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PHARMACOCORRECTION OF MITOCHONDRIAL DYSFUNCTION AND ENERGY IMBALANCE OF SUBSTANTIA NIGRA NEURONS IN EXPERIMENTAL PARKINSON'S DISEASE IN RATS

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Abstract

Parkinson's disease (PD) is a progressive disease and the incidence increases markedly with age, treatment has significantly improved the quality of life of patients with PD, but statistics show that these patients continue to show shorter life expectancies compared to the general population.

Aim of the study. To investigate the features of changes in energy balance and mitochondrial dysfunction on the basis of experimental studies in rats in the modeling of PD and justify the development of possible treatment regimens with specific neuroprotective effects on the dopaminergic system.

Materials and methods. The study was carried out on 90 Wistar rats at the age of 6 months weighing 220-290 grams. Parkinsonism was induced by the administration of the

neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to experimental rats with neuroprotective treatment: I – Intact (passive control); II – animals with experimental Parkinson's disease (PD, active control); III – PD + Amantadine (AM) IV – PD + AM + Cerebrocurin; V – PD + AM + Pramistar; VI – PD + AM + Gliatilin; VII – PD + AM + Noofen; VIII – PD + AM + Pronoran; IX – PD + AM + Melatonin.

Results. Under conditions of formation of experimental PD in rats develops energy deficiency of neuronal cells and dysfunction of the Krebs cycle with a predominance of anaerobic oxidation of the substrate, as well as the development of mitochondrial dysfunction. Prescribed neuroprotective drugs significantly improved energy metabolism in the brain of rats with PD within statistical significance, increased levels of ATP and ADP, enzymatic activity of mCPK and ATPase, decreased levels of AMP, especially pronounced drugs melatonin, cerebrocurin, gliatilin and pronoran. Neuroprotective therapy of experimental PD in rats contributed to a statistically significant increase in the values of pyruvate, malate, LDH and MDG, and also led to a decrease in the values of lactate and lactic acidosis in brain tissues. The appointment of neuroprotective therapy with basic amantadine therapy led to an increase in energy charge, energy potential, phosphorylation index and thermodynamic control of respiration in the mitochondria of neurons, especially in the groups of melatonin, cerebrocurin, gliatilin and pronoran.

Conclusions. Pharmacocorrection of mitochondrial dysfunction and energy imbalance of dopaminergic neurons in experimental PD in rats may indirectly or directly inhibite to the progression of pathology in PD.

Keywords: **Parkinson's disease; neuroprotection; mitochondrial dysfunction; energy balance; melatonin.**

Prevalence. Parkinson's disease (PD) is a progressive disease with an average age of 55 years, and the incidence increases markedly with age, from 20/100 000 in general to 120/100 000 at the age of 70 years [1]. In approximately 95% of cases of PD there is no obvious genetic link (so-called "sporadic" PD), but in other cases the disease is inherited. Subsequently, the symptoms worsen, and before the introduction of levodopa in therapeutic regimens, the mortality rate among patients with PD was three times higher than in normal people of the same age [2]. Although levodopa has significantly improved the quality of life of patients with PD, statistics show that these patients continue to show shorter life expectancies compared to the general population. In addition, most patients with PD suffer

from significant movement disorders after 5-10 years of illness, even with qualified treatment with available symptomatic drugs [3].

Clinically, any disease, including dopamine deficiency in the striatum or direct damage to the striatum, can lead to "parkinsonism", a syndrome characterized by tremor at rest, rigidity, slowness or lack of voluntary movement, postural instability and "freezing" (immobility). PD is the most common cause of parkinsonism, accounting for ~ 80% of cases [4].

