



# Neuroprotective Effect of L-carnitine. Focus on Changing Mitochondrial Function

Andrey V. Voronkov<sup>1†</sup>, Dmitry I. Pozdnyakov<sup>2</sup>

1 *Volgograd State Medical University, 1 Pavshikh Bortsov St, Volgograd 400131 Russia*

2 *Pyatigorsk Medical and Pharmaceutical Institute, 11 Kalinina Ave., Pyatigorsk 357532 Russia*

Corresponding author: Dmitry I. Pozdnyakov ([pozdniackow.dmitry@yandex.ru](mailto:pozdniackow.dmitry@yandex.ru))

---

Academic editor: Mikhail Korokin ♦ Received 9 August 2020 ♦ Accepted 30 September 2020 ♦ Published 17 November 2020

---

Citation: Voronkov AV, Pozdnyakov DI (2020) Neuroprotective Effect of L-carnitine. Focus on Changing Mitochondrial Function. Research Results in Pharmacology 6(4): 29–42. <https://doi.org/10.3897/rrpharmacology.6.57419>

---

## Abstract

**Introduction:** In this study, the neuroprotective effect of L-carnitine administered *per os* in a dose of 25 mg/kg – 800 mg/kg was evaluated. The effects of L-carnitine on changes in mitochondrial function were also studied.

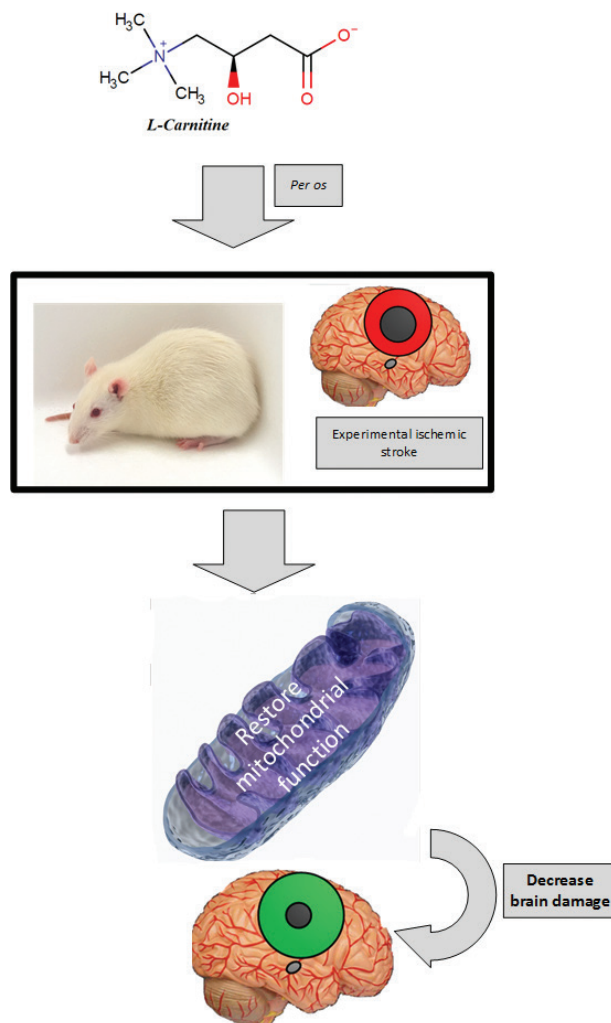
**Materials and Methods:** The neuroprotective effect and mitochondrial function were evaluated in a model of permanent focal ischemia in Wistar-line rats. L-carnitine was administered to rats orally for 72 hours from the moment of modeling ischemia. On the 4<sup>th</sup> day after the ischemia simulation, the change in the respiratory function of the mitochondria, the opening time of the mitochondrial permeability transition pore, the mitochondrial membrane potential, the concentration of intracellular calcium and the size of the cerebral necrosis zone were determined in rats' brain supernatant.

**Results:** As a result, it was found that the administration of L-carnitine contributed to the restoration of mitochondrial function and a decrease in the size of the brain necrosis zone. At the same time, the administration of L-carnitine in low doses (25 mg/kg – 100 mg/kg) did not have a significant effect on the change in the concentration of intracellular calcium. It should be noted that an increase in the dose of L-carnitine from 200 mg/kg to 800 mg/kg was not accompanied by a significant increase in the therapeutic effect.

**Discussion:** L-carnitine is one of the key biomolecules that directly affect metabolic processes. It is known that L-carnitine acts as a "shuttle" for long-chain fatty acids and thus can affect the alteration of mitochondrial function. However, the detailed nature of the mitochondriotropic action of L-carnitine has not been yet established. This was the focus of this study, which showed that the mitochondrion-oriented effect of L-carnitine is dose-dependent and expressed in the form of restoring the respiratory function of mitochondria, restoring the mitochondrial potential and increasing the latent opening time of the mitochondrial permeability transition pore, reducing the level of intracellular calcium.

**Conclusion:** The study allowed us to expand our understanding of the L-carnitine neuroprotective effect and the effect of this compound on changes in mitochondrial function.

### Graphical abstract



### Keywords

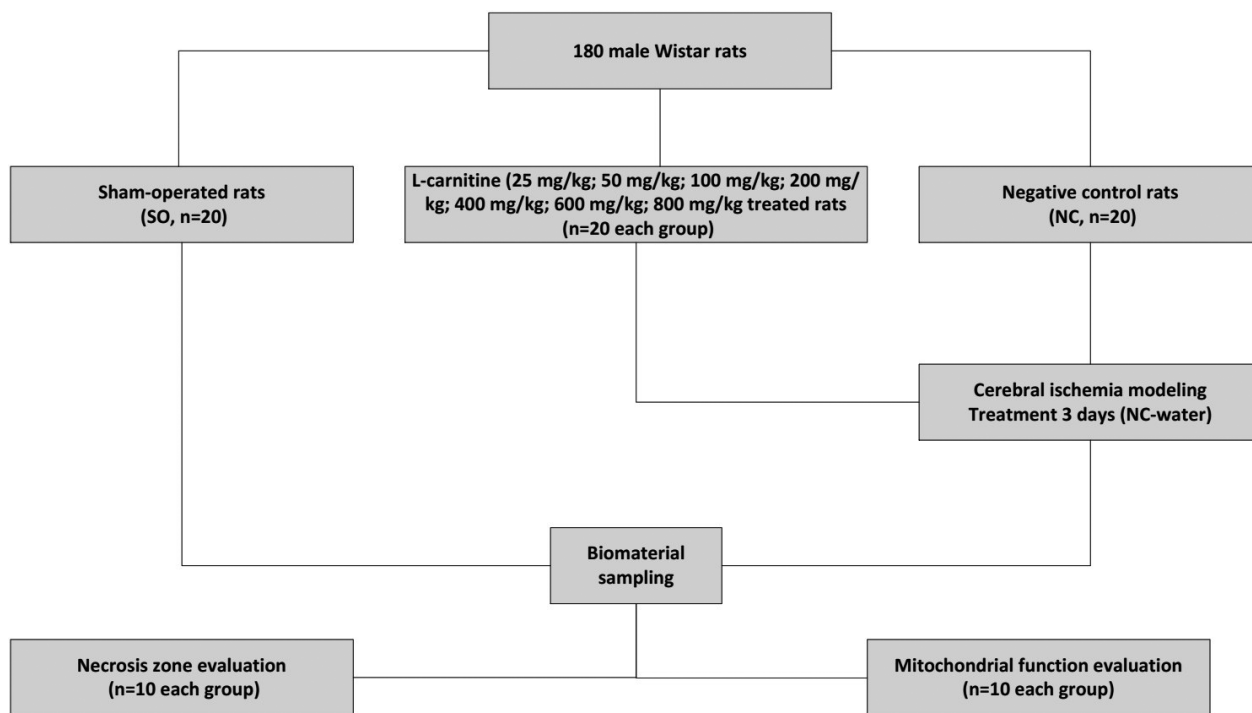
ischemic stroke, mitochondrial dysfunction, L-carnitine, neuroprotective effect.

### Introduction

Mitochondria are primarily known as intracellular energy sources that generate it in the form of ATP during oxidative phosphorylation reactions (OXPHOS) (Sazanov 2015). However, recent studies have shown that the role of mitochondria in the cell is wider than previously thought. To date, it has been established that mitochondria are directly involved in the regulation of calcium homeostasis, programmed apoptotic cell death, and redox reactions. The role of mitochondria in maintaining cell growth and proliferation is also significant (Bonneau et al. 2016). Thus, based on the significant role of mitochondria in cell physiology, it can be assumed that disruption of mitochondrial function dramatically affects the activity of many organs and systems, primarily with high metabolic activity (Grimm and Eckert 2017). It has been established that the brain, making up no more than 2% of body

weight, consumes approximately 20% of the total level of ATP synthesized in the body, and therefore the development of mitochondrial dysfunction will extremely negatively affect the optimal functional state of the brain (Joshi and Mochly-Rosen 2018).

