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MITOCHONDRIAL CEREBRAL DYSFUNCTION IN RATS WITH SCOPOLAMINE-INDUCED NEURODEGENERATION UNDER ENALAPRIL EFFECT

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Resume. Objective. *Neurodestructive diseases are characterized by complex pathobiochemical cascades in the neuron, which cause disturbances in energy metabolism and the formation of mitochondrial dysfunction. The renin-angiotensin system plays an important role in the physiological functioning of mitochondria, the excessive activity of which increases the risk of neurodegenerative diseases of the brain. Although angiotensin-converting enzyme inhibitors are now considered as means of prevention and treatment of ischemic lesions of the central nervous system, their corrective properties in the development of central neurodegeneration continue to be refined. The objective of our study was investigation of enalapril effect, as an angiotensin-converting enzyme inhibitor, in case of mitochondrial dysfunction of the cerebral cortex and hippocampus of rats under conditions of scopolamine-induced neurodegeneration reproducing development of Alzheimer's disease in the experiment.*

Material and methods. *Scopolamine hydrochloride (Sigma, USA) was injected in rats through the peritoneum at a dose of 1 mg/kg for 27 days to simulate Alzheimer's disease. Starting from the 28th day of the experiment, enalapril was introduced through the peritoneum at a dose of 1 mg/kg, once a day for 14 days.*

Results. *Under conditions of scopolamine-induced Alzheimer's disease in the mitochondrial fraction of the cerebral cortex and hippocampus of rats free radical oxidation of lipids and proteins increases, and activity of Krebs cycle enzymes decreases – α-ketoglutarate dehydrogenase and succinate dehydrogenase; light dispersion decreases and a relative rate of mitochondrial swelling increases. After enalapril administration for 14 days to rats with scopolamine-induced Alzheimer's disease the content of products reacting with 2-thiobarbituric acid and protein oxidation modification decreases in the mitochondrial fraction of the cerebral cortex and hippocampus; in both examined structures, the activity of catalase, α-ketoglutarate dehydrogenase, succinate dehydrogenase increases, and superoxide dismutase – only in the cerebral cortex; a gradual decrease of light dispersion and relative rate of mitochondrial swelling occurs.*

Conclusion. *Improvement of the antioxidant system state and energy supply of mitochondria, decreased intensity of mitochondrial swelling in the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer's disease are indicative of the protective properties of enalapril.*

МІТОХОНДРІАЛЬНА ДИСФУНКЦІЯ ГОЛОВНОГО МОЗКУ ЩУРІВ ЗІ СКОПОЛАМІН-ІНДУКОВАНОЮ НЕЙРОДЕГЕНЕРАЦІЄЮ ТА ПІД ВПЛИВОМ ЕНАЛАПРИЛУ

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Ключові слова: *еналаприл, скополамін-індукована хвороба Альцгеймера, функціональний стан мітохондрій.*

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Резюме. Мета роботи. *Для нейродеструктивних захворювань характерними є складні патобіохімічні каскади в нейроні, які спричиняють порушення енергетичного метаболізму та формування мітохондріальної дисфункції. У фізіологічному функціонуванні мітохондрій важливу роль відіграє ренін-ангіотензинова система, надмірна активність якої підвищує ризик нейродегенеративних захворювань головного мозку. Хоча сьогодні інгібітори ангіотензин-перетворювального ферменту розглядаються як засоби профілактики і лікування ішемічного ураження центральної нервової системи, їх корегувальні властивості при розвитку центральної нейродегенерації продовжують уточнюватись.*

Мета дослідження *– вивчення впливу інгібітору ангіотензин-*

перетворювального ферменту еналаприлу при мітохондріальній дисфункції кори головного мозку та гіпокампа щурів за умов скополамін-індукованої нейродегенерації, яка відтворює розвиток хвороби Альцгеймера в експерименті.

