Ultrastructure of Synapses in Cerebral Cortex Layer I in Rats with Low and High Resistance to Hypoxia

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Quantitative analysis of synapses in layer I of the sensorimotor cortex in rats with low resistance to hypoxia revealed pronounced changes in the number of synaptic vesicles docked at the presynaptic membrane in active synaptic zones under conditions of acute hypobaric hypoxia. In high-resistant animals the number of docked synaptic vesicles under these conditions remained unchanged. In was hypothesized that high sensitivity to hypoxia in low-resistant rats is determined by high reactivity of the synaptic transmission system.

Key Words: *synapses; synaptic vesicles; cerebral cortex; hypobaric hypoxia; hypoxic resistance*

Elucidation of the mechanisms of endogenous resistance of the nervous system to hypoxia is important for the treatment of hypoxic and ischemic states in clinical practice.

Biochemical, physiological, and behavioral peculiarities of animals with different resistance to hypobaric hypoxia are now studied in detail [2,4,5,9]. Special attention in elucidation of the mechanisms of hypoxic resistance was focused on disturbances in the oxidative bioenergetic chain [2], while primary disturbances in the cell-cell signaling systems were less studied. However, hypoxic resistance can differ in males and females. Some female rats are more resistant to hypoxia than males [9], which confirms the important role of hormonal regulation in resistance to hypoxia. It cannot be excluded that in low-resistant animals primary disturbances in the nervous system during hypoxia can result from dysfunction of signaling mechanisms related to synaptic regulation.

Our aim was to study the response of synaptic structures in layer I of the cerebral cortex to acute hypobaric hypoxia. Layer I is highly sensitive to hypoxia and plays a key role in general activation processes [3,7].

MATERIALS AND METHODS

The study was carried out on 16 random-bred male rats weighing 250-300 g kept in individual cages under standard vivarium conditions. Acute hypobaric hypoxia was modeled in a pressure chamber at a simulated altitude of 11,500 m. (180 m/sec elevation rate). The animals exhibited behavioral and autonomic reactions of excitation followed by a decrease in motor activity, loss of consciousness, and sharp decrease in the respiratory rate. One month before the experiment, the animals were divided into two groups depending on the time to a drastic drop of muscle tone and apnea under conditions of hypobaric hypoxia: in high-resistant (HR) rats this period was >10 min, and in low-resistant (LR) animals it was <3min. After 1 month, the animals were again subjected to acute hypoxia. The specimens for electron microscopy were taken during acute hypobaric hypoxia before the appearance of apnea respiration. HR and LR rats examined under normobaric conditions without hypoxia served as the control. The specimens for electron microscopy from the left sensorimotor cortex were fixed in 2.5% glutaraldehyde on 0.1 M phosphate buffer (pH 7.4), stai-

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ned in osmium tetroxide, dehydrated in increasing alcohol concentrations, and embedded in Araldite. Morphological analysis of synapses in layer I of the cerebral cortex was performed using an image analysis system. The data were analyzed statistically using Statistica software. The following parameters of the synaptic apparatus were evaluated: length of active synaptic zone; size of the synaptic cleft; number, length, and orientation of filaments in the pre- and post-synaptic densities; the number of synaptic cleft filaments oriented perpendicularly and in parallel to the membrane; the number of vesicles connected by filaments with the active zone; the number of vesicles docked at the active synaptic zone; area of synaptic end-plate; area of postsynaptic densities; and the number of active zones.

