Effect of Cerebronorm on Energy Metabolism and Lipid Peroxidation in Rat Brain during Hypoxia

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Cerebronorm prevents de-energization of mitochondria, limitation of succinate- and NAD-dependent energy production, and oxidation-phosphorylation uncoupling and inhibits LPO processes in the brain of rats under conditions of hypoxia.

Key Words: *hypoxia; brain mitochondria; cerebronorm*

Hypoxia is a typical pathological process accompanying various diseases. Irrespective of the type of hypoxia, characteristic hypoxic disorders are determined by insufficient aerobic energy production in mitochondria [2]. Energy deficiency causes a variety of metabolic shifts, including activation of free radical oxidation in cells [2].

Hypoxia plays a very important role in the pathogenesis of cerebral abnormalities: acute cerebrovascular disorders, chronic cerebral ischemia, toxic and posthypoxic encephalopathy, brain damage, neurotropic intoxication, coma. *etc.* [2,5]. Therefore, the use of drugs correcting energy deficit and stabilizing secondary negative shifts, including LPO activation, is justified in combined therapy of cerebral disorders.

Mitochondrion (MC) substrate-based agents and substances modulating mitochondrial processes are perspective agents for prevention and correction of hypoxic aftereffects [4].

We studied antihypoxic activity of cerebronorm (CN), a complex drug developed in our laboratory. The composition of CN tablets (0.5 g) is as follows: succinic acid (SA; 0.1 g), riboxin (0.1 g), nicotinamide (0.05 g), and riboflavin (0.01 g). The metabolic composition of CN components is designed to maintain oxidative phosphorylation processes in the brain under conditions of hypoxia.

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MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (200-250 g). Hypercapnic hypoxia was induced by placing the animals into a sealed vessel (3 liters) for 1.5 h, which, according to our data corresponds to medium severe hypoxia [4]. CN was used as a preventive drug in a single daily dose of 260 mg/kg intragastrically for 5 days, which corresponded to 50 mg/kg (antihypoxic dose) of SA, 50 mg/kg riboxin, 25 mg/kg nicotinamide, and 5 mg/kg riboflavin. Controls received an equivalent volume of the solvent (1% starch gel).

Mitochondrial function was evaluated by respiratory activity of brain homogenate using the polarographic method. The rates of O_2 consumption in metabolic states before (V_{4p}), during (V_3), and after (V_{4o}) phosphorylation of 1×10^{-4} M ADP during oxidation of 5×10^{-3} M succinate or NAD-dependent substrates malate and glutamate (3×10^{-3} M each) in the presence of SDH inhibitor (malonate; 2×10^{-3} M) or aminotransferase inhibitor (aminooxyacetate; 5×10^{-4} M). Respiration stimulation (V_3/V_{4p}), respiratory control (V_3/V_{4o}), and oxidation-phosphorylation coupling (ADP/O) coefficients were calculated. Inhibition of LPO by CN was evaluated by its effect on the development of spontaneous and ascorbate-dependent LPO in brain

homogenate [2]. The significance of differences was evaluated using Mann—Whitney nonparametric test at 5% significance.

RESULTS

The rate of O_2 consumption by brain MC under conditions of hypoxia in our experiment increased during utilization of endogenous substrates before and during ADP phosphorylation cycle, while the respiration stimulation coefficient decreased (Fig. 1). Hypoxia appreciably increased MC respiration rates under regulated conditions (V_{4p} and V_{4o}) during utilization of exogenous substrates (succinate and NAD-dependent substrates). The rate of MC phosphorylating respiration (V_3) increased during succinate utilization under regulated conditions, this being paralleled by a reduction of V_3/V_{4p} coefficient. Oxidation of NAD-dependent substrates was also associated with an increase in MC respiration rates and a decrease in respiration stimulation and respiratory control coefficients (Fig. 1). Hypoxia was associated with a reduction of the ADP/O coefficient in rat brain MC during oxidation of all types of substrates (Fig. 1), which indicated uncoupling of oxidative phosphorylation. Activation of SDH in hypoxia was paralleled by the development of compensatory limitation of enzyme activity. Malonate, a competitive SDH inhibitor, contrasted the signs of energy deficiency in the MC during oxidation of NAD-dependent substrates: reduced the level of malonate-sensitive respiration and oxidation-phosphorylation coupling in animals exposed to hypoxia (Fig. 1).

These data indicate a strong effect of this hypoxic model on cerebral energy homeostasis. Mitochondrial energy deficiency developing under conditions of O_2 deficit and most demonstrative during oxidation of NAD-dependent substrates, manifested predominantly in increased rates of regulated respiration and less so by increased rate of phosphorylating respiration in the presence of oxidation-phosphorylation uncoupling (reduced respiration stimulation, respiratory control, and ADP/O values). It seems that detected activation of MC respiration in animals exposed to medium severe hypoxia results from transition of organelles from hypoxic to hyperoxic status during *in vitro* incubation [7], which masks the actual status of the object. The possible cause of changes in the metabolic status of MC is impaired permeability of their membranes [8] during *in vivo* hypoxia and subsequent *in vitro* reoxygenation. Increased total number and rate of lipoperoxide production in brain homogenate of animals exposed to hypoxia really indicates activation of free radical formation (Fig. 2).