Матеріал і методи. *Експерименти проводили на нелінійних лабораторних білих щурах-самцях масою 0,18-0,20 кг. Модель хвороби Альцгеймера створювали внутрішньоочеревинним уведенням 27 днів скополаміну гідрохлориду (Sigma, США) дозою 1 мг/кг. Починаючи з 28-ї доби експерименту, еналаприл уводили внутрішньоочеревинно дозою 1 мг/кг, один раз у день упродовж 14 днів.*

Результати. *Встановлено, що при моделюванні скополамін-індукованої хвороби Альцгеймера в мітохондріальній фракції кори головного мозку та гіпокампа щурів підвищується вільнорадикальне окиснення ліпідів, білків та знижується активність ензимів циклу Кребса – α-кетоглутаратдегідрогенази та сукцинатдегідрогенази; знижується розсіювання світла та зростає відносна швидкість набухання мітохондрій. Після 14-денного введення еналаприлу щурам зі скополамін-індукованою хворобою Альцгеймера в мітохондріальній фракції кори головного мозку та гіпокампа знижується вміст продуктів, що реагують із 2-тіобарбітуровою кислотою та окисної модифікації білків; в обох досліджуваних структурах зростає активність каталази, α-кетоглутаратдегідрогенази, сукцинатдегідрогенанзи, а супероксиддисмутази – лише в корі головного мозку; спостерігається поступове зниження світлорозсіювання та зниження відносної швидкості набухання мітохондрій.*

Висновок. *Покращення стану антиоксидантної системи та енергозабезпечення мітохондрій, а також зниження інтенсивності набухання мітохондрій кори головного мозку та гіпокампа щурів зі скополамін-індукованою хворобою Альцгеймера вказує на протективні властивості еналаприлу.*

Introduction. Neurodestructive diseases are characterized by complicated pathobiochemical cascades in the neuron, which cause disorders of energy metabolism and the formation of mitochondrial dysfunction. Mitochondria are a primary source of reactive oxygen forms that can regulate calcium balance, intracellular oxidation-reduction processes and influence the cellular signals. Since neurons are limited in their glycolytic abilities, their functional activity depends on mitochondrial energy production more than on other body cells. A significant amount of mitochondria accumulates in the area of synapses. They provide the mechanisms to transmit nerve impulses. Therefore, mitochondrial dysfunction is one of the main pathogenic chains of neurodegenerative processes and Alzheimer's disease in particular.

According to current scientific data renin-angiotensin system (RAS) plays an essential role in the physiological functioning of mitochondria. Excessive activity of the system increases the risk of neurodegenerative diseases of the brain. Nowadays, the existence of intra-mitochondrial RAS is a well-known fact [1]. Though, in spite of the fact that a considerable amount of results is indicative of the participation of RAS components in central neurodegeneration, the efficacy of pharmacological modulators of RAS remains a subject of scientific investigations [2]. In case of cerebral ischemia, RAS blockers and angiotensin-converting enzyme inhibitors (ACEI), in particular, are known to decrease apoptosis in the hippocampus and considerably improve spatial learning and memory [3]. Although today ACEI are considered as means of prevention and treatment of ischemic injury of the central nervous system, their correcting properties with the development of central neurodegeneration are still being specified [4].

Objective. The objective of our study was to investigate the enalapril effect as an angiotensin-converting enzyme inhibitor in case of mitochondrial dysfunction of the cerebral cortex and hippocampus of rats under conditions of scopolamine-induced neurodegeneration reproducing development of Alzheimer's disease in the experiment.

Material and methods. The experiments were conducted on nonlinear albino male rats with a bodyweight of 0.18-0.20 kg, kept under standard vivarium conditions with natural day and night changes. Scopolamine hydrochloride (Sigma, USA) was injected into rats through the peritoneum at the dose of 1 mg/kg once a day for 27 days to create Alzheimer's disease model [5]. On the 28th day of the experiment, a group of rats with Alzheimer's disease (7 rats) began a course of treatment (14 days) with enalapril introduced through the peritoneum at the dose of 1 mg/kg. A solvent was introduced through the peritoneum to the comparison groups in the same regimen: the control rats and rats with simulated pathology (7 rats each).