Mitochondrial dysfunction is an integral component of the pathogenetic cascade of ischemic brain damage. It is known that with the development of cerebral ischemia and associated diseases, for example, ischemic stroke in the brain, there are two main damage zones – the zone of cerebral infarction and the area of ischemic penumbra, with neurons localized in the latter being the main target of neuroprotective therapy (Guan et al. 2018). In the focus of ischemic penumbra, there is a decrease in the metabolic activity of cells to the lowest possible physiological level, an increase in which can stabilize the state of neuronal cells and reduce the area of cerebral infarction (Dong et al. 2015). Mitochondria located in the penumbra



**Figure 1.** Study design. **Note:** A model of irreversible focal ischemia in rats was used in this study. L-carnitine was administered orally at the indicated doses. The defined parameters were: cerebral necrosis zone; ATP-generating ability; maximum level of respiration; respiratory capacity; intensity of glycolysis; glycolytic capacity; glycolytic reserve; mitochondrial complexes activity; latent time of mitochondrial permeability transition pore; mitochondrial membrane potential; citrate synthase activity; and content of ionized calcium.

zone, due to oxygen deficiency, are unable to ensure the synthesis of macroergic phosphates at the proper level, which leads to a redistribution of the electron flux in the mitochondrial respiratory chain (inversion of the activity of  $F_1F_0$  ATP synthase). In addition, a decrease in the proton gradient leads to reduced mitochondrial membrane potential and the formation of a mitochondrial permeability transitional pore, which is the “no return point”, after which the cell reaches the irreversible apoptotic stage (Broughton et al. 2009). Also, due to the impaired functioning of mitochondrial calcium transporters, the concentration of ionized calcium in the cell increases, which can lead to an increase in the secondary cascade of neuronal damage. It is worth noting that neurons and, accordingly, mitochondria are sensitive to targeted therapeutic effects for several days, which significantly increases the chances of a more favorable prognosis of the disease (Broughton et al. 2009). For example, the study by Mondal et al. (2019) showed a future outlook on correcting mitochondrial dysfunction to achieve a neuroprotective effect. In that work, the administration of tetrahydrocurcumin to mice with ischemic-reperfusion brain damage restored mitochondrial ATP synthesis, reduced the degree of mitochondrial permeability transitional pore opening, and increased basal oxygen consumption (Mondal et al. 2019). At the same time, *L-carnitine* is a well-known metabolic agent, and it is reasonable to assume that the use of this compound is likely to have a positive effect on the change in the functional activity of mitochondria (Koeth et al. 2019).

## Material and methods

### Laboratory animals

This work was performed on 180 male Wistar rats weighing 200–220 grams obtained from the Rappolovo Laboratory Animal Nursery (Leningrad region, Russia). All the animals underwent microbiological control and two-week quarantine before being included in the study. For the duration of the experiment, the rats were kept in Macrolon type III cages, with 5 animals each, which prevented huddling and stress. Granulated wood fraction, changed daily, was used as bedding. The animals had free access (ad libitum) to water and complete pelleted feed. The maintenance conditions (ambient temperature  $20 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 5\%$ , with a natural light-dark change and normal atmospheric pressure), as well as all the manipulations with the rats, were in compliance with the generally accepted standards for working with laboratory animals (Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes, September 22, 2010). The research concept was approved by the local ethics committee (minutes No. 14 dated 12/23/2019).

### Focal cerebral ischemia model

Cerebral ischemia in rats was modeled by the modified Tamura method. The rats were anesthetized with chloral hydrate (350 mg/kg, intraperitoneally); the skin was removed

above and to the right of the eye, after which an incision was made, and the soft tissues were dissected, exposing the zygomatic process. Next, the zygomatic process was removed, and a burr hole was made with a high-speed drill above the intersection of the middle cerebral artery and the olfactory tract (the dura mater was removed). Then, coagulation of the middle cerebral artery was performed using a thermocoagulator, and the termination of blood flow through the artery could be observed. If possible, the topography of the soft tissues was restored, the wound was sutured in layers and treated by an antiseptic solution (Benzylidimethyl [(3-myristoilamine) propyl] ammonium chloride monohydrate; 0.01% solution). Until awakening, the animals were kept under a warming lamp (Pozdnyakov et al. 2019).

### Study design

During the study, nine equal experimental groups with 20 animals in each were formed. The first group included the sham-operated (SO) animals. In the remaining rats, ischemic brain damage was modelled, in addition, the negative control group (NC) was deprived of pharmacological support, and animal groups №№. 3–9 received **L-carnitine** (“Elkar”, manufactured by PIK-PHARMA, Russian Federation) at various doses (25 mg/kg; 50 mg/kg; 100 mg/kg; 200 mg/kg; 400 mg/kg; 600 mg/kg, and 800 mg/kg). **L-carnitine** was administered per os through an atraumatic catheter 30 minutes after modeling cerebral ischemia and then once a day for three days. On the fourth day after the operation, the rats were decapitated, and the brain was removed under chloral hydrate anesthesia. Moreover, in 10 animals from the group, the change in the size of their brain necrosis zone was evaluated, and the state of mitochondrial function was studied in the remaining rats. The study design is shown in Fig. 1.

### Biomaterial sampling

The animals’ brain was used in this work as test-material. The rats were decapitated; their crania were opened, and the brain was removed. Moreover, in 10 animals from the group, the brain was homogenized in PBS (phosphate-buffered saline) with a pH of 7.4 in a ratio of 1:7 in a Potter mechanical homogenizer (the change in the size of the brain necrosis zone was evaluated). In the remaining 10 rats, the brain was homogenized in an isolation medium (1 mmol EDTA, 215 mmol mannitol, 75 mmol sucrose, 0.1% BSA solution, 20 mmol HEPES, with a pH of 7.2). Next, the resulting homogenate was centrifuged at 1,000 g for 3 minutes, then the post-nuclear supernatant was re-centrifuged at 13,000 g for 15 minutes. The mitochondrial fraction was resuspended in the isolation medium. All the procedures were performed at 4 °C (Folbergrová et al. 2016)

### Necrosis zone evaluation

The size of the necrosis zone was determined by the triphenyltetrazolium method. The brain was removed, with

cutting off the cerebellum and separating the hemispheres. Both hemispheres were weighed, then separately homogenized and placed in cups with 10 ml of a 1% solution of triphenyltetrazolium chloride in phosphate buffer (pH 7.4). The samples were placed in a water bath for 20 minutes at 37 °C. The samples were centrifuged at 5000 RPM/10 min. Next, 3 ml of cooled chloroform was added to the obtained supernatant and incubated for 15 minutes at 40 °C, periodically stirring. The resulting mixture was re-centrifuged, and the optical density of the chloroform extract of formazan was measured at 492 nm against pure chloroform. The calculation of the necrosis zone was expressed as a percentage of the total mass of the hemispheres by the formula (Voronkov et al. 2019):

$$x = 100 - \frac{\varepsilon_1 M_1 + \varepsilon_2 M_2}{\varepsilon_1 (M_1 + M_2)} 100$$

where x is the size of the necrosis zone as a percentage of the total mass of the brain;

$\varepsilon_1$  is the optical density of the sample with an intact hemisphere;

$\varepsilon_2$  is the optical density of the sample with a damaged hemisphere;

$M_1$  is the mass of the intact hemisphere;

$M_2$  is the mass of the damaged hemisphere.

### Respirometric analysis

An analysis of the state of the respiratory function of mitochondria was carried out by the method of respirometry using an AKPM1-01L laboratory respirometer system (Alfa Bassens, Russia). The mitochondrial respiratory function was assessed by the change in oxygen consumption in the medium against the introduction of mitochondrial respiratory uncouplers. The last in the work were: oligomycin 1 µg/ml; 4-(trifluoromethoxy) phenyl) hydrazono) malononitrile (FCCP-1 µmol/ml); rotenone – 1 µM/ml; and sodium azide – 20 mmol/ml. The oxidation substrates were: glucose – 15 mmol/ml; pyruvic acid – 10 mmol/ml; malate – 1 mmol/ml; succinate – 10 mmol/ml; ascorbate – 2 mmol/ml; ADP – 1 mmol/ml; N, N, N', N'-tetramethyl-1,4-phenylenediamine (TMPD) – 0.5 mmol/ml. The overall assessment of mitochondrial function was determined by the level of oxygen consumption in the medium after sequential addition of oligomycin, FCCP and rotenone to the medium, and the ATP-generating ability was determined (by the difference in oxygen consumption after the addition of FCCP and oligomycin); the maximum level of respiration (by the difference in oxygen consumption after the addition of FCCP and rotenone) and the respiratory capacity (by the difference in oxygen consumption after the addition of FCCP and the basal level of oxygen consumption). The activity of glycolysis processes was evaluated when glucose was used as an oxidation substrate during the registration of oxygen consumption under the conditions of sequential addition of glucose, oligomycin and sodium azide to the

medium. The intensity of glycolysis was determined (by the difference in oxygen consumption after adding glucose and the basal level of oxygen consumption), glycolytic capacity (by the difference in oxygen consumption after adding oligomycin and glucose), and glycolytic reserve (by the difference in oxygen consumption after adding glucose and sodium azide). Additionally, the activity of complexes I, II, IV, and V of the mitochondrial respiratory chain was evaluated. The activity of complex I was determined by the difference in oxygen consumption after adding the malate/pyruvate and rotenone mixture to the medium. The activity of complex II was evaluated by the difference in oxygen consumption after adding succinate and oligomycin to the medium. The activity of complex IV was determined by the difference in oxygen consumption after adding the mixture of rotenone/TMPD/ascorbate and sodium azide to the medium. The activity of complex V was evaluated by the difference in oxygen consumption after adding rotenone and ADP to the medium. During the analysis, the biosample volume was 275  $\mu$ l, and that of the injected analyzers – 25  $\mu$ l. Oxygen consumption was determined in ppm. The oxygen consumption in the sample was converted to the protein concentration in the sample, which was determined by the Bradford method, as described in (Carlsson et al. 2011).

