

# BDNF-Mediated Regulation of the Brain Mitochondria Functional State in Hypoxia

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**The aim of the study** was to study the effect of TrkB-mediated action of the brain-derived neurotrophic factor (BDNF) on animal survival and mitochondrial respiratory chain activity in acute hypobaric hypoxia model *in vivo*.

**Materials and Methods.** *In vivo* experiments were performed on mature male CBA mice weighing 20–25 g. In order to modulate acute hypobaric hypoxia, the animals were placed in the hypobaric chamber (220–240 mm Hg) which simulates conditions corresponding to the altitude of 10 000 m above sea level. The oxygen consumption rate by the brain mitochondria under the hypoxic influence was evaluated using a high-resolution OROBOROS Oxygraph-2k respirometer (OROBOROS Instruments, Austria).

**Results.** Preventive BDNF application has been established to increase the survival of the CBA-line animals after acute hypobaric hypoxia modeling and to influence favorably the work of mitochondrial respiratory chain complex I.

**Conclusion.** BDNF increases animal resistance to acute hypobaric hypoxia and influences the work of mitochondrial respiratory chain through TrkB-signaling mechanisms. Antihypoxic effect of BDNF is realized by maintaining the activity of NADH-dependent pathway of substrate oxidation and ATP synthesis.

**Key words:** brain-derived neurotrophic factor; BDNF; TrkB-signaling; acute hypobaric hypoxia; oxidative phosphorylation; neuroprotection.

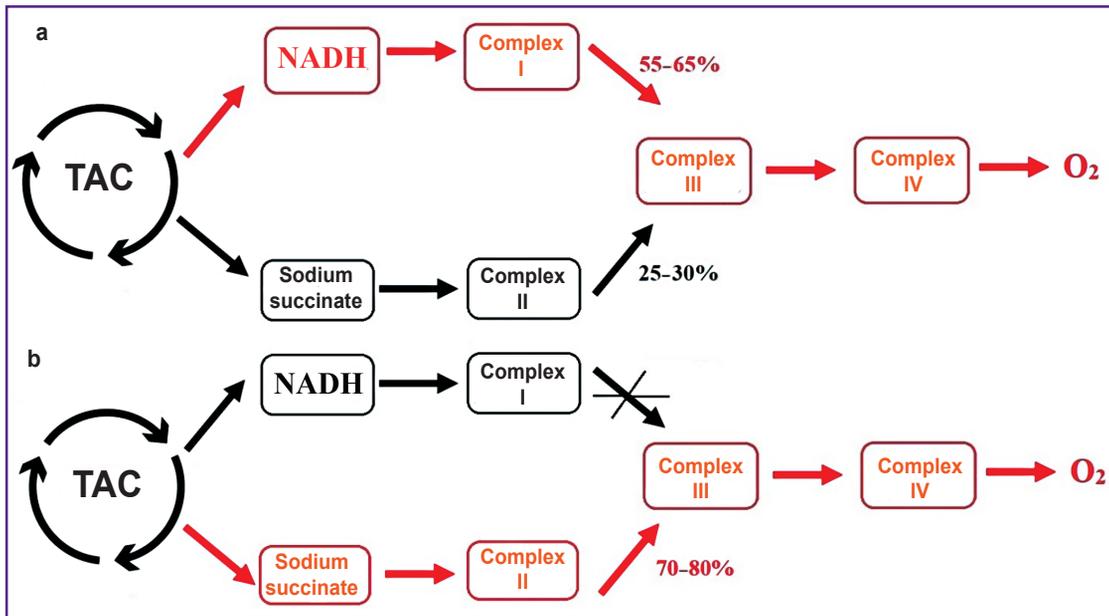
## Introduction

Hypoxia is one of the leading factors of brain cell damage accompanying traumatic and ischemic processes. Increased sensitivity of the brain tissue to oxygen deficiency is determined by great energy consumption necessary for its functioning. Mitochondria serve as a target for hypoxia/ischemia. Reduction of oxygen concentration causes derangements in the electron transport function of their respiratory chain

and is a phase process (Figure 1). These damages are non-specific as they always start in the area of mitochondrial enzyme complex I (NAD-dependent area of the respiratory chain) and involve thereafter the area of cytochromes B, C and cytochrome oxidase [1–8].