Against the background of the development of PD tremor occurs at rest, but decreases with arbitrary movements, so it usually does not affect daily activity. Rigidity means increased resistance (stiffness) to the passive movement of the patient's limbs. Bradykinesia (slowness of movement), hypokinesia (decreased range of motion) and akinesia (lack of normal unconscious movements, such as rocking the arm while walking) manifest as many symptoms, including lack of normal facial expression (hypomimia), decreased voice (hypophonia), saliva (inability to swallow without thinking), reduction in size (micrography) and writing speed, as well as reduction in stride length while walking. Bradykinesia can significantly impair quality of life, as performing daily tasks such as dressing or eating takes much longer [5]. Patients with PD also tend to have a stooped posture and may lose normal postural reflexes, leading to falls and sometimes wheelchair confinement. Freezing, the inability to start random movements, such as walking (patients "stick" to the ground when they try to start moving), is a common symptom of parkinsonism. Anomalies of affect and cognition also often occur; patients may become passive or withdrawn without initiative; they may behave too quietly if they are not encouraged to participate. Answers to questions are delayed, cognitive processes are slowed down (bradyphrenia). Depression is common, and dementia is much more common in PD, especially in elderly patients [6].

The possibility that the oxidative phosphorylation defect plays a role in the pathogenesis of PD was caused by the discovery that MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) blocks the mitochondrial electron transfer chain, inhibiting complex I. Further studies found anomalies in the activity of complex I in PD. In vitro studies show that such a defect in complex I can expose cells to oxidative stress and energy deficiency [7]. Disorders of oxidative phosphorylation detected in PD are not limited to the brain, as reduced activity of complex I was found in platelets of patients with PD and in hybrid cells (cell lines designed to contain mitochondria derived from platelets of patients with PD).

This finding suggests that complex I deficiency is inherited from the mitochondrial genome or that some systemic toxicity leads to mutations in mitochondrial DNA. However, mutations in mitochondrial DNA in patients with PD have not yet been identified [8].

Almost 100% of molecular oxygen is consumed by mitochondrial respiration, and powerful oxidants, including hydrogen peroxide and superoxide radicals, are usually formed as by-products. Inhibition of complex I increases the production of reactive oxygen species (ROS) superoxide, which can form toxic hydroxyl radicals or react with nitric oxide to form peroxynitrite [9]. These molecules can cause cell damage by reacting with nucleic acids, proteins and lipids. One of the targets of these reactive particles may be the electron transfer chain itself, which leads to damage to mitochondria and the subsequent formation of ROS. Elevation of biological markers of oxidative damage was detected in the substantia nigra of the brain in PD. In addition, the content of antioxidant glutathione was reduced in the substantia nigra of the brain in PD, which is consistent with an increase in ROS, although this may also indicate a primary decrease in protective mechanisms against ROS [10].

Factors that potentially cause mitochondrial damage in PD have not yet been well studied. Numerous studies using various genetic disorders and models of toxic damage in the formation of PD have contributed to a better understanding of the pathogenesis of the disease, many of these studies indicate mitochondrial dysfunction as an important point in PD development and determines the relevance of this study.

Aim of the study. To investigate the features of changes in energy balance and mitochondrial dysfunction on the basis of experimental studies in rats in the modeling of PD and justify the development of new treatment regimens with specific neuroprotective effects on the dopaminergic system.

Materials and methods. The study was conducted in accordance with Directive 2010 / 63EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, as well as with the national "Common Ethical Principles for Animal Experiments" (Ukraine, 2001) and the guidelines set out in in "Basic principles of studying the toxicity of potential pharmacological drugs" (State Enterprise «Ukrainian Pharmaceutical Quality Institute», K., 2000). The experiment was approved by the Commission on Bioethics of Zaporizhia State Medical University. All experiments were conducted during 2018-2020 on the basis of the Training Medical and Laboratory Centre of Zaporizhia State Medical University, certified by the Ministry of Health of Ukraine (certificate № 039/14).

The study was performed on 90 Wistar rats 6 months weighing 220-290 grams. The animals were kept in standard vivarium conditions (12-hour light cycle, temperature 22^0C). For experiments, animals were subjected to food deprivation. Parkinsonism was induced by administration of the neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to experimental rats. The intact group received a single intraperitoneal saline solution of 1 ml per 100 g of weight, and the control group after the introduction of MPTP – a single intraperitoneal saline solution in a similar dosage.