#### Evaluation of mitochondrial permeability transition pore opening

The latent time of mitochondrial permeability transition pore opening was evaluated by a spectrophotometric method. The incubation medium contained: 0.5 ml of the analyzed supernatant, 200 mM of KCl, and 0.5 ml of a 1  $\mu$ M solution of cyclosporin A. The resulting mixture was adjusted to 2 ml with HEPES buffer solution with a pH of 7.4. The optical density of the mixture was recorded at  $\lambda = 540$  nm, then the resulting solution was incubated for 25 min at room temperature with constant stirring. At the same time, the latent time of opening of the mitochondrial pore was evaluated in seconds (by changing the optical density of the incubation medium) (Zhyliuk et al. 2015).

#### Mitochondrial membrane potential evaluation

Mitochondrial membrane potential was evaluated by a spectrophotometric method. The incubation medium contained: 0.5 ml of the analyzed supernatant and 0.5 ml of a 9  $\mu$ M solution of safranin O. The resulting mixture was adjusted to 2 ml with HEPES buffer solution with a pH of 7.4. The optical density of the mixture was recorded at  $\lambda = 515$  nm and  $\lambda = 525$  nm. The proton moving force (transmembrane electrochemical gradient,  $\Delta\Psi$ ) was determined by the difference of the optical density:  $\Delta\Psi = A_{515} - A_{525}$  (Zhyliuk et al. 2015).

#### Evaluation of citrate synthase activity

The citrate synthase activity was evaluated according to the method proposed by Shepherd and Garland based

on spectrophotometric determination of colored decay products of 5,5'-di-thiobis-(2-nitrobenzoic acid) in the presence of acetyl-CoA and oxaloacetate. The reaction mixture contained: 5,5'-di-thiobis-(2-nitrobenzoic acid) – 100 mM; Tris-HCl buffer with a pH of 7.8–100 mM; acetyl CoA – 100 mM; 0.1% Triton-X – 100  $\mu$ l, and the investigated supernatant – 4  $\mu$ l. The reaction was started by adding 100  $\mu$ l of oxaloacetate. The change of absorbance was recorded at a wavelength of 412 nm for 3 minutes at room temperature. Citrate synthase activity was expressed in U/mg protein. Protein concentration was estimated by the Bradford method (Shepherd and Garland 1969).

#### Evaluation of the calcium ions concentration

The content of ionized calcium in the analyzed samples was evaluated by the fluorescence method using Fura-2/AM as a reporter. The incubation medium contained equimolar amounts (100  $\mu$ l) of the studied supernatant and fluorescent reporter. The excitation wavelength of Fura-2/AM is 340 nm. The emission wavelength of Fura-2/AM is 380 nm. The concentration of calcium in the sample was calculated as the difference between the fluorescence signals at 340 nm and 380 nm, with a 510 nm filter installed (Hitachi MPF-4 spectrofluorimeter). The calcium content was converted to the protein concentration in the sample (Field et al. 1994).

#### Statistical analysis

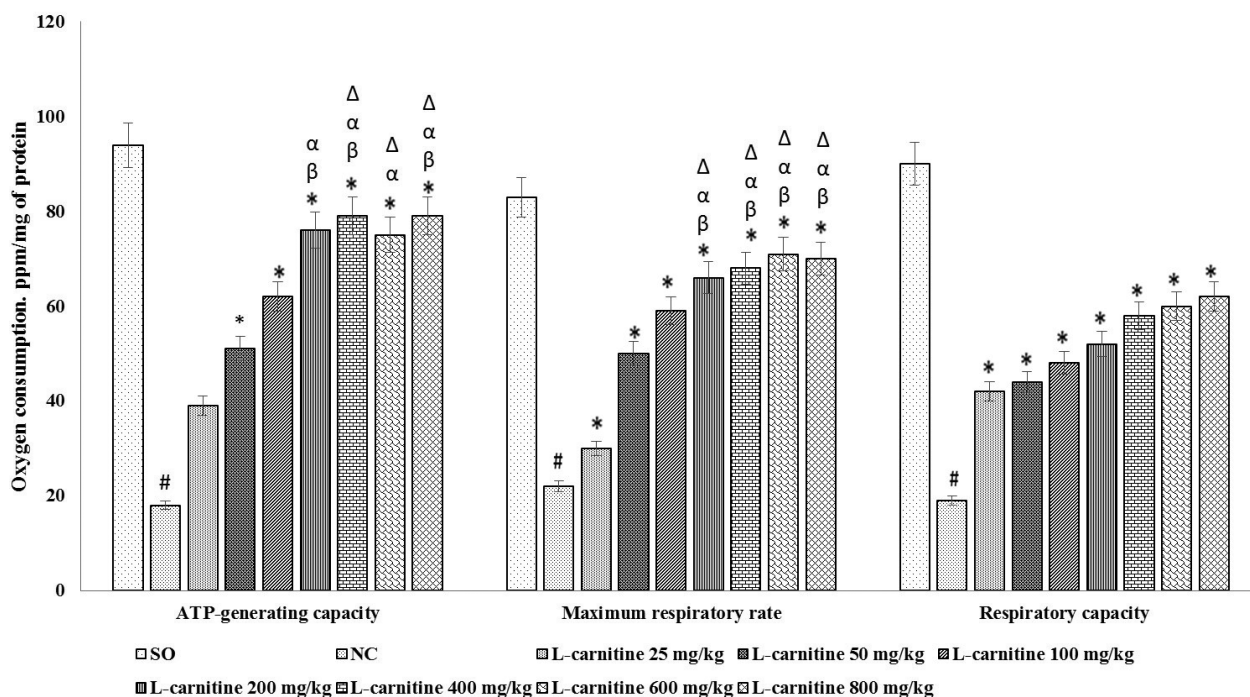
The obtained results were statistically processed and expressed as  $M \pm SEM$ . A comparison of groups of means was carried out using the ANOVA method with post-processing by means of Newman-Keuls test for multiple comparisons. The differences were considered statistically significant at  $p < 0.05$ . For the statistical analysis, the STATISTICA 6.0 application package (StatSoft, USA) for Windows was used.

## Results

#### The results of the L-carnitine effect on the change in the total respiratory function of neuronal mitochondria in rats under the cerebral ischemia conditions

In the course of this block of the experimental work, it was found that in the animals of the NC group, in comparison with the SO (sham-operated) rats, the ATP-generating ability, the maximum respiration rate and respiratory capacity decreased by 5.9 times ( $p < 0.05$ ), 3.47 times ( $p < 0.05$ ), and 4.78 times ( $p < 0.05$ ), respectively (Fig. 2).

Against the L-carnitine administration to the animals, dose-dependent changes in the studied parameters were registered. For instance, with an increase in the administered dose of L-carnitine from 200 mg/kg to 800 mg/kg, the studied indicators of the mitochondria total respiratory function did not change significantly and were