Euthanasia of rats was conducted under light ether narcosis. The brain was removed and washed thoroughly with cooled 0.9 % NaCl solution. According to the stereotaxic atlas, the hippocampus and the cerebral cortex were isolated [6].

Mitochondrial fraction was isolated by means of the

differentiation centrifugation method of homogenates of the structures examined. For this purpose, the cerebral cortex and hippocampus were washed with cooled (2-4°C) 0.9 % KCl solution, grinded and homogenized in 10-fold volume of the buffer pH 7.4: sucrose 250 mM, ethylene diamine tetraacetate 1 mM, tris-HCl 10 mM [7].

The state of lipid peroxide oxidation in the mitochondria was evaluated by the levels of active products of thiobarbituric acid (AP TBA); carbonylation of mitochondrial proteins – by the amount of derivatives of 2,4-dinitrophenylhydrazone with the formation of carboxylphenylhydrazone (CPH) and expressed in nmol of carbonyl derivatives per 1 mg of protein [8]. The state of the antioxidant protection system in the mitochondria was evaluated by the activity of superoxide dismutase (SOD) enzymes [EC 1.15.1.1] and catalase [EC 1.11.1.6] [9, 10]. The activity of enzymes of α-ketoglutarate dehydrogenase (α-KGDH) [EC 1.2.4.2] and succinate dehydrogenase (SDH) [EC 1.3.5.1] by means of the spectrophotometric method was determined to assess the energy supply of the mitochondria [11, 12]. Swelling of the mitochondria was registered by their ability to extension-contraction and changes in optic density [13, 14]. Changing of E 520 parameters in the incubation medium was used to calculate a relative rate of mitochondrial swelling. This parameter characterizes changes in the permeability of the organelle's internal membrane (massive swelling and depolarization of mitochondria) resulting from mitochondrial pore formation due to the overloading of cells with calcium ions [15]. The protein content in the mitochondria was determined by the Lowry method [16].

The results of the study were statistically processed using the Student criterion. The Mann-Whitney nonparametric criterion of comparison was also used to confirm the reliability of the conclusions. It demonstrated similar results of calculations made applying the Student criterion concerning the p value. Differences were considered statistically valuable with $p \leq 0.05$.

Results. The results of our investigations showed (Table) that scopolamine-induced Alzheimer's disease is characterized by disorders of the prooxidant-antioxidant balance in the mitochondria of the cerebral cortex and hippocampus. 70.3 % increase in AP TBA content in the cerebral cortex and 100.9 % increase in the hippocampus were determined in comparison with the parameters of the control group. CPH content in the mitochondria of both examined structures was 46.5 % increased. The data obtained were indicative of the intensification of free radical oxidation processes of lipids and proteins that promoted injury of biological membranes. At the same time, functional disorders of the antioxidant protection enzymatic systems were found by the indices of SOD and catalase activity.

Thus, SOD activity in the cerebral cortex 27.9 % decreased, and only the tendency to its activity decrease was found in the hippocampus. Catalase activity in both examined structures decreased: 25.4 % in the cerebral cortex and 45.6 % in the hippocampus. The data obtained are first of all indicative of cerebral cortex injury in rats with simulated Alzheimer's disease. Second, they are indicative of greater injury of the hippocampus as an important chain in the development of neurodestructive pathology.

Moreover, on the basis of the investigations conducted decrease in dehydrogenase activity was found in the group of rats with simulated pathology, which determines the efficacy of the mitochondrial energy supply of the brain (Fig. 1, 2). In fact, α-KGDH activity (NAD^+-dependent enzyme) 35.4 % decreased in the cerebral cortex and 43 % – in the hippocampus. At the same time, the activity of FAD^+-dependent SDH in both examined structures was 63.6 and 54.8 % decreased, respectively. According to the literary data, disorders of oxidation processes and excessive formation of free radicals at the early stages of the development of Alzheimer's disease can result in the irreversible opening of a mitochondrial pore, promoting the apoptosis process [17].