Preventive treatment with CN before hypoxia eliminates energy deficit in cerebral MC by transferring the organelles in animals of the studied group into the reduced respiratory activity mode ("economy strategy") under conditions of low Po_2. CN treatment before hypoxia reduces MC respiration rates under regulated conditions (V_{4p} and V_{4o}) during oxidation of substrates of all types, this indicating elimination of hypoxic changes in metabolism and can be explained by the stabilizing effect of the drug on MC membranes. CN promotes the increase in the respiration stimulation coefficients during oxidation of endogenous substrates, succinate, and NAD-dependent substrates by MC (Fig. 1); during succinate oxidation CN promotes the increase of the respiratory control coefficient, which fact indicates a wider range of MC respiratory activity (V_3-V_4). Normalization of the ADP/O coefficient during oxidation of all types of substrates was noted in MC of animals CN in comparison with the parameters in untreated animals.

The decrease in LPO intensity in brain tissue under conditions of hypoxia after CN treatment (Fig. 2) presumably reflects normalization of oxidative phosphorylation, prevention of the cascade of free-radical cell damage [2] as a result of organelle energization. Presumably, the antioxidant effect of CN is explained by the formation of reduced glutathione [8] at the expense of electron return in the respiratory chain as a result of its monopolization by succinate.

Activation of SDH by exogenous succinate contributes to the antihypoxic effect of the drug [3]. Enzyme activity does not depend on the deficit of oxidized NAD^+ forms in hypoxia, due to which the energy-producing function of MC is preserved in the presence of disturbed NAD-dependent respiration of organelles. Presumably, the substrate effect of SA is realized predominantly in the liver during primary passage of the substance through it, due to intense metabolism of the substrate [3]. The close neurohumoral relationship between the organs and strain irradiation in the energy production systems [6] suggest that the antihypoxic mechanism of the drug in the brain does not depend on the substrate effect of SA. Presumably, it is caused by the receptor direct and indirect effect of the drug [1,9]: the concentration of SA, received with the drug dose of 50 mg/kg, does not reach the Michaelis' constant for SDH (0.35 mM) even in the plasma. The antioxidant effect of riboxin is realized via stimulation of NAD synthesis in MC from nicotinamide, xanthine oxidase inhibition, and suppression of the