At present, questions of pharmacological correction of brain hypoxia are referred to high-priority medicobiological problems. In spite of the variety of medicines, antihypoxants, there are different limitations for their use in brain pathologies because of a great number of

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**Figure 1. A scheme of mitochondrial respiratory chain functioning:**  
(a) in normoxia; (b) in hypoxia; TAC — tricarboxylic acid cycle

side-effects and difficulties of their overcoming through blood–brain barrier. It should be noted that endogenous molecules present a great scientific interest today, as they are capable of realizing intercellular molecular cascades with participation of a cell genetic apparatus; besides, they not only trigger adaptive mechanisms in cells but also support them by changing the energy metabolism. This property enables potential molecules to activate the necessary processes, e.g. neuroprotection or neuroplasticity.

Numerous studies [9, 10] showed that among chemical substances potentially capable of controlling cell metabolism level in decreased oxygen concentrations, brain-derived neurotrophic factor (BDNF) attracts special attention. In particular, BDNF has been found to influence the respiratory control index of brain mitochondria (the rate of oxidative phosphorylation increases). This effect is mediated by the work of a major TrkB-signaling pathway (a signaling cascade induced by BDNF binding with TrkB receptors via mitogen-activated protein kinase, MAP) [9]. Preventive introduction of the neurotrophic factor has also been shown to improve animal resistance to acute hypobaric hypoxia by increasing the lifetime at altitude and to prevent spatial memory impairment in the post-hypoxic period.

The present work focuses on the study of BDNF possibilities to modulate the hypoxic effects during regulation of mitochondria functional state via the BDNF-TrkB interaction.

**The aim of the study** was to study the effect of TrkB-mediated action of the brain-derived neurotrophic factor on animal survival and activity of mitochondrial

respiratory chain complexes I, II in acute hypobaric hypoxia model *in vivo*.

## Materials and Methods

*In vivo* experiments were performed on mature CBA male mice weighing 20–25 g. The animals were kept in a certified vivarium of the National Research Lobachevsky State University of Nizhni Novgorod. The studies were performed in compliance with the requirements of Orders No.1179 of the Ministry of Health of the USSR of 11.10.1983 and No.267 of the Ministry of Health of the Russian Federation of 19.06.2003, and also in compliance with the international rules Guide for the Care and Use of Laboratory Animals, met the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 2006), and approved by Bioethics Committee of Lobachevsky State University.

All animals were divided into the following groups: group 1 — intact mice; group 2 — control animals exposed to acute hypobaric hypoxia without introduction of the examined substances; group 3 — mice exposed to acute hypobaric hypoxia with preventive intraperitoneal injection of selective blocker of TrkB receptors — ANA-12 at a concentration of 0.5 mg/kg; group 4 — mice exposed to acute hypobaric hypoxia with preventive intranasal injection of BDNF at a concentration of 4 mg/kg; group 5 — a comparison group of animals exposed to acute hypobaric hypoxia with preventive introduction of 1% dimethyl sulfoxide (DMSO) in combination with Tween 20 to exclude the influence

of ANA-12 solvents on the examined parameters. The doses of the applied substances were chosen according to the results of the previous studies [10, 11]. The preparations were introduced 45 min before acute hypobaric hypoxia modeling.

In order to modulate acute hypobaric hypoxia, the animals were placed in the hypobaric chamber (220–240 mm Hg) which simulates conditions corresponding to the altitude of 10,000 m above sea level [12].