Further investigations showed that after enalapril administration the content of AP TBA 23.4 % decreased in the cerebral cortex, and 33.9 % - in the hippocampus. At the same time, the content of CPH in both structures 29.6 % decreased in the cerebral cortex and 31.1 % – in the hippocampus. The results obtained are indicative of inhibition of lipid and protein peroxidation processes.

A positive effect of enalapril on the activity of antioxidant protection enzymes should be mentioned.

Table

Enalapril effect on free radical lipid and protein oxidation, energy supply in the mitochondria of the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer's disease (M±m, n=7)

Indices	Examined cerebral structures	Control	Alzheimer's disease	Alzheimer's disease + enalapril
The content of AP TBA, nmol/mg of protein	Cortex	12.78±1.251	21.77±1.502*	16.71±0.872* **
	Hippocampus	11.63±0.652	23.34±1.301*	15.39±0.930**
The content CPH, nmol/mg of protein	Cortex	24.67±1.391	36.17±2.623*	25.54±1.182**
	Hippocampus	18.29±1.102	26.68±1.209*	18.42±1.011**
The activity of SOD, units /mg of protein	Cortex	0.428±0.0271	0.307±0.0173*	0.497±0.0251**
	Hippocampus	0.379±0.0449	0.257±0.0541	0.351±0.0462
The activity of catalase, mcmol H_2O_2/min of mg of protein	Cortex	175.87±10.576	131.17±10.018*	184.57±15.898**
	Hippocampus	170.21±10.990	92.56±15.159*	157.29±10.547**

*Note. * – reliability of differences compared with the control group of rats; ** – reliability differences compared with the group of rats with Alzheimer's disease.*

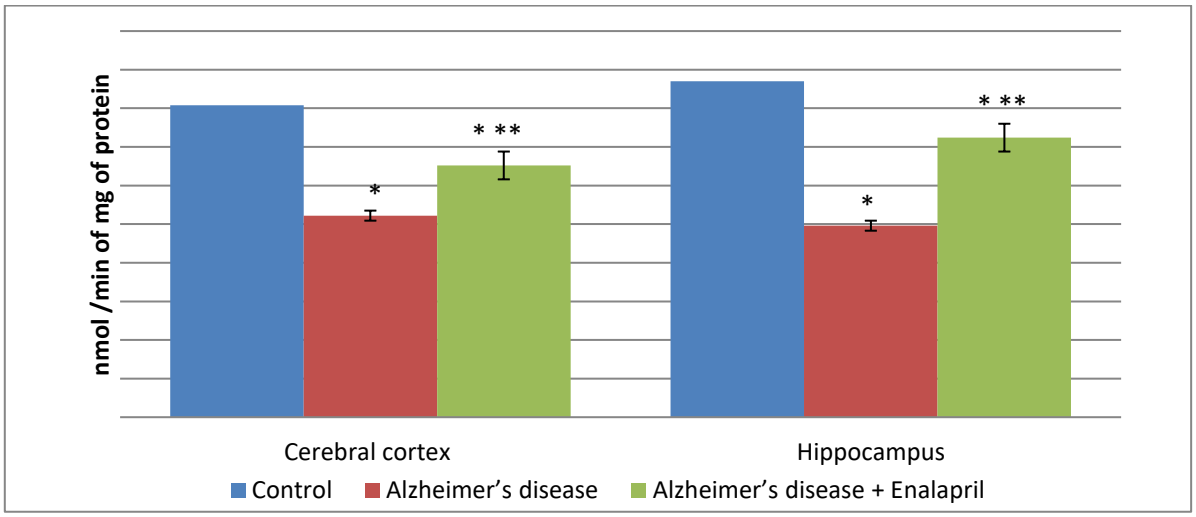

Fig. 1. *Enalapril effect on the activity of α-ketoglutarate dehydrogenase in the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer's disease (M±m, n=7). Note. * – reliability of differences compared with the control group of rats, ** – reliability of differences compared with the group of rats with Alzheimer's disease*