We have developed a concept for the treatment of PD, which consists in the selective effect on the leading targets of neurodegeneration – nitrosative stress, deprivation of endogenous neuroprotection factors (HSP70), disorders of the thiol disulfide system, mitochondrial dysfunction, inhibition of dopamine transmission, neuroapoptosis, triggering IL-1b pathways of neurodegeneration. To confirm the prospects of the selected target units, we prefer drugs whose mechanism involves the impact on these target units. Cerebrocurin contains neurotophic factors and protein reelin, reduces the symptoms of primary and secondary mitochondrial dysfunction, regulates transcriptional processes, noofen can increase dopamine levels, activates Roberts' compensatory shunt, pramistar is a nootropic drug of racemates, exhibits dopamine agonist properties, increases the density of nicotine cholinergic receptors, stimulates energy metabolism, pronoran has the properties of a dopamine agonist, melatonin in therapeutic doses stimulates the expression of HSP70, normalizes the thiol disulfide system, modulates the mitochondrial pore activity, stimulates the compensatory malate-aspartate shunt, regulates NO \ SH-mechanisms of gene transcription, gliatilin is a nootropic drug that has a specific mitoprotective effect and cholinomimetic effect.

The verification for the strategy of rational therapy in PD was based on studying the activity of drugs in groups of animals: I – Intact (passive control); II – animals with experimental Parkinson's disease (PD, active control); III – PD + Amantadine (AM) IV – PD + AM + Cerebrocurin; V – PD + AM + Pramistar; VI – PD + AM + Gliatilin; VII – PD + AM + Noofen; VIII – PD + AM + Pronoran; IX – PD + AM + Melatonin.

All drugs were administered to animals intragastrically using a metal probe. The average effective dose (ED50) of drugs was determined in the model of PD on the ability of drugs to affect the level of reduced glutathione, glutathione reductase, glutathione peroxidase and nitrotyrosine in the cytosolic fraction of the brain of experimental animals. The course of study drugs in experimentally justified doses was as follows: amantadine – 1 mg / kg, cerebrocurin – 150 mkl / kg, pramistar – 10 mg / kg, gliatilin – 250 mg / kg, noofen – 100 mg / kg, pronoran – 2 mg / kg, melatonin – 10 mkg / kg in animals with experimental PD.

For biochemical studies, blood was rapidly removed from the brain, separated from the meninges, and the test pieces were placed in liquid nitrogen. It was then ground in liquid nitrogen to a powdery state and homogenized in 10 times the volume of medium as described above. For long-term storage, the material was frozen and stored at -80 ° C. To determine the rate of mitochondrial pore opening, a suspension of 0.5-1.0 mg protein / ml was used. Protein-free extract was obtained by adding a precise sample of brain tissue homogenate in perchloric acid (0.6), followed by neutralization with 5 M potassium carbonate.

The state of energy metabolism was determined by the level of the most important intermediates – ATP, ADP, AMP, lactate, pyruvate, malate.

The content of pyruvic acid in brain tissue samples was determined spectrophotometrically by the Umbright method. Pyruvic acid reacts with 2,4-dinitrophenylhydrazone to form a hydrazone, the solution of which in an alkaline medium has a brownish-red color, the intensity of which is directly proportional to the content of pyruvate in the sample. The pyruvate content was calculated according to the calibration schedule. The pyruvate content was expressed in mkM / g tissue.

The lactic acid content was determined by the methods of Hohorst. In the presence of lactate dehydrogenase, lactate is converted to pyruvate, and the binding that is formed during the reaction of pyruvate with hydrazine-glycine buffer, promotes the complete oxidation of lactate. The amount of NADH formed during the reaction equimolarly to the amount of lactate was recorded spectrophotometrically at a wavelength of 340 nm. The lactate content was expressed in mkM per 1 g of tissue.

The amount of malate was determined by methods of Hohorst by reducing NADH at 340 nm. The method consists in the fact that in the presence of malate dehydrogenase (MPH) malate is converted into oxaloacetic acid. Binding of oxaloacetic acid with hydrazine-glycine buffer provides complete oxidation of malate. The formation of the reduced form of NADH is equivalent to the amount of oxidized malate, the increase of which was recorded at 340 nm spectrophotometrically. The malate content was expressed in mkM / g of tissue.