Mitochondria functional activity was determined 24 h after the acute hypobaric hypoxia modeling. Mitochondria were isolated using standard differential centrifugation method [13]. All manipulations were performed on ice. The equipment and isolation media were also cooled. After animal decapitation, the cranium was quickly opened, the brain dissected (within 20 s), placed into the precooled porcelain mortar, and washed with ice-cold isolation medium composed of 70 mM saccharose, 210 mM mannitol, 30 mM HEPES, 0.1 mM EDTA (pH 7.4), whereupon the cerebellum was removed. The cerebral hemispheres and brainstem were put into isolation medium and then subjected to homogenization in the glass homogenizer placed in ice. An electrically-driven Teflon pestle had a clearance excluding mitochondria destruction. The ratio between tissue mass and the isolation media was 1:7. The obtained brain homogenate was centrifuged at 2700 rpm (the temperature range from –3 to 0°C) for 10 min. The supernatant was poured into the tube and centrifuged for 15 min at 8500 g. The precipitated mitochondria were washed with the cold isolation medium (+4°C) and resuspended in the medium containing 210 mM mannitol, 70 mM saccharose, 0.1 mM EGTA, 10 mM

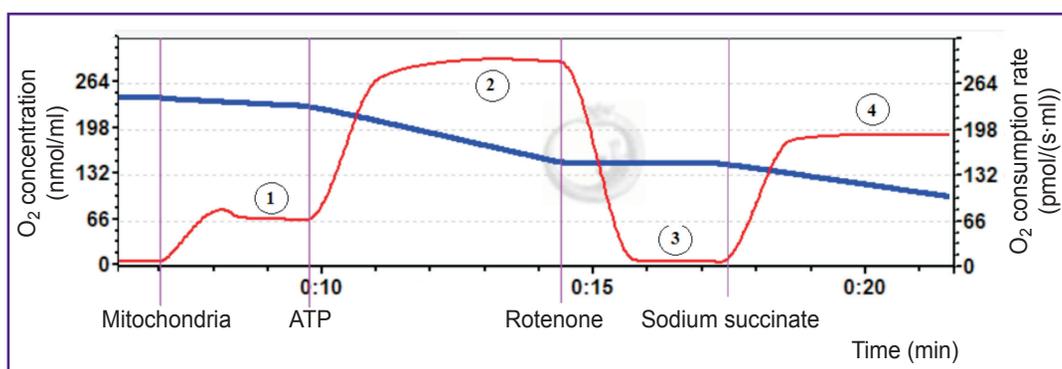
HEPES (pH 7.4), and centrifuged again for 15 min at 8500 g. The obtained suspension of mitochondria was stored on ice avoiding its freezing. The Bradford method was used for quantitation of protein in the isolated mitochondria.

Oxygen consumption by the isolated mitochondria was registered polarographically using a high-resolution respirometer OROBOROS Oxygraph-2k (OROBOROS Instruments, Austria) in 2 ml of the incubation medium (210 mM mannitol, 70 mM saccharose, 0.1 mM EGTA, 10 mM HEPES, pH 7.4) with constant stirring. Oxygen consumption rate was expressed in picomols/s/1 mg of mitochondrial protein.

Oxygen consumption in the chamber was fixed using DatLab5 software (OROBOROS Instruments, Austria).

The state of the mitochondrial respiratory chain was evaluated according to the following parameters: the rate of oxygen consumption by the mitochondria at a high content of substrates of glutamate 5 mM and malate 5 mM (substrates of complex I) in the incubation medium (Figure 2, state 1); oxidative phosphorylation rate of the respiratory chain in the presence of 5 mM adenosine diphosphate (ADP) (Figure 2, state 2); inhibition of complex I activity with 0.5 μM rotenone (Figure 2, state 3); and work intensity of the respiratory chain after the stimulation of complex II with 10 mM sodium succinate (Figure 2, state 4).

The obtained results are presented as the mean ± standard error of the mean (M±SEM). The significance of the differences between the experimental groups was determined by Mann–Whitney test using SigmaPlot 11.0 program (Systat Software Inc., USA). The differences were considered statistically significant at p≤0.05.