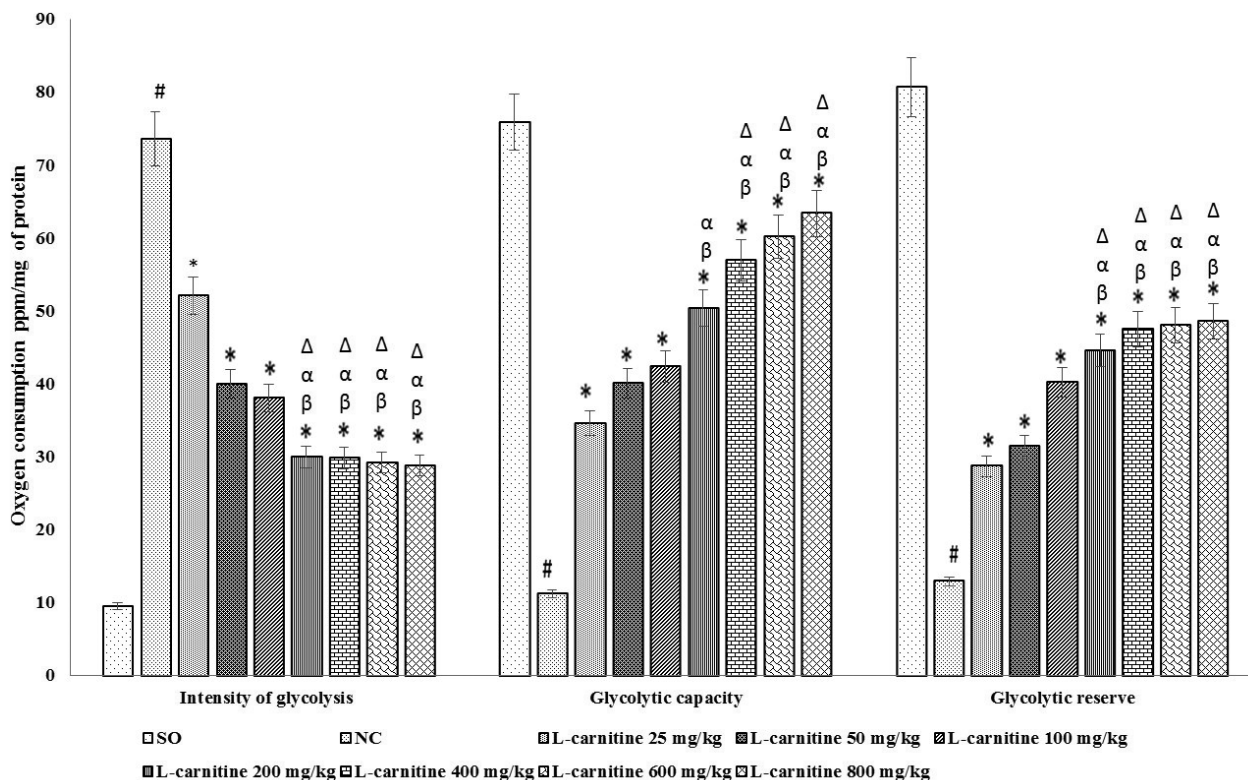


**Figure 2.** The effect of L-carnitine at various doses on the change of overall mitochondrial function in rats with cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats; β – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg; α – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg; Δ – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .

higher than the similar parameters of the NC group. At the same time, in the groups of animals treated by L-carnitine at the doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg, the ATP-generating ability, maximum respiration rate and respiratory capacity were also higher compared to those of the NC group (Fig. 2). For example, in the group of rats administered with L-carnitine at a dose of 25 mg/kg, the ATP-generating ability, the maximum respiration rate and respiratory capacity were 2.33 times ( $p < 0.05$ ), 1.63 times ( $p < 0.05$ ), and 2.36 times ( $p < 0.05$ ), respectively higher than the corresponding parameters of the NC group. With the administration of L-carnitine at a dose of 50 mg/kg, these indicators increased in comparison with those of the NC group of animals: the ATP-generating ability – by 3.36 times ( $p < 0.05$ ), the maximum respiration rate – by 2.04 times ( $p < 0.05$ ), and respiratory capacity – by 2.61 times ( $p < 0.05$ ). In the rats treated by L-carnitine at a dose of 100 mg/kg, there was an increase in the ATP-generating ability, maximum respiration rate and respiratory capacity by 4.18 times ( $p < 0.05$ ), 2.26 times ( $p < 0.05$ ), and 2.79 times ( $p < 0.05$ ), respectively. When administering L-carnitine at a dose of 200 mg/kg to the animals when compared to the NC group of rats, there was an increase in the ATP-generating ability – by 4.76 times ( $p < 0.05$ ); the maximum respiration rate – by 2.83 times ( $p < 0.05$ ), and respiratory capacity – by 2.96 times ( $p < 0.05$ ). At the same time, an increase in the dose of L-carnitine from 200 mg/kg to 800 mg/kg was not accompanied by an increase in the therapeutic effect (Fig. 2).

### The results of the L-carnitine effect on the change in the activity of anaerobic processes in the rats' brain under the cerebral ischemia conditions

Assessing the change of the anaerobic processes activity in rats' brain under the cerebral ischemia conditions, it was found that in the animals of the NC group, there was observed an increase in glycolysis intensity by 8.7 times ( $p < 0.05$ ), accompanied by a decrease in glycolytic capacity and glycolytic reserve by 6.11 times ( $p < 0.05$ ) and 5.54 times ( $p < 0.05$ ), respectively (Fig. 3). Against the administration of L-carnitine to the rats within the studied dose range, there was a decrease in the intensity of glycolysis (with the administration of 25 mg/kg L-carnitine – by 29.5% ( $p < 0.05$ ), 50 mg/kg – by 45.6% ( $p < 0.05$ ), 100 mg/kg – 52.4% ( $p < 0.05$ ), and 200 mg/kg – 59.3% ( $p < 0.05$ )). At the same time, as in the case of evaluating the total respiratory function, an increase in the dose of L-carnitine from 200 mg/kg to 800 mg/kg did not lead to a significant increase in the pharmacological effect (Fig. 3). With the use of L-carnitine, there was observed an increase in glycolytic capacity and glycolytic reserve (the most pronounced and stable results were observed with administering L-carnitine at a dose of 200 mg/kg: glycolytic capacity increased by 4.1 times ( $p < 0.05$ ) and glycolytic reserve – by 3.1 times ( $p < 0.05$ )). However, it is worth noting that with an increase in the dose of L-carnitine from 200 mg/kg to 800 mg/kg, there was a slight tendency to an increase in glycolytic capacity with stable glycolytic reserve (Fig. 3).



**Figure 3.** The effect of L-carnitine at various doses on the change in the intensity of anaerobic processes in rats with cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats; β – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg; α – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg; Δ – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .

**The results of the L-carnitine effect on changes in the activity of mitochondrial complexes in rats brain under the cerebral ischemia conditions**

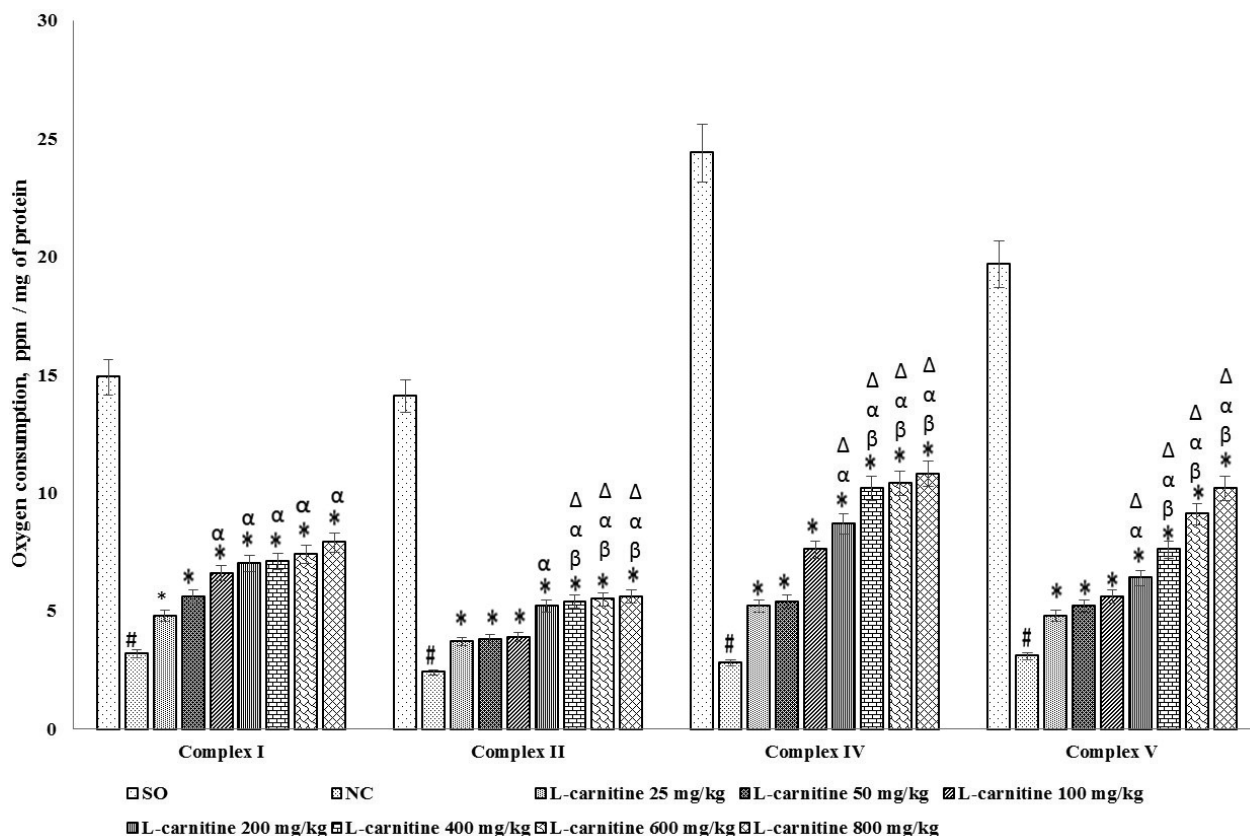
Assessing the change in the activity of mitochondrial complexes in the rats under the conditions of cerebral ischemia, it was found that in the animals of the NC group, there was a decrease in the activity of complex I – by 6.6 times ( $p < 0.05$ ), complex II – by 10.1 times ( $p < 0.05$ ), complex IV – by 7.3 times ( $p < 0.05$ ), and complex V – by 8 times ( $p < 0.05$ ) compared to the similar results of the SO group of rats (Fig. 4).