Adenyl nucleotides were determined by thin layer chromatography. The method is based on the separation of ATP, ADP and AMP in the system dioxane-isopropanol-water-ammonia on a thin layer of sorbent, followed by quantification by direct spectrophotometry at 260 nm. Nucleotides are arranged in order from bottom to top: ATP, ADP, AMP. The content of nucleotides is calculated according to the calibration graphs compiled for each nucleotide. Based on the obtained data on the content of ATP, ADP and AMP, additional indicators were

calculated: energy charge (EC), energy potential (EP), phosphorylation index (PI), thermodynamic respiration control (TRC).

In order to establish the possibility of the studied drugs to influence the phenomena of mitochondrial dysfunction, we studied the degree of mitochondrial pore opening and mitochondrial transmembrane potential (Δψm). The opening of the mitochondrial pore was initiated by the introduction of cyclosporine-A (0.5 ml) and was determined spectrophotometrically at λ = 540 nm and at 25° C with constant stirring for 25 minutes. Studies of the mitochondrial transmembrane potential (Δψm) were performed by adding 9 μM of safronin O to the medium. Spectrophotometry was performed at a wavelength of 515 nm and 525 nm. Δψm was determined by the difference in light absorption at 515 nm and 525 nm (ΔA515-525 nm).

Statistical processing of research data was performed using the software package "Statistica® for Windows 6.0" (StatSoftInc., №AXXR712D833214FAN5). Descriptive statistics included calculations of arithmetic mean values (M), median (Me), standard errors of the mean (± m) and interquartile range (interval) – values of the 25th and 75th percentiles. Before applying the statistical criteria, the hypothesis of a normal law of distribution of random variables was tested (according to the Shapiro-Wilk test). Under the conditions of the normal distribution, the reliability of intergroup differences according to the obtained experimental data was established using the Student's parametric t-test. In the case where the data did not correspond to the laws of normal distribution, the comparative analysis was performed using the nonparametric Mann-Whitney U-test. To compare the independent variables in more than two samples, analysis of variance (ANOVA) was used in the normal distribution, or the Kruskal-Wallis test for a distribution other than normal. Comparison of groups on a qualitative basis was performed using the criterion χ^2 with the analysis of conjugation tables. To analyze the patterns of the relationship between individual indicators, a correlation analysis was performed using the Pearson or Spearman correlation coefficient. Differences $p < 0.05$ (95%) were considered statistically significant for all types of analysis.

Research results. As a result of the formation of experimental PD in rats, the levels of ATP and ADP in the control group decreased by 33.59 and 47.27%, respectively, relative to the intact group, and the level of AMP increased in the brain by 22.73% as a result of energy deficiency of neuronal cells and disorders functioning of the Krebs cycle with the dominance of anaerobic oxidation of the substrate. Also in the control group there was a decrease in the activity of the enzyme mCPK by 59.09%, and inhibition of ATPase activity by 30.11% compared with healthy rats (Table 1).

Table 1

The effect of the studied drugs on the production, transport and utilization of energy in the brain of rats with experimental Parkinson's disease (M ± m; n = 10)

Indicator	ATP, mkM / g tissue	ADP, mkM / g tissue	AMP, mkM / g tissue	mitochondrial creatine phosphokinase (mCPK), mkM / mg protein / min	ATPase activity, mkM / mg protein / min
Intact (passive control)	3,87±0,21	0,55±0,06	0,17±0,02	1,98±0,19	25,11±2,06
Parkinson's disease (PD, active control)	2,57±0,23	0,29±0,02	0,22±0,03	0,81±0,07	17,55±2,48
PD+Amantadine (AM)	2,81±0,33	0,35±0,03	0,21±0,02	1,15±0,21	20,39±1,98
PD+AM+Cerebrocurin	3,49±0,27*	0,44±0,05*	0,15±0,02*	1,78±0,21*	23,82±2,95*
PD+AM+Pramistar	3,19±0,41	0,40±0,05	0,19±0,02	1,42±0,18	21,36±1,57
PD+AM+Gliatilin	3,39±0,29*	0,46±0,04*	0,20±0,01*	1,81±0,19*	22,14±3,05*
PD+AM+Noofen	2,89±0,31	0,39±0,05	0,21±0,03	1,35±0,21	19,26±2,18
PD+AM+Pronoran	3,53±0,26*	0,47±0,32*	0,19±0,01*	1,85±0,19*	23,35±3,76*
PD+AM+Melatonin	3,80±0,42*	0,51±0,04*	0,18±0,01*	1,91±0,22*	24,12±3,48*