**Figure 2. A typical example of registration of the rate of oxygen consumption by mitochondria**

A successive introduction of the respiratory chain components to the suspension of mitochondria being in the incubation medium: (1) the rate of oxygen consumption by mitochondria in the incubation medium containing a high concentration of complex I substrates: 5 mM glutamate and 5 mM malate (transfer of electrons from hydrogen atoms of NADH to the respiratory chain enzymes); (2) stimulation of the oxidative phosphorylation of the mitochondrial respiratory chain by the introduction of exogenous 5 mM of ADP into the incubation medium (the process of oxidation of the reduced NADH equivalents by the respiratory chain enzymes followed by the ATP synthesis); (3) inhibition of the complex I work with 0.5 μM rotenone solution (blockage of electron transfer in complex I from the iron-sulphur cluster to the oxidized ubiquinon); (4) succinate-dependent pathway of substrate oxidation (introduction of succinate substrate to the incubation medium of the respiratory chain complex II)

Results

At the first stage of the study, a neuroprotective effect of BDNF in acute hypoxic conditions and the role of TrkB receptors in its realization were assessed.

The animal survival in modeled hypoxia has been found to be 27%; in the group of mice with ANA-12 application 13% (2 times lower than the control values); in the group of animals with the BDNF administration, the survival was equal, on average, to 40% (1.4 times higher than in the control group) (Figure 3). The percentage of the survived mice with the DMSO injection did not differ from the values of the control group and amounted to 27%.

The next stage was devoted to the determination of the oxygen consumption rate by the mitochondria in normoxia and in the modeled acute hypoxic conditions. The results showed that the basal rate of oxygen consumption during oxidation of glutamate and malate substrates in the control group significantly reduces by 25.5% compared to the intact values and makes  $2096.99 \pm 200.90$  pmol/(s·ml)/1 mg of protein (Figure 4). Application of TrkB receptor blocker ANA-12 in modeled hypoxia maintains the parameters of NADH oxidation within the control values. Preventive BDNF administration influences positively the process of NADH oxidation in hypoxia. Significant differences between the values of the intact group ( $2814.74 \pm 188.20$  pmol/(s·ml)/1 mg of protein) and BDNF group ( $2340.92 \pm 147.50$  pmol/(s·ml)/1 mg of protein) have not been found.

Oxidative phosphorylation rate of the mitochondrial respiratory chain in the control group after modeled hypoxia significantly decreases by 24% relative to the intact values and amounts to  $12,565.1 \pm 80.4$  pmol/(s·ml)/1 mg of protein (Figure 5). However, increase of the substrate phosphorylation rate of ADP in the groups with application of ANA-12 and BDNF was noted: the rate was  $18,466.8 \pm 474.9$  and  $17,909.5 \pm 954.6$  pmol/(s·ml)/

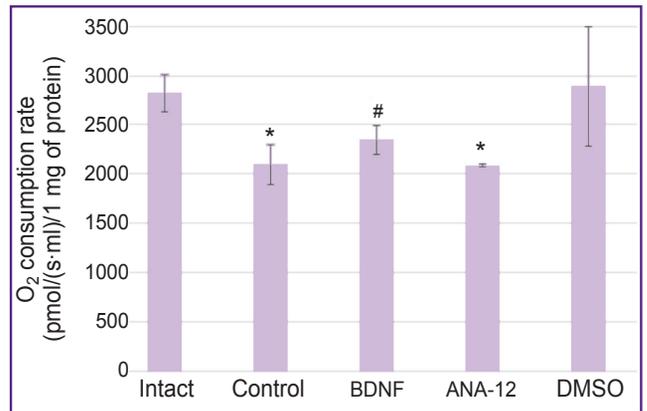


Figure 4. Basal rate of oxygen consumption by mitochondria under the excess of glutamate and malate substrates

\* Statistically significant differences with the intact group,  $p \leq 0.05$ ; # with the control group,  $p \leq 0.05$ ; Mann–Whitney test