Against the background of the L-carnitine administration to the animals, there was observed an increase in the activity of the mitochondrial respiratory chain complexes, but when administering L-carnitine at a dose of 25 mg/kg, there was an increase (relative to the NC group of animals) of the activity of complex I, complex II, complex IV, and complex V by 1.9 times ( $p < 0.05$ ), 1.5 times ( $p < 0.05$ ), 1.8 times ( $p < 0.05$ ), and 1.7 times ( $p < 0.05$ ), respectively. At the same time, when L-carnitine was used at a dose of 50 mg/kg and 100 mg/kg, the changes in the activity of mitochondrial complexes were comparable (Fig. 4). It should be noted that when administering L-carnitine at the doses of 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg, the most pronounced change in the activity of mitochondrial respiratory chain complexes was observed. So, with the

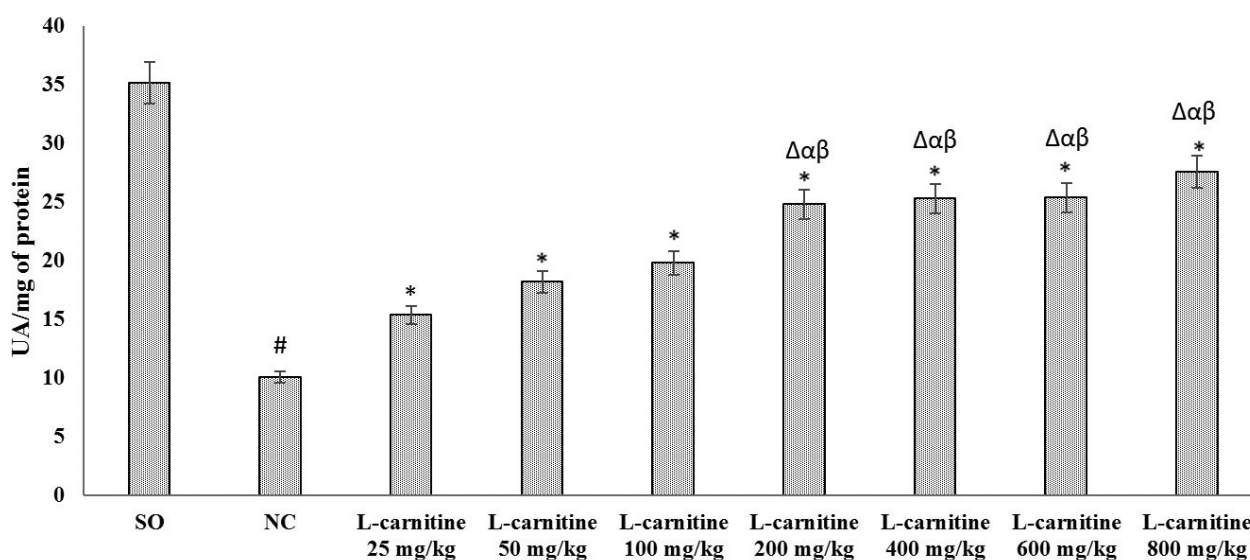
use of L-carnitine at a dose of 200 mg/kg, the activity of complex I increased (relative to the NC group of rats) by 3.4 times ( $p < 0.05$ ); complex II – 4.1 times ( $p < 0.05$ ), complex IV – 2.9 times ( $p < 0.05$ ), complex V – 3.2 times ( $p < 0.05$ ), while when administering L-carnitine at a dose of 800 mg/kg, the activity of complexes I; II; IV, and V increased by 3.7 times ( $p < 0.05$ ), 4.6 times ( $p < 0.05$ ), 3.6 times ( $p < 0.05$ ), and 4.2 times ( $p < 0.05$ ), respectively (Fig. 4). However, it is worth noting that there were no statistically significant differences in the activity of complex I in the animals treated by L-carnitine at the doses of 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg. Also, the activity of complex IV and V with the use of L-carnitine at a dose of 100 mg/kg did not statistically significantly differ from those of the rats that were treated by L-carnitine at the doses of 200 mg/kg, 400 mg/kg, 600 mg kg, and 800 mg/kg (Fig. 4).

**The results of the L-carnitine effect on the change of the citrate synthase activity in rats’ brain under the cerebral ischemia conditions**

When assessing the changes in the activity of citrate synthase in the brain supernatant in rats under the cerebral ischemia conditions, it was found that in the NC group there was a decrease in the activity of this enzyme in comparison to the SO rats by 3.4 times ( $p < 0.05$ ). At the

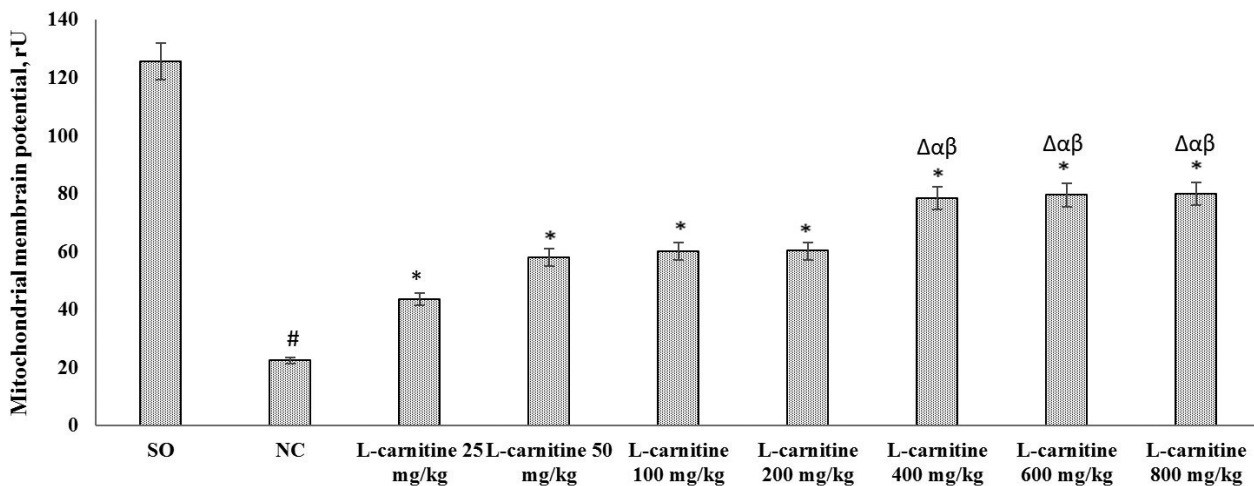


**Figure 4.** The effect of L-carnitine at various doses on the change in the activity of mitochondrial respiratory chain complexes in rats with cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats;  $\beta$  – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg;  $\alpha$  – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg;  $\Delta$  – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .



**Figure 5.** The effect of L-carnitine at various doses on the change in the activity of citrate synthase in rats with cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats;  $\beta$  – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg;  $\alpha$  – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg;  $\Delta$  – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .





**Figure 6.** The effect of L-carnitine at various doses on the change in the mitochondrial membrane potential in rats with cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats; β – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg; α – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg; Δ – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .

same time, the administration of L-carnitine within the studied dose range contributed to the restoration of the citrate synthase activity. For instance, administering L-carnitine at the doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg, the activity of citrate synthase increased in comparison with that in the NC group of animals by 1.4 times ( $p < 0.05$ ), 1.8 times ( $p < 0.05$ ), and 1.9 times ( $p < 0.05$ ), respectively. More significant changes were observed when administering L-carnitine at high doses: the activity of citrate synthase with the use of L-carnitine at a dose of 200 mg/kg increased by 2.3 times ( $p < 0.05$ ) relative to that in the NC group of animals; at a dose of 400 mg/kg – by 2.4 times ( $p < 0.05$ ); 600 mg/kg – 2.5 times ( $p < 0.05$ ), and 800 mg/kg – 2.8 times ( $p < 0.05$ ). Moreover, it should be noted that statistically significant differences in the activity of citrate synthase in the groups of rats treated by L-carnitine at the doses of 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg have not been established (Fig. 5).