Notes: p is the level of statistical significance when comparing samples using ANOVA analysis of variance (Kraskel-Wallis test), * − $p \leq 0.05$ according to the control group.

After the introduction of the drug amantadine, there is a certain positive dynamics in the energy system of neurons in rats with PD. Thus, the level of ATP and ADP increased by 8.54 and 17.14%, respectively, the enzymatic activity of mCPK and ATPase increased by 29.57 and 13.93%, respectively.

The following results were recorded with the combined administration of amantadine with neuroprotective drugs of different mechanism of action: statistically significant elevation of ATP and ADP by 26.36 and 34.09% in the cerebrocurin group ($p \leq 0.05$); by 19.44 and 27.50%, respectively, in the group of the pramistar; by 24.19 and 36.96% in the gliatilin group ($p \leq 0.05$); by 11.07 and 25.64% in the noofen group; by 27.20 and 38.30% in the pronoran group ($p \leq 0.05$); by 32.37 and 43.14%, respectively, in the melatotin group ($p \leq 0.05$). Also, in each of these groups after the appointment of therapy there were a simultaneous decrease in AMP levels in the brain of rats with PD.

The studied activity of mCPK and ATPase were statistically significantly increased in the cerebrocurin group by 54.49 and 26.32%, respectively ($p \leq 0.05$), in the pramistar group by 42.94 and 17.84%, in the gliatilin group by 55.25 and 20.73% ($p \leq 0.05$), in the noofen group by 40.00 and 8.89%, in the pronoran group by 56.22 and 24.84% ($p \leq 0.05$), and in the melatonin group by 57.60 and 27.24%, respectively ($p \leq 0.05$).

Thus, the prescribed neuroprotective drugs significantly improved the energy metabolism in the brain of rats with PD within the statistical significance, especially pronounced effect were recorded for the drugs melatonin, cerebrocurin, gliatilin and pronoran.

We also investigated the effect of PD and neuroprotective therapy on the concentration of pyruvate, malate, lactate, isocitrate and the activity of succinate dehydrogenase (SDG), malate dehydrogenase (MDG) enzymes in rat neurons (Table 2).

Table 2

The effect of the studied drugs on the indicators of carbohydrates and energy metabolism in the brain of rats with experimental Parkinson's disease (M ± m; n = 10)

Indicator	Pyruvate, mkM / g tissue	Lactate, mkM / g tissue	Malate, mkM / g tissue	Isocitrate, mkM / g tissue	SDG, nM / mg protein / min	MDG, mkM / mg protein / min
Intact (passive control)	0,49±0,05	2,27±0,28	0,47±0,03	0,60±0,09	6,46±0,80	12,45±2,06
Parkinson's disease (PD, active control)	0,32±0,04	4,89±0,52	0,30±0,05	0,39±0,06	2,95±0,34	6,28±0,70
PD+Amantadine (AM)	0,39±0,04	4,02±0,55	0,36±0,03	0,48±0,05	3,94±0,51	7,81±0,88
PD+AM+Cerebrocurin	0,42±0,06	3,17±0,41*	0,44±0,07*	0,55±0,41*	5,15±0,62*	11,02±1,45*
PD+AM+Pramistar	0,40±0,05	3,89±0,37	0,42±0,05*	0,51±0,41*	4,39±0,46*	9,84±1,07*
PD+AM+Gliatilin	0,41±0,04	3,27±0,25	0,43±0,06	0,53±0,50*	5,48±0,41*	10,92±1,15*
PD+AM+Noofen	0,40±0,06	3,72±0,39*	0,36±0,05	0,52±0,07	3,98±0,41*	8,36±0,91
PD+AM+Pronoran	0,42±0,04	3,41±0,32	0,44±0,03	0,54±0,04*	5,31±0,47*	10,42±0,99*
PD+AM+Melatonin	0,45±0,04*	2,98±0,19*	0,45±0,04*	0,56±0,06*	5,62±0,60*	11,48±1,15*

Notes: p is the level of statistical significance when comparing samples using ANOVA analysis of variance (Kraskel-Wallis test), * − $p \leq 0.05$ according to the control group.