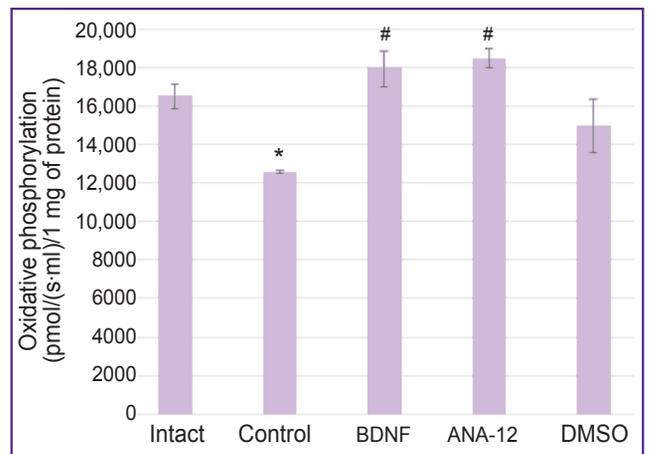


Figure 5. Oxidative phosphorylation of the mitochondrial respiratory chain

\* Statistically significant differences with the intact group,  $p \leq 0.05$ ; # with the control group,  $p \leq 0.05$ ; Mann–Whitney test

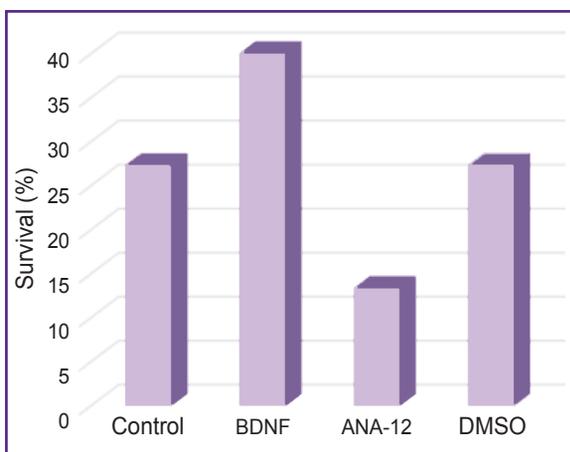


Figure 3. Survival of CBA mice after acute hypobaric hypoxia modeling

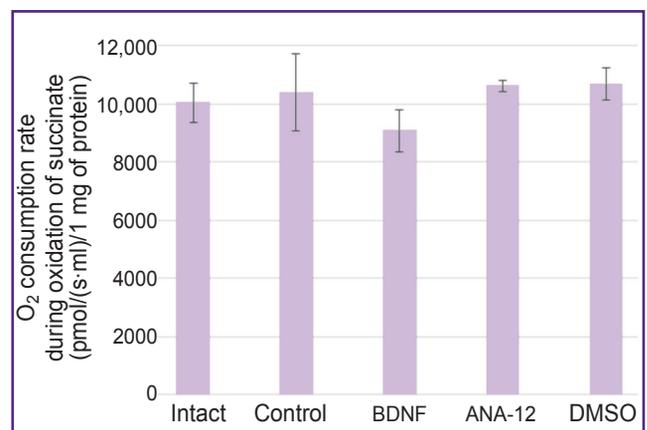


Figure 6. Succinate-dependent pathway of substrate oxidation (active work of the mitochondrial respiratory chain complex II when succinate is oxidized)

1 mg of protein, respectively, being by 47 and 43% higher than the control values.

It is interesting to note that significant differences between the parameters of the mitochondrial respiratory chain complex II activity have not been found (Figure 6). Respiration intensity of the brain mitochondria in succinate oxidation in the groups did not significantly differ from the appropriate control values (10,409.4±1329.1 pmol/(s·ml)/1 mg of protein).

