactivity caused by the injection of dopamine into the same areas and motor activity increased after the systemic administration of amphetamine (32). Thus, low concentrations of GABA, which are apparently associated with a decrease in glutamate decarboxylase activity, may be of protective and

adaptive nature. The role of glycine in the pathogenesis of Parkinson's disease is poorly understood. However, based on reports about the diminished rate of degradation of biogenic amines under the influence of glycine (33), it can be assumed that low concentrations of this amino acid contribute to the more rapid development of the disease.

It is obvious that drugs that normalize not only the content of dopamine but also noradrenaline, acetylcholine, and neurotransmitter amino acids can be considered effective in the treatment of Parkinson-like syndrome (and Parkinson's disease). The efficiency of neurotrophic factors-containing stem cells transplanted into the brain structures of rats has been proven in experiments (34). The peptide complex (PC-2) developed by the *Institute of Bioregulation and Gerontology* (St. Petersburg, Russian Federation) also contains neurotrophic factors. Thus, we have hypothesized that it might have protective properties in experimental Parkinson-like syndrome in rats.

It was found that the PC-2 peptide complex (as stated by the manufacturer, it exerts tissue-specific regulatory effects on the brain cells) normalized levels of noradrenaline (on the 20th day after the beginning of its administration). However, it was less effective for dopamine. In addition, the peptide complex contributes to the normalization of all the neurotransmitter amino acids studied in our research and the activity of mitochondrial enzymes on the 20th day from the beginning of its intake.

The results of our research strongly suggest that more profound researches will allow developing a method for alleviating the course of Parkinson's disease in humans.

LIST OF ABBREVIATIONS

GABA – gamma-aminobutyric acid
6-OHDA – 6-hydroxydopamine
RF – retardation factor

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