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# Structural/Functional Modifications in the Mitochondria of Brainstem Cells in Rat Offspring Subjected to Prenatal Hypoxia

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We examined changes in the morphofunctional state of the mitochondria (MCh) and immunohistochemical peculiarities of brainstem neurons in rat offspring exposed to experimental prenatal (intrauterine) hypoxia of different severity, moderate and strong. This was provided by exposure of pregnant females to $O_2/N_2$ respiratory mixtures containing 12 and 7% $O_2$, respectively. Experimental groups included 20 one-month-old rats (offspring of 9 females, control and subjected to hypoxia). We estimated the ultrastructural characteristics of the MCh and also expression of the *CD95 APO-1*/*Fas* and *Bcl-2* genes modulating the intensity of apoptosis and mitoptosis in these cells. Severe intrauterine hypoxia resulted in the development of structural distress in the MCh of brainstem cells; all stages of MCh degradation, from swelling to complete dissipation, were observed. Juvenile forms of these organelles were absent. Mosaic-like destruction of myelin with manifestations of edema was observed. After the moderate prenatal hypoxia, about half of the changes in the MCh ultrastructure could be qualified as directed toward an increase in the compensatory capabilities of the MCh apparatus. In rats after moderate hypoxic influence, levels of expression of the *CD95 APO-1*/*Fas* and *Bcl-2* genes were indicative of a greater readiness of the neurons to apoptosis and decrease in the probability to inhibition of the respective MCh pathway in brainstem neurocites. At the same time, the MCh and neurocites of animals subjected to severe intrauterine hypoxia demonstrated decreased trends toward mitoptosis and apoptosis, respectively. The obtained results characterizing the effects of intrauterine hypoxia of different levels on the formation of structural/functional changes in the MCh of brainstem cells can be taken into account in the process of development of novel approaches to the treatment of MCh diseases.

**Keywords**: rat offspring, brainstem cells, prenatal (intrauterine) hypoxia, mitochondria (MCh), genes *CD95 APO-1*/*Fas* and *Bcl-2*, electron microscopy, immunohistochemistry.

## INTRODUCTION

Perinatal hypoxia is one of the most important trigger factors initiating a chain of pathological reactions resulting in a broad spectrum of neurosomatic deviations in newborns and children. These deviations are based, to a significant extent, on disorders of energy metabolism. At present, an enormous volume of data on physiological, biochemical, and molecular mechanisms of hypoxia-related effects has been accumulated. The dynamics of this process are very complicated, and numerous mechanisms controlling this process on the organismal, cellular, and molecular levels are involved. This is why a number of pathogenetic aspects of hypoxia-related disorders, questions related to antihypoxic defense of the organism in general, and those related to the functioning of separate organs and systems under conditions of hypoxia, remain at present unresolved despite of about a century-long history of studies within the respective field [1, 2].

It is obvious that elucidation of the peculiarities of energy metabolism under conditions of oxygen insufficiency is of crucial value for both fundamental physiology and practical medicine. The parameters of energy metabolism are the main prognostic criteria of the severity of hypoxic disorders. The importance of studies within the

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above-mentioned field increases because there are strong interrelations between the characteristics of energy metabolism and normal/impaired functional/metabolic status of the organism [1].

The main part of energy metabolism is realized on the membranes of mitochondria (MCh) in the process of aerobic cellular respiration and oxidative phosphorylation; this is why mitochondrial dysfunction (MChD) at the development of the hypoxic states plays a leading role in disorders and decompensation of the energy supply of various cells, including neurons [3, 4]. Mitochondrial disorders that were earlier believed to be rare phenomena became at present rather frequent pathologies, in particular in children. A "classic" MCh disease is found in a few cases of autism; these deviations are usually determined by genetic abnormalities and disorders of the respiratory MCh pathway. This disease is also related to perinatal damages of the brain; it is manifested in disorders of cognitive and associative functions under cerebral pathologies [3, 5, 6]. In these cases, researchers frequently find symptoms of MChD, but without some classical phenomena typical of the MCh disease. Among such phenomena, reversible re-uptake of the transmitters (catecholamines, dopamine, and serotonin), rapid depletion of the energetic stores in the nerve cells, formation of a specific complex of mitochondrial pores (MChPs), and initiation of mitoptosis should be mentioned. The *CD95 APO-1*/*Fas* gene functions as the best-known inductor of the latter process in the brain, while the *Bcl-2* gene is responsible for inhibition of the above process [3, 7]. Opening of the MChPs transforms these organelles from energy-producing units into consumers of the oxidation substrates, but with no ATP formation [3].

The dynamics of formation of structural and functional changes in different organs and tissues under conditions of hypoxia are determined, to a considerable extent, by the rate of development of hypoxia, nature of the factors initiating this state, and peculiarities of the compensatory/adaptation reactions in one organ or another. The resistivity of tissues of different organs and systems with respect to hypoxia varies within broad limits. The nervous system is most sensitive to hypoxia; as early as in 10–15 min, neurons of the medulla and upper parts of the brainstem demonstrate profound structural modifications significantly influencing intracellular organelles [7, 8]. Activation of the process of free-radical oxidation accompanied by intensification of lipid peroxidation is a general reaction of the brain to most damages. Toxic products of peroxidation formed under the respective conditions evoke destruction of the biological membranes and disturb their functions. Precisely the membranes of the neuronal MCh suffer primarily in this case. The ion permeability of these membranes increases, while the voltage on these membranes necessary for ATP synthesis from ADP and orthophosphate drops [9, 10]. Damages to the MCh ultrastructure result in their functional inadequacy; this, in turn, results in disorders of energy metabolism of the brain cells. Under conditions of oxygen deficiency in the tissue, a deficiency of macroergic compounds produced in the course of phosphorylation reactions is readily formed. The latter reactions are realized on the internal MCh membranes and are coupled with oxidation/reduction processes [8, 11]. Such disorders especially intensely influence the brain tissues because the latter are characterized by especially high energy requirements and high sensitivity to disorders in the production of energy. The presence of only 20% of mutant DNA in the MCh of brain neurons can result in the development of clearly expressed functional disorders in the CNS; for comparison, such changes are not observed in the MCh of hepatocites even under conditions of the presence of 80% of mutant DNA [11, 12].

Structural modifications of MCh related to hypoxia begin from swelling and changes in the electron microscopic characteristics of the membranes. At severe hypoxia (a dramatic drop in the $O_2$ tension in the tissue), the number of cristae in the MCh decreases significantly, intense vacualization of these organelles develops, and morphogenesis of MCh is modified. The latter shift can result in either a decrease or an increase in their total number. Four main directions of modifications of the MCh under conditions of tissue hypoxia have been differentiated. These are (i) changes in the number and dimensions of the MCh, (ii) formation of the so called megaMCh, (iii) modifications of the shapes of the MCh, and (iv) changes in the structure of the cristae. These processes determine certain energy-dependent configurational states of these organelles, namely orthodoxal, deenergized, energized, and the so called energized/twisted. These states significantly affect the activity of synthetic processes in the cell [10, 13–15].

Despite the great number of publications describing morphofunctional states of the MCh in neurons, posthypoxic structural MCh changes