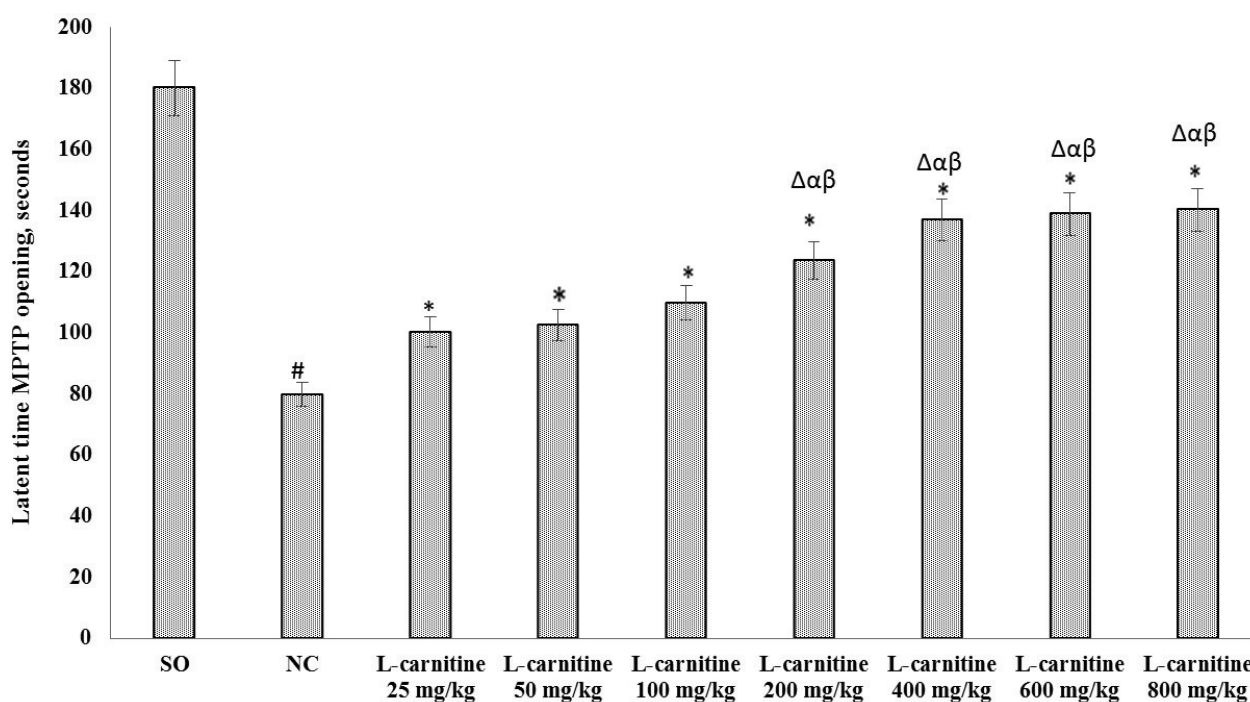
#### The results of the L-carnitine effect on changes in the mitochondrial membrane potential in rats' brain under the cerebral ischemia conditions

Under the conditions of modeling cerebral ischemia in NC rats, there was observed a 5-time decrease in the value of the mitochondrial membrane potential (Fig. 6) relative to the parameter of the SO animal group ( $p < 0.05$ ). When administering L-carnitine to the rats, there was an increase in the membrane potential of mitochondria relative to that in the NC group of animals, while with the use of L-carnitine at a dose of 25 mg/kg, the mitochondrial membrane potential increased by 1.8 times ( $p < 0.05$ ). With the administration of L-carnitine at the doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg,

the membrane potential of mitochondria increased by 2.1 times ( $p < 0.05$ ), 2.3 times ( $p < 0.05$ ), and 2.4 times ( $p < 0.05$ ), respectively. It is worth noting that the most significant changes in the mitochondrial membrane potential were registered when administering L-carnitine at the doses of 400 mg/kg, 600 mg/kg, and 800 mg/kg, by which the membrane potential of mitochondria increased by 2.9 times ( $p < 0.05$ ), 3.0 times ( $p < 0.05$ ), and 3.1 times ( $p < 0.05$ ), respectively (Fig. 6).

#### The results of the L-carnitine effect on the change in the latent time of mitochondrial permeability transitional pore opening in rats' brain under the cerebral ischemia conditions

Assessing the change in the latent opening time of the mitochondrial permeability transitional pore, it was found that in the NC group of animals, this indicator decreased by 2.36 times ( $p < 0.05$ ) relative to that in the SO group of rats. When using L-carnitine at the doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg, there was observed an increase in the latent time of opening of the mitochondrial permeability transitional pore by 1.3 times ( $p < 0.05$ ), 1.4 times ( $p < 0.05$ ), and 1.47 times ( $p < 0.05$ ), respectively. At the same time, when animals were treated by L-carnitine at the doses of 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg, the latent opening time of the mitochondrial permeability transitional pore was higher by 1.69 times ( $p < 0.05$ ), 1.79 times ( $p < 0.05$ ), 1.81 times ( $p < 0.05$ ), and 1.83 times ( $p < 0.05$ ), respectively, than that in the NC group. It is worth noting that no statistically significant differences between the groups of animals treated by L-carnitine at the doses of 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg were found (Fig. 7).



**Figure 7.** The effect of L-carnitine at various doses on the change in the latent time of mitochondrial permeability transition pore opening in rats with cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats; β – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg; α – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg; Δ – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .

### The results of the L-carnitine effect on changes in the concentration of calcium in the brain supernatant in rats under the cerebral ischemia conditions

In the course of assessing the change in the concentration of calcium ions (Fig. 8) in the supernatant of the animal brain, it was found that in the NC group, there was observed an 83% increase in the content of intracellular calcium ( $p < 0.05$ ). When administering L-carnitine at the doses of 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg, the concentration of ionized calcium was less by 25.1% ( $p < 0.05$ ), 29% ( $p < 0.05$ ), 30% ( $p < 0.05$ ), and 31% ( $p < 0.05$ ), respectively, than the corresponding indicator of the NC rat group (Fig. 8). It should be noted that the administration of L-carnitine to the animals at the doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg did not significantly affect the change in the content of calcium ions in the supernatant of the rats' brain.

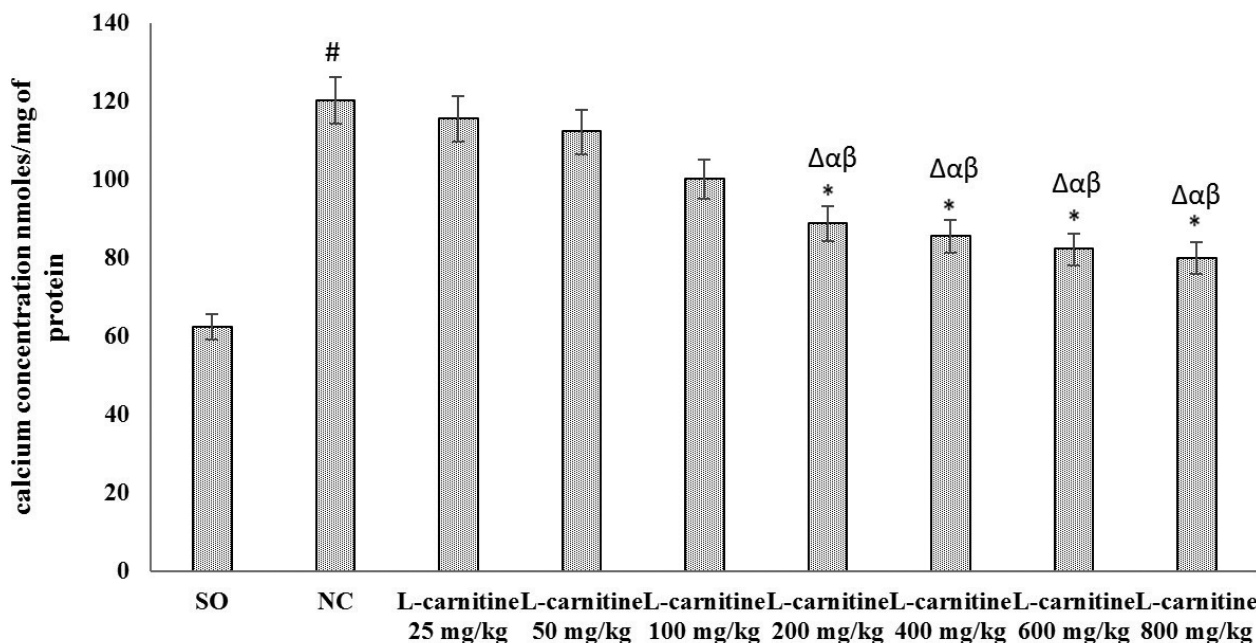
### The results of the L-carnitine effect on the change in the size of the brain necrosis zone in rats under the cerebral ischemia conditions

In the NC group of rats, the zone of cerebral necrosis was  $45.2 \pm 1.298\%$  (Fig. 9). With the administration of L-carnitine at a dose of 25 mg/kg, there was observed a 19.6% decrease in the zone of cerebral infarction ( $p < 0.05$ ) in comparison with that in the NC group. When administering L-carnitine at the doses of 50 mg/kg, 100 mg/kg,