## Discussion

The investigation of the BDNF effect on CBA mice resistance to the acute hypoxic influence has shown that this neurotrophin at concentration of 4 µg/kg improves the animal survival. However, this effect is neutralized by blockade of TrkB receptors. Protective mechanisms of BDNF determined by the ability of a mature protein to bind with TrkB receptor may be supposed to activate the intracellular signaling cascades increasing the survival of the nervous cells in hypoxia. Thus, when a protein complex NF-κB (nuclear factor kappa B) is activated, antiapoptotic proteins of the Bcl2 and IAP families are expressed. This effect together with the activation of other factors (e.g. c-jun, cIAP1) serves as the main agent inhibiting apoptosis [10, 14, 15].

Molecular mechanisms of any hypoxia form are connected with the inhibition of aerobic energy synthesis, energy-dependent functions, and, as a consequence, formation of functional and metabolic disorders which cause dysfunction of the mitochondrial apparatus, displaying itself in phase alterations of the activity of mitochondrial enzymatic complexes [16]

Reduction of the oxidizing ability of NADH-dependent link of the mitochondrial respiratory chain under oxygen deficiency has been shown by us to be mediated by BDNF-TrkB-signaling. In acute hypobaric hypoxia, the oxygen consumption rate in the process of glutamate and malate oxidation has been found to correlate with the values of the oxygen consumption rate with the application of the selective blocker of the TrkB receptors (ANA-12). However, preventive BDNF administration restores the oxygen consumption rate actually to the intact values. Similar data were obtained in the studies of Markham et al. [9]. The researchers have shown that BDNF influences the work of the respiratory chain complex I of the brain mitochondria when oxidizing glutamate and malate by MAP-kinase (one of the three signaling cascades activated by BDNF-TrkB interaction). This effect has been reported by them to be specific for the brain mitochondria (similar alterations were absent on liver specimens).

Brain-derived neurotrophic factor affects also the oxidative phosphorylation via BDNF-TrkB-signaling which is expressed as a respiratory control enhancement. The results of our investigations show that in acute hypobaric hypoxia, BDNF maintains the rate of ATP synthesis within the limits of the intact values but blockade of TrkB

receptors does not affect the parameters of oxidative phosphorylation. When TrkB receptors are inactivated due to oxygen deficiency, regulation of endogenous BDNF may be supposed to be mediated by TrkA and TrkC receptors which are in the active state.

Absence of reliable changes in the values of respiration intensity via succinate-dependent pathway of substrate oxidation may be connected with the time index of the post-hypoxic period. There are numerous reports confirming the impairment of electron-transport function of the respiratory chain complex I in hypoxia which persists for the first 30 min–2 h of reoxygenation. The work of the respiratory chain complex II was shown by us to become normal 24 h after the simulation of acute hypobaric hypoxia [1, 17].

Thus, the ability of BDNF to influence favorably the survival and functioning of the respiratory chain of the brain mitochondria demonstrate its exclusive importance as a component of the endogenous antihypoxic protective system.

## Conclusion

BDNF neurotrophic factor increases animal resistance to acute hypobaric hypoxia and influences the work of mitochondrial respiratory chain through TrkB-signaling. Antihypoxic effect of BDNF is realized by maintaining the activity of NADH-dependent pathway of ATP substrate oxidation and synthesis.

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**Conflicts of interest.** The authors have no conflicts of interest to declare.

## References

1. Lukyanova L.D., Kirova Yu.I., Sukoyan G.V. Signaling mechanisms of adaptation to hypoxia and its role in systemic regulation. *Biologicheskie membrany* 2012; 29(4): 238–252.
2. Duchon M. Mitochondria in health and disease: perspectives on a new mitochondrial biology. *Mol Aspects Med* 2004; 25(4): 365–451, <https://doi.org/10.1016/j.mam.2004.03.001>.
3. Wheaton W.W., Chandel N.S. Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am J Physiol Cell Physiol* 2011; 300(3): 385–393, <https://doi.org/10.1152/ajpcell.00485.2010>.
4. Hernansanz-Agustín P., Ramos E., Navarro E., Parada E., Sánchez-López N., Peláez-Aguado L., Cabrera-García J.D., Tello D., Buendía I., Marina A., Egea J., López M.G., Bogdanova A., Martínez-Ruiz A. Mitochondrial complex I deactivation is related to superoxide production in acute hypoxia. *Redox Biol* 2017; 12: 1040–1051, <https://doi.org/10.1016/j.redox.2017.04.025>.
5. Görlach A., Dimova E.Y., Petry A., Martínez-Ruiz A., Hernansanz-Agustín P., Rolo A.P., Palmeira C.M., Kietzmann T. Reactive oxygen species, nutrition, hypoxia and diseases: problems solved? *Redox Biol* 2015; 6: 372–385, <https://doi.org/10.1016/j.redox.2015.08.016>.
6. Olmez I., Ozyurt H. Reactive oxygen species and