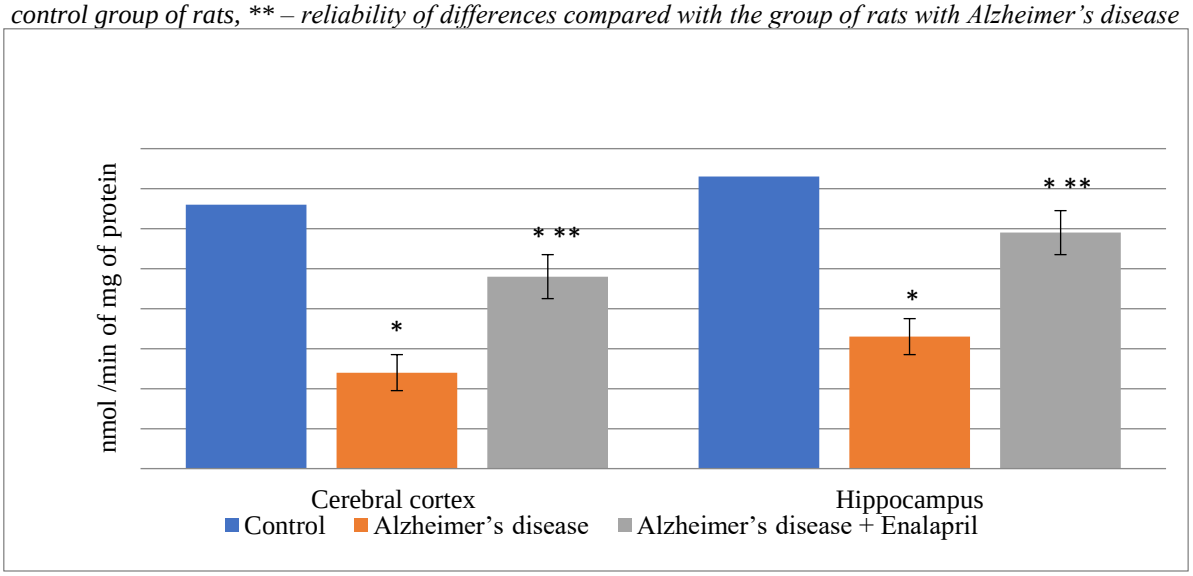

Fig. 2. *Enalapril effect on the activity of succinate dehydrogenase in the mitochondria of the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer's disease (M±m, n=7). Note. * – reliability of differences compared with the control group of rats; ** – reliability of differences compared with the group of rats with Alzheimer's disease*

Thus, activity of SOD and catalase in the cerebral 29 and 40.7 % increased. In the hippocampus 69.9 % increase of catalase was found only, which confirms primary injury of hippocampus with scopolamine-induced Alzheimer's disease. At the same time, increased activity of Krebs cycle enzymes was found: α-KGDH and SDH activity 24.9 and 100 % increased in the cerebral cortex, and in the hippocampus – 45.9 and 78.8 % respectively.

As it was mentioned above, synapses are the first "indicator" of disorders in case of Alzheimer's disease, since they require considerable energy supply. Therefore, we got interested in functional changes in mitochondria as energy producing structures in the cerebral cortex and hippocampus under conditions of neurodegeneration development and assess enalapril effect on their functional state.

Fig. 3a, b presents the dynamics of changes in the mitochondrial swelling intensity of the examined brain structures. In rats of the control group, after 60-minute incubation of mitochondrial suspension of the cerebral cortex and hippocampus, the level of light dispersion 1.1 times decreased. In rats with Alzheimer's disease, light dispersion of mitochondrial suspension 60 minutes later 1.2 times decreased in the cerebral cortex and 1.3 times – in the hippocampus. Enalapril administration for 14 days to rats with Alzheimer's disease led to a gradual decrease in light dispersion in the cerebral cortex and the hippocampus – 1.0 and 1.1 times, respectively.

Further analysis of investigations showed that a relative rate of mitochondrial swelling which is measured in UN/min/mg of protein in rats with scopolamine-induced Alzheimer's disease increase in comparison with the control group in the cerebral cortex from 1.6±0.014 to 1.9±0.008 and in the hippocampus – from 1.8±0.009 to

2.1±0.012. Though after enalapril administration a relative rate of mitochondrial swelling decreased in comparison with the parameters of rats with stimulated pathology in both examined structures: to 1.75±0.011 – in the cerebral cortex and 2.0±0.007 – in the hippocampus.