The decrease in the levels of pyruvate, malate and the increase in lactic acidosis on the background of PD in the control group was 34.69; 36.17 and 53.58%, respectively, relative to the intact group, with simultaneous depression of the activity of LDH and MDG by 54.33 and 49.56%, respectively.

Administration of cerebrocurin in combination with amantadine to experimental animals contributed to a statistically significant increase in the values of pyruvate, malate, LDH and MDG by 23.81; 31.82; 42.72 and 43.01%, respectively ($p \leq 0.05$), and also led to a decrease in lactate values by 35.17% ($p \leq 0.05$).

The drug pramistar in combination with amantadine statistically significantly increased pyruvate, malate, LDH and MDG by 20.00; 28.57; 32.80 and 36.18%, respectively ($p \leq 0.05$), and inhibited lactic acidosis of the brain by 20.45%.

Gliatilin statistically significantly increased pyruvate, malate, LDH and MDG by 21.95; 30.23; 46.17 and 42.49%, respectively ($p \leq 0.05$), and inhibited lactic acidosis of the brain by 33.13%.

Administration of noofen to animals in combination with amantadine contributed to a statistically significant increase in the values of pyruvate, malate, LDH and MDG by 20.00; 16.67; 25.88 and 24.88%, respectively ($p \leq 0.05$), and also led to a decrease in lactate values by 23.93% ($p \leq 0.05$).

The drug pronoran in combination with amantadine statistically significantly increased pyruvate, malate, LDH and MDG by 23.81; 31.82; 44.44 and 39.73%, respectively ($p \leq 0.05$), and also inhibited lactic acidosis of the brain by 30.27%.

Melatonin statistically significantly increased pyruvate, malate, LDH and MDG by 28.89; 33.33; 47.51 and 45.30%, respectively ($p \leq 0.05$), and inhibited lactic acidosis of the brain by 39.06% ($p \leq 0.05$).

The effects of the studied drugs on key links in the pathogenesis of mitochondrial dysfunction in animals with experimental PD are presented in table 3.

Table 3

The effect of the studied drugs on the parameters of energy metabolism in the cytosolic fraction of the brain of rats with experimental Parkinson's disease

(M ± m; n = 10)

Indicator	Energetic charge	Energetic potential	Phosphorylation index	Thermodynamic respiration control
Intact (passive control)	0,89±0,07	5,86±0,52	4,54±0,50	3,48±0,29
Parkinson's disease (PD, active control)	0,71±0,11	3,78±0,28	1,90±0,13	1,25±0,11
PD+Amantadine (AM)	0,79±0,08	4,56±0,50	2,76±0,19	2,19±0,20*
PD+AM+Cerebrocurin	0,86± 0,10*	4,88±0,36*	3,65±0,48*	3,11±0,26*
PD+AM+Pramistar	0,81±0,07*	4,70±0,35*	2,89±0,31*	2,35±0,23*
PD+AM+Gliatilin	0,87± 0,10*	4,95±0,47*	3,80 ± 0,30*	3,27 ± 0,26*
PD+AM+Noofen	0,84± 0,11*	4,20± 0,31	3,04±0,51*	2,50±0,53*
PD+AM+Pronoran	0,86±0,09	4,61±0,55	3,81±0,31	3,31±0,22*
PD+AM+Melatonin	0,87±0,88*	5,02±0,49*	3,93±0,41*	3,37±0,28*

Notes: p is the level of statistical significance when comparing samples using ANOVA analysis of variance (Kraskel-Wallis test), * − $p \leq 0.05$ according to the control group.