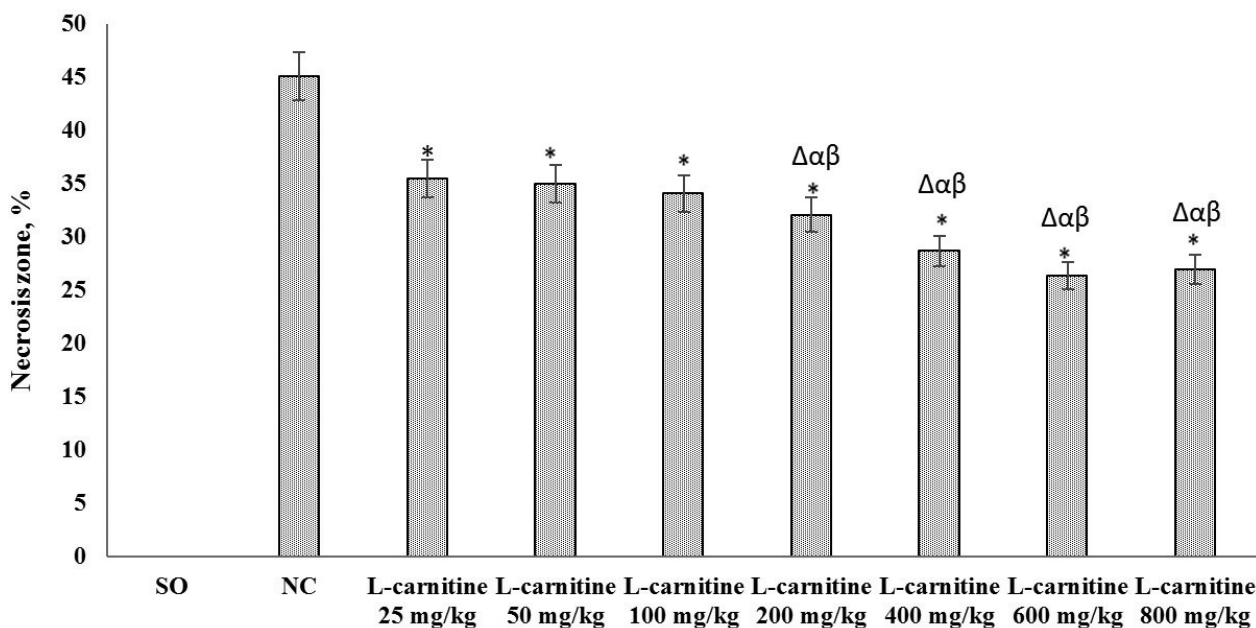
and 200 mg/kg, the brain necrotic area was less by 21.5% ( $p < 0.05$ ), 23.5% ( $p < 0.05$ ), and 28.8% ( $p < 0.05$ ), respectively, than the same indicator for the NC group. The most pronounced changes in the size of the brain necrosis zone happened with the use of L-carnitine at the doses of 400 mg/kg, 600 mg/kg, and 800 mg/kg, which decreased the necrotic area in relation to the NC group of animals by 36.9% ( $p < 0.05$ ), 39.4% ( $p < 0.05$ ), and 38.1% ( $p < 0.05$ ), respectively (Fig. 9).

## Discussion

Carnitine (L-3-hydroxy-4-N, N, N-trimethylaminobutyrate, vitamin B11) is an essential water-soluble nutrient. One of the main functions of L-carnitine is to transport long-chain fatty acid residues through the mitochondrial membrane from the cytosol to the mitochondrial matrix with the goal of β-oxidation and, accordingly, the formation of macroergic compounds and generation of cellular energy (Pietrocola et al. 2015). In addition, L-carnitine is involved in metabolic reactions that maintain the balance of acyl-CoA/CoA, which is also necessary to maintain the proper energy status of the cell (Bene et al. 2018). The direct precursor of L-carnitine in the human body is 6-N-trimethyllysine, formed from lysine and methionine, which are donors of the hydrocarbon skeleton and 4-N-methyl groups. Some endogenous proteins containing N-methyl lysine residues in their structure can also be precursors



**Figure 8.** The effect of L-carnitine at various doses on the change in the intensity of anaerobic processes in rats with cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats; β – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg; α – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg; Δ – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .



**Figure 9.** The effect of L-carnitine at various doses on the change in the size of the brain necrosis zone in rats with conditions of cerebral ischemia. **Note:** SO – sham-operated rats; NC – group of animals of negative control; # – statistically significant relative to the SO group of rats; \* – statistically significant relative to the NC group of rats; β – statistically significant relative to the group of rats treated by L-carnitine at a dose of 25 mg/kg; α – statistically significant relative to the group of rats treated by L-carnitine at a dose of 50 mg/kg; Δ – statistically significant relative to the group of rats treated by L-carnitine at a dose of 100 mg/kg. In all cases, the Newman-Keuls test,  $p < 0.05$ .

of L-carnitine (in this case, L-carnitine is synthesized in transmethylation reactions, in which S-adenosylmethionine acts as a methyl group donor). In the classical version, the biosynthesis of L-carnitine consists of four sequential reactions, where in the end L-carnitine is synthesized

from  $\gamma$ -butyrobetaine under the influence of  $\gamma$ -butyrobetaindeoxygenase (Rebouche 2004).

The main food sources of L-carnitine are meat, fish, and milk products. In plant foods, the content of L-carnitine is not high. True vegetarians have more than 90% of total

**L-carnitine** synthesized endogenously (Rebouche 2004). The bioavailability of **L-carnitine** from food is about 54–86%. The highest content of **L-carnitine** can be found in the intracellular space, in which about 99% of the total level of **L-carnitine** in the body is localized. In this regard, the concentration of **L-carnitine** in the blood is relatively low and is approximately 22–50  $\mu\text{mol/L}$  (26–53  $\mu\text{mol/L}$  in men and 19–44  $\mu\text{mol/L}$  in women) (Opalka et al. 2001).

**L-carnitine** is one of the key molecules necessary for the optimal functioning of mitochondria. As mentioned above, **L-carnitine** provides for  $\beta$ -oxidation reactions, transporting residues of fatty acids to the mitochondrial matrix during the reactions of the carnitine-acyl carnitine cycle, which consists of three successive stages. At the first stage, long-chain fatty acid fragments interact with carnitine palmitoyl transferase I to form acyl carnitine equivalents of fatty acids, which are transported to the mitochondrial matrix by means of carnitine acylcarnitine translocase (stage II). At the final stage, carnitine palmitoyl transferase II reduces the acyl carnitine derivatives on the inner mitochondrial membrane to the starting products (Yan et al. 2015).

Several studies have shown that L-carnitine is a potentially effective neuroprotective agent. Ueno et al. (2015) showed that the oral administration of **L-carnitine** at high doses (600 mg/kg) under the conditions of chronic brain hypoperfusion in animals caused by bilateral occlusion of the common carotid arteries contributed to the restoration of the memory trail in rats, and also a decrease in the oxidative modification of cell structures by lipid peroxidation type. The neuroprotective effect of **L-carnitine** was supposed to be associated with the regulation of the PTEN/Akt/mTOR signaling pathway, improved myelination of nerve fibers, and reduction of oxidative stress. The study by Binienda et al. (2006) found that intraperitoneal prophylactic administration of **L-carnitine** at a dose of 100 mg/kg reduced the negative metabolic changes in the striatum of rats under hypoxia conditions, which were expressed in the up-regulation of the UCP2 protein and D1 dopamine receptors (administration of **L-carnitine** suppressed these changes). The study by Al-Majed et al. (2006) demonstrated that the administration of 300 mg/kg **L-carnitine** to rats with cerebral ischemia contributed to the normalization of the restored glutathione concentration, an increase in ATP concentration and a decrease in TBARS (2-thiobarbituric acid active products). However, according to (Ferreira and McKenna 2017), more detailed studies are needed to evaluate other aspects of the **L-carnitine** neuroprotective effect. Conducted study showed that therapeutic administration of **L-carnitine** at a dose range of 25 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg contributed to the

restoration of mitochondrial function in rats under conditions of permanent cerebral ischemia. At the same time, the use of **L-carnitine** at low doses (25 mg/kg – 100 mg/kg) improved the state of the respiratory function of mitochondria, but had less effect on the change in the level of intracellular calcium. The administration of **L-carnitine** within the dose range of 200 mg/kg – 800 mg/kg stabilized the respiratory function of mitochondria, which was reflected in an increase in the ATP-generating ability, maximum respiration level, respiratory capacity, glycolytic capacity, and glycolytic reserve, as well as a decrease in the intensity of anaerobic processes. In addition, the use of high-doses of **L-carnitine** restored the activity of complexes I, II, IV, and V of the mitochondrial respiratory chain, increased the mitochondrial membrane potential, and decreased the latent time of formation of the mitochondrial permeability transition pore and the level of intracellular calcium. Moreover, in a number of experimental studies, there are data proving that the compounds capable of suppressing the formation of mitochondrial permeability transition pore are promising neuroprotectors (Kalani et al. 2018). As a result, the observed changes in mitochondrial function contributed to a decrease in the cerebral infarction zone (it is most pronounced when **L-carnitine** was administered at the doses of 400 mg/kg, 600 mg/kg, and 800 mg/kg). It should be noted that a consistent increase in the dose of **L-carnitine** from 200 mg/kg to 800 mg/kg (*per os*) did not lead to an increase in the pharmacological effect. However, one should not deny other possible mechanisms of the **L-carnitine** neuroprotective effect, for example, the antioxidant or endothelioprotective effect (Fielding et al. 2018), which, together with the restoration of mitochondrial function, potentiate the neuroprotective effect of **L-carnitine**.

## Conclusion

The study showed the promise of the use of **L-carnitine** at a dose above 200 mg/kg (orally) as a neuroprotective agent aimed at restoring mitochondrial function under conditions of permanent focal cerebral ischemia. At the same time, a neuroprotective effect is achieved when administering **L-carnitine** at low doses (25 mg/kg – 100 mg/kg), but without a significant effect on the change in the concentration of intracellular calcium.

## Conflict of interest

Authors declare no conflict of interest.