Discussion. Thus, in rats with scopolamine-induced Alzheimer's disease the pore of non-specific permeability of the mitochondria is activated, as it is a regulator of ion and protein transport. Respectively, the intracellular Ca^{2+} pool increases and imbalance between the cytosolic and mitochondrial ion levels occurs. These processes induce disorders of prooxidant-antioxidant balance in the mitochondria.

Under conditions of enalapril administration in rats with Alzheimer's disease the activity of the antioxidant system enzymes and Krebs cycle increased, the content of products of lipid and protein peroxidation and intensity of mitochondrial swelling decreased. Inhibition of oxygen reactive forms production is a probable mechanism of the agent action, which is stimulated by angiotensin II. It results in the decrease of AP TBA and CPH content and increase of antioxidant enzymes activity both in the cerebral cortex and hippocampus. The above processes inhibit development of mitochondrial dysfunction due to the blockade of NADN-oxidase of the endothelial cells and inhibition of peroxinitrite formation, which promotes improvement of the cerebral circulation and inhibits ischemic-hypoxic effects on the central nervous system [18].

One of the important mechanisms in enalapril action is the fact that blockade of angiotensin II stimulates K^+-mediated release of acetylcholine – one of the primary neurotransmitters of the cholinergic system. Clinical signs of Alzheimer's disease are associated with the loss of cholinergic innervation in the cerebral cortex [19], which is confirmed by clinically evidenced efficacy of

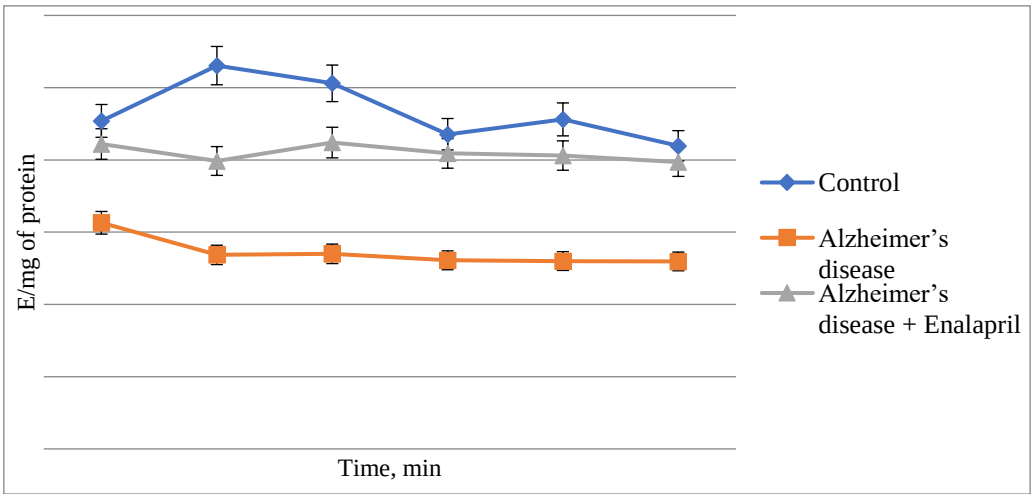

Fig. 3a. *Intensity of mitochondrial swelling in the cerebral cortex of rats with scopolamine-induced Alzheimer's disease after enalapril administration during 14 days in the dose of 1 mg/kg.*
Note. The resalts are represented as Me[min-max], n=7, p≤0.05