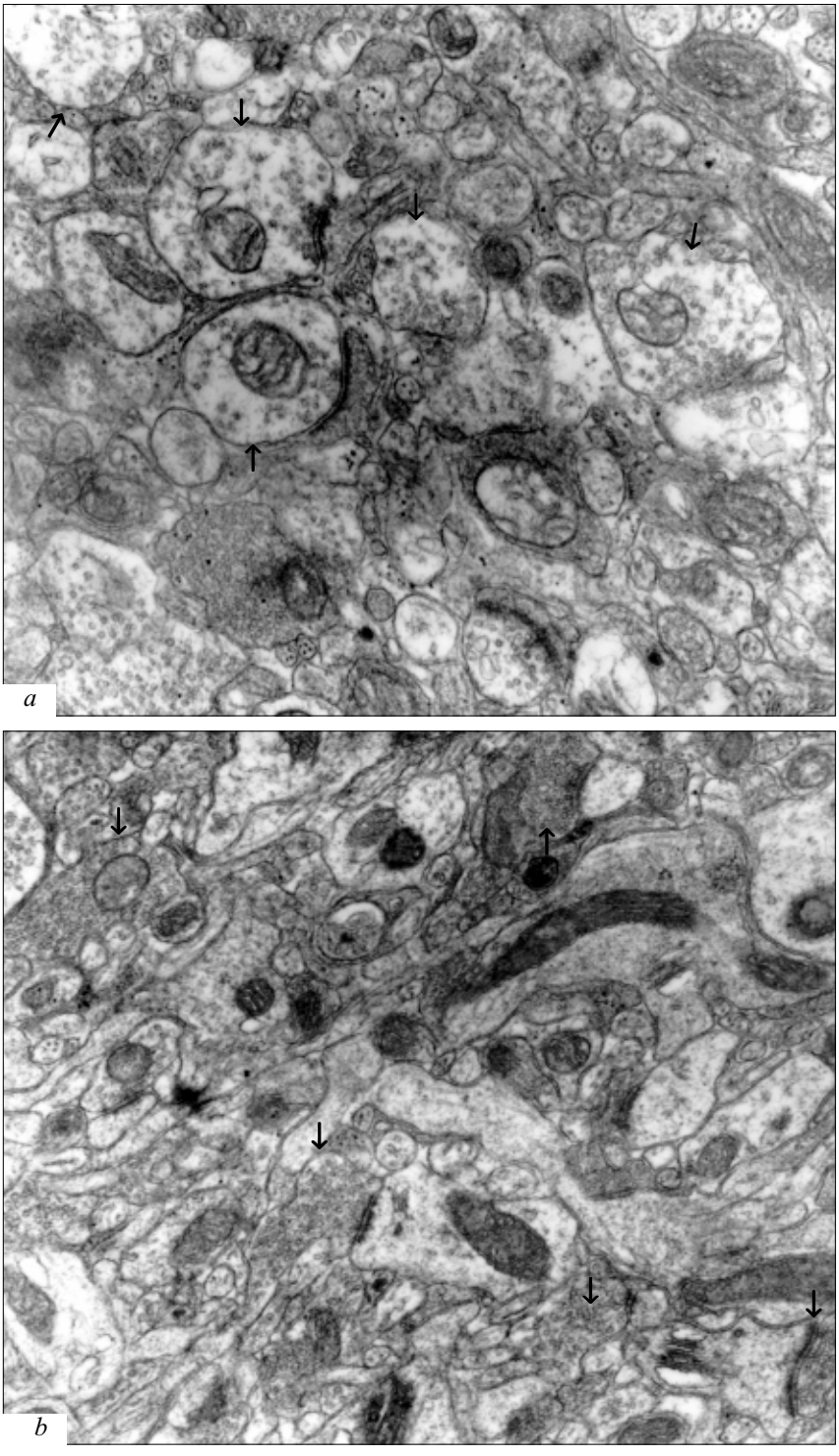

Fig. 1. Ultrastructural changes in layer I of cerebral cortex in rats highly resistant to hypoxia during acute hypobaric hypoxia modeled in a pressure chamber at a simulated altitude of 11,500 m, ×20,000. *a*) experimental group with swollen synapses and mitochondria; *b*) control group, no alterations. Synapses are shown by arrows.

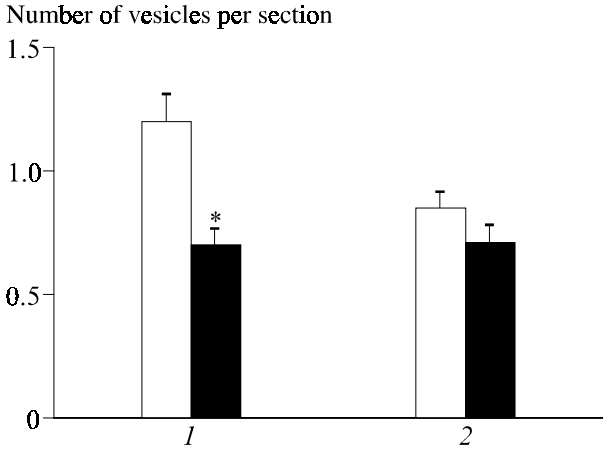

Fig. 2. Effect of hypoxia on the number of synaptic vesicles docked at the active zone membrane in synapses in layer I of the sensorimotor cortex from low- (1) and high-resistant (2) rats. Open bars: control; shaded bars acute hypobaric hypoxia, respectively. *p<0.01 compared to the control.

RESULTS

Morphological changes in layer I of the cerebral cortex during acute hypobaric hypoxia were different in LR and HR rats. In HR, but not in LR rats marked selective swelling of synapses and structural changes in mitochondrial crystae were noted (Fig. 1). These changes can be explained by longer exposure of HR rats to hypoxia in comparison with LR rats.

Despite the absence pronounced edematous alterations in synapses of LR rats (due to short-term exposure to hypoxia) rearrangements in the synaptic transmission apparatus were noted in LR, but not in HR rats. For instance, in LR rats the number of synaptic vesicles docked at the active zone decreased by 60% during the acute phase of hypobaric hypoxia in comparison with the control (significant difference), while in HR rats this parameter decreased by only 20% (insignificant). In the control, the number of docked vesicles was higher in LR rats (Fig. 2). This peculiarity of synapses in LR rats is probably a structural basis of their enhanced sensitivity to hypoxia. Other parameters of synaptic apparatus in neurons, in particular, the length of synaptic zones and the area of postsynaptic densities (published data suggest that these parameters change in the postischemic and posthypoxic periods) remained unchanged.

The number of vesicles docked at the membrane in active zones is directly related to the degree of synaptic activation and reflects the process of the induced (not spontaneous) release of the transmitter [8,10]. At the initial stage, hypoxia produces excitation characterized by hyperactivation of the impulse activity in the brain [1,6]. A higher number of the docked vesicles in LR rats (in comparison with that in HR rats) can also contribute to hypoxia-induced hyperexcitation followed by inhibitory effects. Hypoxic excitation assessed by EEG gradually increases in LR animals, while in HR animals it alternates with compensatory inhibitory periods [5].

High reactivity of the vesicular docking system in LR animals can explain the increase in motor and exploratory activity during long-term moderate hypoxia, behavioral parameters of HR animals under these conditions remained unchanged [4].

We hypothesized that the response of layer I synapses to acute hypoxia in LR and HR animals are mediated via different mechanisms. LR rats are characterized by rapid functional excitation and inhibition due to high sensitivity of their synaptic transmission apparatus. In HR rats functional inhibition to a lesser extent depends on synaptic transmission. Therefore, this study revealed morphological peculiarities of cortical synapses in LR rats manifested in enhanced reactivity of the vesicular docking system at the active zone membrane. This mechanism probably underlies low resistance to hypoxia in these animals. These differences are probably genetically determined.

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