In the formation of mitochondrial dysfunction in PD rats in the control group in neurons there are decrease in energy charge and energy potential by 20.23 and 35.50%, respectively, compared with the intact group, as well as a decrease in phosphorylation index and thermodynamic control of respiration by 58.15 and 64.08% respectively.

The appointment of neuroprotective therapy in the form of the drug cerebrocurin with basic therapy with amantadine led to an increase in energy charge, energy potential, phosphorylation index and thermodynamic control of respiration by 17.44; 22.54; 47.95 and 59.81%, respectively, statistically significant relative to control ($p \leq 0.05$).

Pramistar had a similar tendency to influence as cerebrocurin, but less pronounced. Thus, the increase in energy charge, energy potential, phosphorylation index and thermodynamic control of respiration was 12.35; 19.58; 34.26 and 46.81%, respectively, statistically significant relative to control ($p \leq 0.05$).

Gliatilin increased energy charge, energy potential, phosphorylation index and thermodynamic control of respiration by 18.39; 23.64; 50.00 and 61.77%, respectively, statistically significant relative to control ($p \leq 0.05$).

Noofen also had a positive effect on the normalization of mitochondrial dysfunction, but not so pronounced. The drug increased energy charge, energy potential, phosphorylation index and thermodynamic control of respiration by 15.48; 10.00; 37.50 and 50.00%, respectively, statistically significant relative to control ($p \leq 0.05$).

Pronoran significantly increased energy charge, energy potential, phosphorylation index and thermodynamic control of respiration by 17.44; 18.00; 50.13 and 62.24%, respectively, statistically significant relative to control ($p \leq 0.05$).

The appointment of neuroprotective therapy in the form of the drug melatonin with basic therapy with amantadine led to an increase in energy charge, energy potential, phosphorylation index and thermodynamic control of respiration by 18.39; 24.70; 51.65 and 62.91%, respectively, statistically significant relative to control ($p \leq 0.05$).

Discussion. A growing body of literature suggests that mitochondria are a major source of ROS, which may contribute to the development of intracellular oxidative stress. In the process of oxidative phosphorylation, complex I (NADH-quinone oxidoreductase) acts as an entry point for electrons from the mitochondrial matrix into the electron transport chain (ETC), catalyzes the transfer of electrons from NADH to ETC subunits. Complex I and III in ETC are considered to be the main areas of ROS production in mitochondria [11]. Superoxide radical is the primary ROS formed in mitochondria as a result of the transfer of one electron to oxygen in the respiratory chain. Superoxide dismutase 2 or MnSOD converts the

superoxide radical into hydrogen peroxide, which is further detoxified by the enzyme catalase. However, in the presence of metal ions such as Fe^{2+} hydrogen peroxide can be converted into a highly reactive hydroxyl radical as a result of the Fenton reaction, which causes severe oxidative damage to cellular components. It is believed that the production of superoxide depends on factors such as the concentration of electron donors, local oxygen concentration and the velocity kinetics of the second order between them [12].

In mitochondrial complex I, the hyperproduction of superoxide radicals is caused by low ATP production, as a consequence, high proton-driving force (ΔpH and Δψ) and a reduced pool of coenzyme Q; high NADH / NAD^+ ratio in the mitochondrial matrix. In addition to the above conditions, the formation of ROS in complex I also increases significantly during the process of reverse electron transport. The reverse transport of electrons occurs when there is a decrease in the pool of ubiquinone, which causes electrons to rise up from ubiquinone into complex I under conditions of high proton-driving force [13].