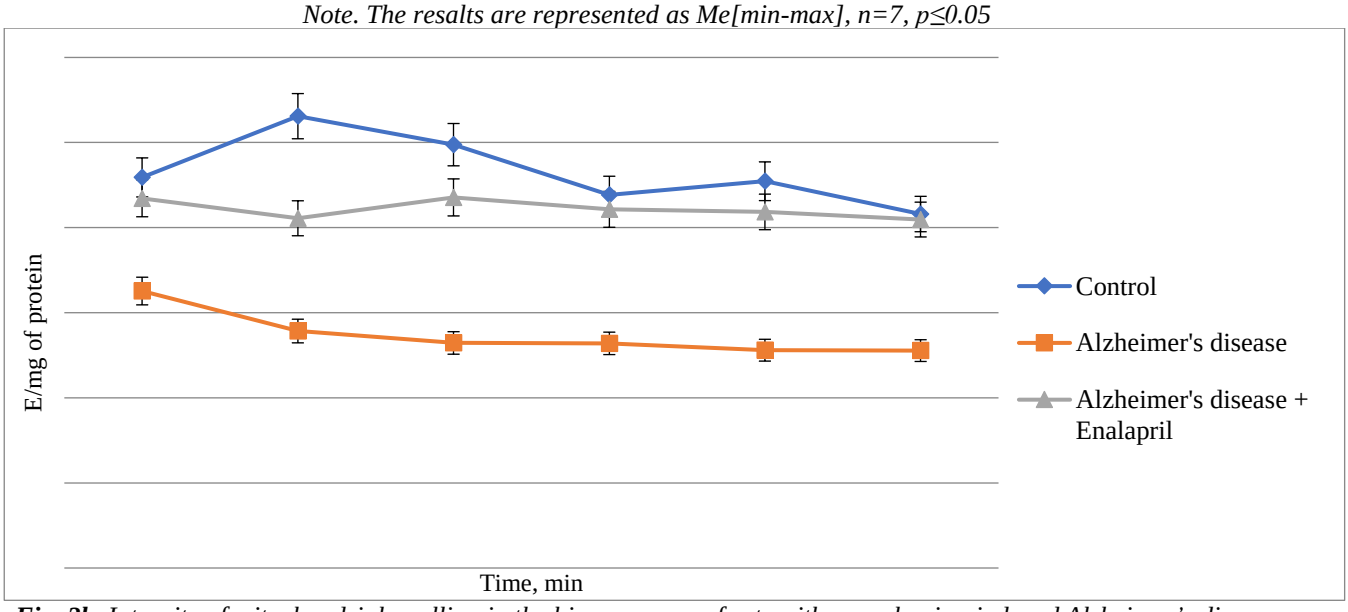

Fig. 3b. *Intensity of mitochondrial swelling in the hippocampus of rats with scopolamine-induced Alzheimer's disease after enalapril administration during 14 days in the dose of 1 mg/kg.*
Note. The results are represented as Me[min-max], n=7, p≤0.05

anticholinesterase preparations of a central action [20].

Therefore, the results obtained are indicative of the fact that enalapril under conditions of scopolamine-induced Alzheimer's disease in rats possesses a protective effect, which is characterized by improvement of a functional state and parameters of the mitochondrial energy metabolism, increased activity of the antioxidant system in the cerebral cortex and hippocampus.

Conclusion. Under conditions of scopolamine-induced Alzheimer's disease in the mitochondrial fraction of the cerebral cortex and hippocampus of rats, free radical oxidation of lipids and proteins increases, and activity of Krebs cycle enzymes decreases – α-ketoglutarate dehydrogenase and succinate dehydrogenase; light dispersion decreases and a relative rate of mitochondrial swelling increases.

After enalapril administration for 14 days to rats with scopolamine-induced Alzheimer's disease, the content of products reacting with 2-thiobarbituric acid and protein oxidation modification decreases in the mitochondrial fraction of the cerebral cortex and hippocampus; in both examined structures the activity of catalase, α-ketoglutarate dehydrogenase, succinate dehydrogenase increases, and superoxide dismutase – only in the cerebral cortex; a gradual decrease of light dispersion and relative rate of mitochondrial swelling occurs.

Improvement of the antioxidant system state and energy supply of mitochondria, decreased intensity of mitochondrial swelling in the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer's disease are indicative of protective properties of enalapril.

Prospects for further research. It is planned to study the effect of enalapril on the morphological state of the cerebral cortex and hippocampus under the conditions of experimental neurodegenerations of different genesis.

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