## References

- Al-Majed AA, Sayed-Ahmed MM, Al-Omar FA, Al-Yahya AA, Aleisa AM, Al-Shabanah OA (2006) Carnitine esters prevent oxidative stress damage and energy depletion following transient forebrain ischaemia in the rat hippocampus. *Clinical and Experimental Pharmacology & Physiology* 33(8): 725–733. <https://doi.org/10.1111/j.1440-1681.2006.04425.x> [PubMed]

- Bene J, Hadzsiev K, Melegh B (2018) Role of carnitine and its derivatives in the development and management of type 2 diabetes *Nutrition & Diabetes* 8(1): 8. <https://doi.org/10.1038/s41387-018-0017-1> [PubMed]
- Binienda ZK, Ali SF, Virmani A, Amato A, Salem N, Przybyla BD (2006) Co-regulation of dopamine D1 receptor and uncoupling protein-2 expression in 3-nitropropionic acid-induced neurotoxicity: neuroprotective role of L-carnitine *Neuroscience Letters* 410(1): 62–65. <https://doi.org/10.1016/j.neulet.2006.09.070> [PubMed]
- Bonneau B, Ando H, Kawai K, Hirose M, Takahashi-Iwanaga H, Mikoshiba K (2016) IRBIT controls apoptosis by interacting with the Bcl-2 homolog, Bcl2l10, and by promoting ER-mitochondria contact. *eLife* 5: e19896. <https://doi.org/10.7554/eLife.19896> [PubMed] [PMC]
- Broughton BR, Reutens DC, Sobey CG (2009) Apoptotic mechanisms after cerebral ischemia. *Stroke* 40(5): e331–e339. <https://doi.org/10.1161/STROKEAHA.108.531632> [PubMed]
- Carlsson N, Borde A, Wölfel S, Kerman B, Larsson A (2011) Quantification of protein concentration by the Bradford method in the presence of pharmaceutical polymers. *Analytical Biochemistry* 411(1): 116–121. <https://doi.org/10.1016/j.ab.2010.12.026> [PubMed]
- Dong W, Qi Z, Liang J, Shi W, Zhao Y, Luo Y, Ji X, Liu KJ (2015) Reduction of zinc accumulation in mitochondria contributes to decreased cerebral ischemic injury by normobaric hyperoxia treatment in an experimental stroke model. *Experimental Neurology* 272: 181–189. <https://doi.org/10.1016/j.expneurol.2015.04.005> [PubMed] [PMC]
- Ferreira GC, McKenna MC (2017) L-Carnitine and acetyl-L-carnitine roles and neuroprotection in developing brain. *Neurochemical Research* 42(6): 1661–1675. <https://doi.org/10.1007/s11064-017-2288-7> [PubMed]
- Field ML, Azzawi A, Styles P, Henderson C, Seymour AM, Radda GK (1994) Intracellular Ca<sup>2+</sup> transients in isolated perfused rat heart: measurement using the fluorescent indicator Fura-2/AM. *Cell Calcium* 16(2): 87–100. [https://doi.org/10.1016/0143-4160\(94\)90004-3](https://doi.org/10.1016/0143-4160(94)90004-3) [PubMed]
- Fielding R, Riede L, Lugo JP, Bellamine A (2018) L-carnitine supplementation in recovery after exercise. *Nutrients* 10(3): 349. <https://doi.org/10.3390/nu10030349> [PubMed]
- Folbergrová J, Ješina P, Kubová H, Druga R, Otáhal J (2016) Status epilepticus in immature rats is associated with oxidative stress and mitochondrial dysfunction. *Frontiers in Cellular Neuroscience* 10: 136. <https://doi.org/10.3389/fncel.2016.00136> [PubMed]
- Grimm A, Eckert A (2017) Brain aging and neurodegeneration: from a mitochondrial point of view. *Journal of Neurochemistry* 143(4): 418–431. <https://doi.org/10.1111/jnc.14037> [PubMed]
- Guan R, Zou W, Dai X, Yu X, Liu H, Chen Q, Teng W (2018) Mitophagy, a potential therapeutic target for stroke. *Journal of Biomedical Science* 25(1): 87. <https://doi.org/10.1186/s12929-018-0487-4> [PubMed]
- Joshi AU, Mochly-Rosen D (2018) Mortal engines: Mitochondrial bioenergetics and dysfunction in neurodegenerative diseases. *Pharmacological Research* 138: 2–15. <https://doi.org/10.1016/j.phrs.2018.08.010> [PubMed]
- Kalani K, Yan SF, Yan SS (2018) Mitochondrial permeability transition pore: a potential drug target for neurodegeneration. *Drug Discovery Today* 23(12): 1983–1989. <https://doi.org/10.1016/j.drudis.2018.08.001> [PubMed]
- Koeth RA, Lam-Galvez BR, Kirsop J, Wang Z, Levison BS, Gu X, Copeland MF, Bartlett D, Cody DB, Dai HJ, Culley MK, Li XS, Fu X, Wu Y, Li L, DiDonato JA, Tang WHW, Garcia-Garcia JC, Hazen SL (2019) L-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *The Journal of Clinical Investigation* 129(1): 373–387. <https://doi.org/10.1172/JCI9460> [PubMed]
- Mondal NK, Behera J, Kelly KE, George AK, Tyagi PK, Tyagi N (2019) Tetrahydrocurcumin epigenetically mitigates mitochondrial dysfunction in brain vasculature during ischemic stroke. *Neurochemistry International* 122: 120–138. <https://doi.org/10.1016/j.neuint.2018.11.015> [PubMed]
- Opalka JR, Gellerich FN, Zierz S (2001) Age and sex dependency of carnitine concentration in human serum and skeletal muscle. *Clinical Chemistry* 47(12): 2150–2153. [PubMed]
- Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F, Kroemer G (2015) Acetyl coenzyme A: a central metabolite and second messenger. *Cell Metabolism* 21(6): 805–821. <https://doi.org/10.1016/j.cmet.2015.05.014> [PubMed]
- Pozdnyakov DI, Miroshnichenko KA, Voronkov AV, Kovaleva TG (2019) The administration of the New Pyrimidine Derivative – 4-{2-[2-(3,4-Dimethoxyphenyl)-Vinyl]-6-Ethyl-4-Oxo-5-Phenyl-4H-Pyrimidine-1-Il}Benzsulfamide restores the activity of brain cells in experimental chronic traumatic encephalopathy by maintaining mitochondrial function. *Medicina* 55(7): 386. <https://doi.org/10.3390/medicina55070386>
- Rebouche CJ (2004) Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Annals of the New York Academy of Sciences* 1033: 30–41. <https://doi.org/10.1196/annals.1320.003> [PubMed]
- Sazanov LA (2015) A giant molecular proton pump: structure and mechanism of respiratory complex I. *Nature Reviews. Molecular Cell Biology* 16(6): 375–388. <https://doi.org/10.1038/nrm3997> [PubMed]
- Shepherd D, Garland PB (1969) The kinetic properties of citrate synthase from rat liver mitochondria. *The Biochemical Journal* 114(3): 597–610. <https://doi.org/10.1042/bj1140597> [PubMed]
- Ueno Y, Koike M, Shimada Y, Shimura H, Hira K, Tanaka R, Uchiyama Y, Hattori N, Urabe T (2015) L-carnitine enhances axonal plasticity and improves white-matter lesions after chronic hypoperfusion in rat brain. *Journal of Cerebral Blood Flow and Metabolism* 35(3): 382–391. <https://doi.org/10.1038/jcbfm.2014.210> [PubMed]
- Voronkov AV, Pozdnyakov DI, Nigaryan SA, Khouri EI, Miroshnichenko KA, Sosnovskaya AV, Olokhova EA (2019) Evaluation of the mitochondria respirometric function in the conditions of pathologies of various geneses. *Pharmacy & Pharmacology* 7(1): 20–31. <https://doi.org/10.19163/2307-9266-2019-7-1-20-31>
- Yan S, Yang XF, Liu HL, Fu N, Ouyang Y, Qing K (2015) Long-chain acyl-CoA synthetase in fatty acid metabolism involved in liver and other diseases: an update. *World Journal of Gastroenterology* 21(12): 3492–3498. <https://doi.org/10.3748/wjg.v21.i12.3492> [PubMed]
- Zhyliuk VI, Mamchur VV, Pavlov S (2015) Role of functional state of neuronal mitochondria of cerebral cortex in mechanisms of nootropic activity of neuroprotectors in rats with alloxan hyperglycemia. *Experimental and Clinical Pharmacology [Eksperimental'naia i Klinicheskaia Farmakologija]* 78(2): 10–14. [PubMed] [in Russian]

## Author contributions

- **Andey V. Voronkov**<sup>†</sup>, Doctor Habil. of Medicine, Professor, Director of Medical College, e-mail: [prohor77@mail.ru](mailto:prohor77@mail.ru), **ORCID ID** <https://orcid.org/0000-0001-6638-6223>. The author was engaged in developing the research concept, preparing the final version of the manuscript, and carrying out the statistical analysis of the results.
- **Dmitry I. Pozdnyakov**, PhD in Pharmacy, Associate Professor of the Department of Pharmacology with course of Clinical Pharmacology, e-mail: [pozdniackow.dmitry@yandex.ru](mailto:pozdniackow.dmitry@yandex.ru), **ORCID ID** <https://orcid.org/0000-0003-0889-7855>. The author was engaged in developing the research design, conducting the experiment, and preparing the final version of the manuscript.