The development of oxidative stress as a result of hyperproduction of ROS is one of the putative mechanisms of death of dopaminergic neurons in PD, and mitochondrial complex I is considered one of the main sources of ROS. PD-specific decrease in the activity of mitochondrial complex I or the level of protein in the substantia nigra of patients has long been detected. Studies using purified mitochondria have also shown mitochondrial complex I deficiency in the frontal cortex of patients with PD. A small deficiency in the activity of complex I was also found in the striatum, cortical tissue of the brain, fibroblasts, platelets, skeletal muscle and lymphocytes of patients with PD [14]. Catalytic subunits of complex I have been found to contain oxidized proteins, and in patients with PD there was a correlation between increased protein oxidation and decreased electron transfer capacity, suggesting that oxidative damage to these subunits may lead to disruption of complex I. A recent study shows that the levels of oxidized coenzyme Q-10 and 8-hydroxy-2'-deoxyguanosine in the cerebrospinal fluid of patients with PD were significantly increased, indicating the role of mitochondrial oxidative damage and oxidative DNA damage in the pathology of PD. In addition, a decrease in the activity of complex I was observed in cytoplasmic hybrid cell lines that contain mitochondrial DNA from patients with PD [15].

Subsequent studies also showed that specific knockout in Ndufs4 gene mice in midbrain dopaminergic neurons did not cause overt neurodegeneration, loss of striatal innervation, or symptoms of parkinsonism, although these mice had impaired dopamine homeostasis and increased dopamine breakdown metabolites. Knockouts of dopaminergic neurons Ndufs4 did not lead to loss of substantia nigra neurons, but they were more

vulnerable to neurotoxicity induced by mitochondrial complex 1 and MPTP toxin. These data suggest that a deficiency of complex 1 may contribute to the death of dopaminergic neurons in the presence of other toxic factors [16].

Rotenone, a mitochondrial toxin, can also cause loss of dopaminergic neurons, and this toxicity was significantly attenuated by methylene blue, a compound that functions as an alternative electron transporter that bypasses blockade of complex I / III, emphasizing the role of complex 1 deficiency due to toxicity. It is known that the loss of dopamine homeostasis can affect mitochondrial function, and accordingly another study shows that redox modifications of dopamine can inhibit mitochondrial respiratory chain complexes [17].

Oxidized dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) inhibited the activity of complex I and complex II in a dose-dependent manner, whereas reduced dopamine, but not DOPAC, inhibited the activity of complex II. These data indicate possible targets of dopamine metabolites that could potentially contribute to the high sensitivity of dopaminergic neurons to the development of mitochondrial dysfunction in PD [18]. It is believed that the substantia nigra is more vulnerable to complex I dysfunction than other areas of the brain due to the generation of ROS by nigrostriatal dopaminergic neurons during dopamine metabolism. In addition, mouse dopaminergic neurons also showed a decrease in mitochondrial mass compared with non-dopaminergic neurons and ventral tegmental neurons, suggesting that this deficiency may contribute to the selective vulnerability of these neurons in mouse models with PD [19].

In general, all these literature data suggest that the violation of the mitochondrial respiratory chain, in particular the deficiency of complex I and the subsequent increase in the production of ROS, may indirectly or directly contribute to the progression of pathology in PD.

Limitations in research. Financial resources, method and data collection.

Prospects for further research. Prospects for further research are to study the features of changing the behavioral reactions and cognitive-mnestic functions of rats under experimental Parkinson's disease and the prospects of development of a strategy of pharmacological correction.

Conclusions

1. Under conditions of formation of experimental PD in rats develops energy deficiency of neuronal cells and dysfunction of the Krebs cycle with a predominance of anaerobic oxidation of the substrate, as well as the development of mitochondrial dysfunction.

2. Prescribed neuroprotective drugs significantly improved energy metabolism in the brain of rats with PD within statistical significance, increased levels of ATP and ADP, enzymatic activity of mCPK and ATPase, decreased levels of AMP, especially pronounced drugs melatonin, cerebrocurin, gliatilin and pronoran.

3. Neuroprotective therapy of experimental PD in rats contributed to a statistically significant increase in the values of pyruvate, malate, LDH and MDG, and also led to a decrease in the values of lactate and lactic acidosis in brain tissues.

4. The appointment of neuroprotective therapy with basic amantadine therapy led to an increase in energy charge, energy potential, phosphorylation index and thermodynamic control of respiration in the mitochondria of neurons, especially in the groups of melatonin, cerebrocurin, gliatilin and pronoran.

Conflicts of interest. Neither author has actual or potential conflicts of interest.

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