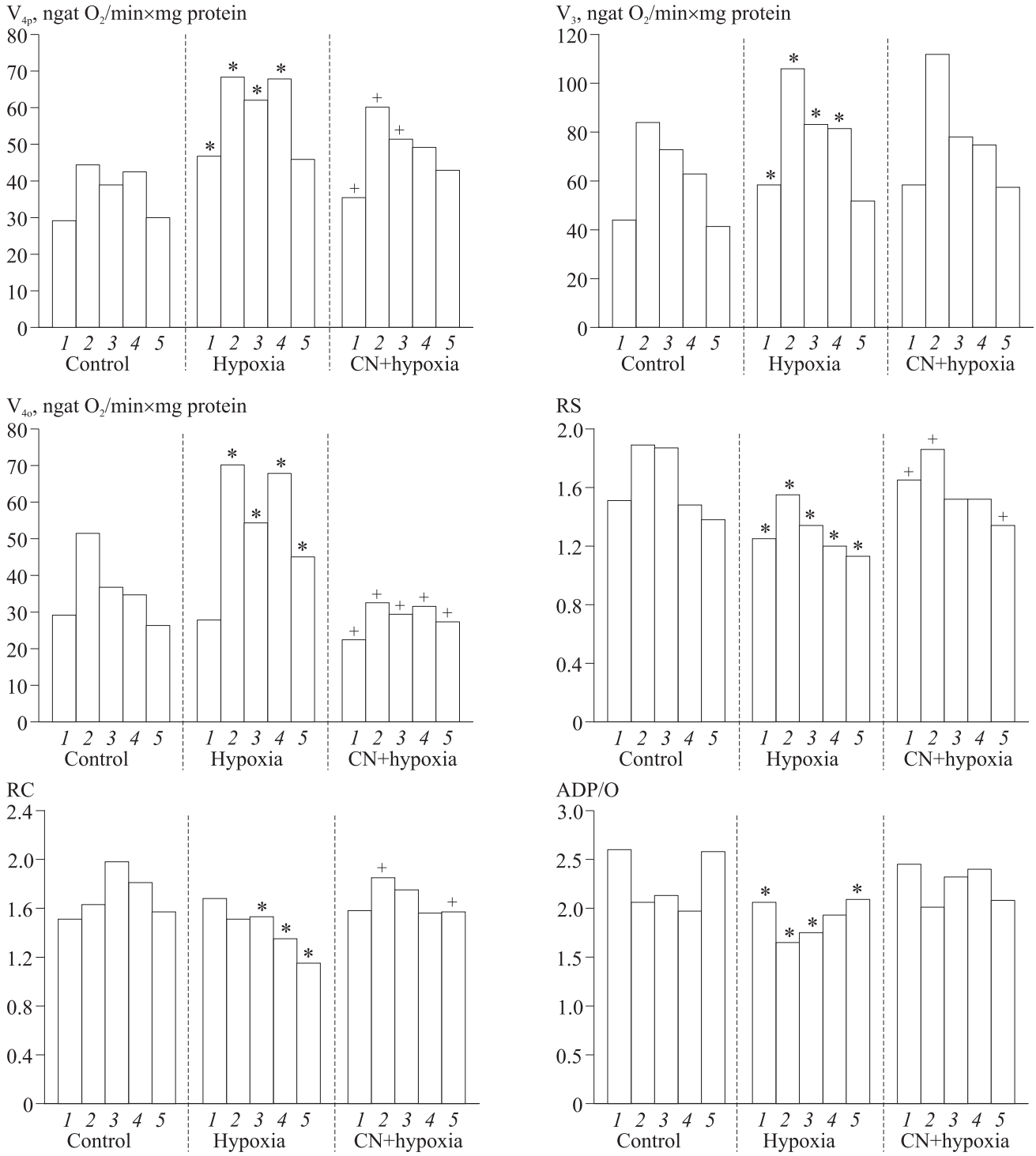

Fig. 1. Function MC from rat brain during hypoxia and after preventive treatment with CN. Oxidation substrates: *1*) endogenous; *2*) succinate; *3*) malate and glutamate; *4*) malate, glutamate, and malonate; *5*) malate, glutamate, and aminooxyacetate. RS: respiration stimulation coefficient; RC: respiration control coefficient. Here and in Fig. 2: $p<0.05$ compared to: *control, +hypoxia.

radical processes. Riboflavin is characterized by antihypoxic effect associated with activation of flavin reductase and reduction of the content of macroergic substances (ATP and creatine phosphate), as well as by antioxidant effects caused by glutathione reduction [8]. Nicotinamide is a component of NAD, it activates cellular NAD-dependent dehydrogenases and antioxidant systems of ubiquinone oxidoreductases, protecting cell membranes from free radicals [10]. As the targets of CN

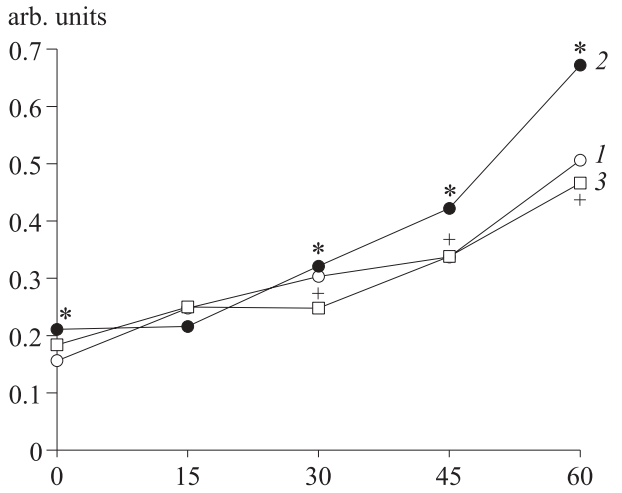

Fig. 2. Effect of CN on the dynamics of accumulation of ascorbate-dependent TBA-reactive products in the brain of rats during hypoxia. *1*) control; *2*) hypoxia; *3*) CN.

components cytoprotective effects are different in hypoxia, presumably, their effects are synergic.

These data suggest clinical use of CN as a cerebroprotector for prevention or therapy of damaged function of the CNS in disorders of the cerebral hemodynamics or hypoxia.

REFERENCES

1. A. M. Babskii, Yu. S. Stefankoff, and M. N. Kondrashova, *Succinic Acid in Medicine, Food Industry, and Agriculture* [in Russian], Pushchino (1996), pp. 14-21.
2. Yu. A. Zozulya, V. A. Baraboi, and D. A. Sutkovoi, *Free-Radical Oxidation and Antioxidant Defense in Cerebral Diseases* [in Russian], Moscow (2000).
3. E. I. Mayevskii, A. S. Rozenfeld, M. V. Vazatashvili, *et al.*, *Succinic Acid in Medicine, Food Industry, and Agriculture* [in Russian], Pushchino (1996), pp. 52-57.
4. R. R. Saifutdinov and V. A. Khazanov, *Eksp. Klin. Farmakol.*, **61**, No. 5, 27-29 (1997).
5. A. V. Smirnov and B. I. Krivoruchko, *Anesteziol. Reanimatol.*, No. 3, 97-98 (1997).
6. V. A. Khazanov, *Byull. Eksp. Biol. Med.*, Suppl. 2, 40-44 (2003).
7. V. A. Khazanov and A. N. Poborskii, *Ibid.*, **112**, No. 9, 258-260 (1991).
8. H. Boxles, *Int. J. Vitam. Nutr. Res.*, **67**, No. 5, 321-328 (1997).
9. W. He, F. Mlao, D. Lin, *et al.*, *Nature*, **429**, No. 6988, 188-193 (2004).
10. T. Kishi, T. Takashi, A. Usiu, and T. Okamoto, *Biofactors*, **10**, Nos. 2-3, 131-138 (1999).