ischemic cerebrovascular disease. *Neurochem Int* 2012; 60(2): 208–212, <https://doi.org/10.1016/j.neuint.2011.11.009>.

7. Chaturvedi R.K., Flint Beal M. Mitochondrial diseases of the brain. *Free Radic Biol Med* 2013; 63: 1–29, <https://doi.org/10.1016/j.freeradbiomed.2013.03.018>.

8. Galkin A., Abramov A.Y., Frakich N., Duchon M.R., Moncada S. Lack of oxygen deactivates mitochondrial complex I: implications for ischemic injury? *J Biol Chem* 2009; 284(52): 36055–36061, <https://doi.org/10.1074/jbc.m109.054346>.

9. Markham A., Cameron I., Franklin P., Spedding M. BDNF increases rat brain mitochondrial respiratory coupling at complex I, but not complex II. *Eur J Neurosci* 2004; 20(5): 1189–1196, <https://doi.org/10.1111/j.1460-9568.2004.03578.x>.

10. Vedunova M.V., Sakharova T.A., Mitroshina E.V., Shishkina T.V., Astrakhanova T.A., Mukhina I.V. Antihypoxic and neuroprotective properties of BDNF and GDNF in vitro and in vivo under hypoxic conditions. *Sovremennye tehnologii v medicine* 2014; 6(4): 38–47.

11. Stragier E., Massart R., Sallery M., Hamon M., Geny D., Martin V., Boule F., Lanfumey L. Ethanol-induced epigenetic regulations at the *Bdnf* gene in C57BL/6J mice. *Mol Psychiatry* 2015; 20(3): 405–412, <https://doi.org/10.1038/mp.2014.38>.

12. *Metodicheskie rekomendatsii po eksperimental'nomu izucheniyu preparatov, predlagaemykh dlya klinicheskogo izucheniya v kachestve antigipoksicheskikh sredstv* [Guidelines on experimental study of drugs offered for clinical study as antihypoxic agents]. Pod red. Lukyanovoy L.D. [Lukyanova L.D. (editor)]. Moscow; 1990.

13. Egorova M.V., Afanasyev S.A. Isolation of mitochondria from cells and tissues of animals and human: modern methodical approaches. *Sibirskij medicinskij zurnal* 2011; 26(1–1): 22–28.

14. Vedunova M.V., Mishchenko T.A., Mitroshina E.V., Mukhina I.V. TrkB-mediated neuroprotective and antihypoxic properties of brain-derived neurotrophic factor. *Oxid Med Cell Longev* 2015; 2015: 453901, <https://doi.org/10.1155/2015/453901>.

15. Skaper S.D. Neurotrophic factors: an overview. *Methods Mol Biol* 2018; 1727: 1–17, [https://doi.org/10.1007/978-1-4939-7571-6\\_1](https://doi.org/10.1007/978-1-4939-7571-6_1).

16. Kristián T. Metabolic stages, mitochondria and calcium in hypoxic/ischemic brain damage. *Cell Calcium* 2004; 36(3–4): 221–233, <https://doi.org/10.1016/j.ceca.2004.02.016>.

17. Lukyanova L.D. Signal role of mitochondria in adaptation to hypoxia. *Fiziologichnyi zhurnal* 2013; 59(6): 141–154.