Posthypoxic Disturbances in Glutamatergic Signal Transduction in Rat Brain Neurons: Correction Effect of Preconditioning

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Severe 3-h hypobaric hypoxia was followed by impairment of Ca^{2+}-mediated glutamatergic signal transduction in the posthypoxic period (no less than 72 h). This impairment manifested in changes in the calcium response to glutamate application in slices of rat brain cortex. Moderate hypoxic preconditioning prevented these disturbances developed over the first day after sever hypoxia.

Key Words: *hypoxia; preconditioning; glutamatergic signal transduction; calcium*

Studies performed during the past decade showed that pathological processes in the brain during severe hypoxia can be prevented by preexposure to weak stressors. Well-tolerable moderate hypoxia is an experimental preconditioning factor promoting adaptation of brain cells to impairment of blood supply [1].

Our *in vivo* experiments on cats showed that severe hypoxia produced by cessation of artificial ventilation for 5 min is followed by prolonged suppression of electrophysiological reactions and changes in the contents of intracellular Ca^{2+} and phosphoinositides in response to stimulation of brain neurons by exogenous glutamate. By contrast, short-term anoxia (1.5-2.0 min) produced a potentiating effect [7]. *In vitro* experiments with slices of rat olfactory cortex showed that long-term anoxia (10 min) is followed by persistent pathological hyperactivation of Ca^{2+} and phosphoinositide regulatory systems and suppression of bioelectric activity in neurons. However, short-term anoxia (2 min) was accompanied by moderate activation of intracellular regulatory systems and potentiation of evoked postsynaptic potentials [3,8,9]. Moreover, short-term anoxia abolished pathological changes produced by long-term anoxia [4].

In vivo and *in vitro* observations indicate that the early neuroprotective mechanisms of hypoxic preconditioning are realized via intracellular Ca^{2+} and phosphoinositide systems mediating glutamatergic signal transduction [1,2]. Long-term neuroprotection initiated by hypoxic preconditioning is of particular interest. Its mechanisms involve not only signal reactions, but also slow and persistent adaptive changes in the regulation of gene expression and production of adaptive proteins [1].

Here we studied the effects of severe hypoxia and hypoxic preconditioning on intracellular Ca^{2+}-mediated glutamatergic signal transduction in slices of rat brain cortex during the early posthypoxic period.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. The animals were divided into 2 groups. Group 1 rats were subjected to severe hypobaric hypoxia (SHH) in a pressure chamber. Pressure was maintained at 160-180 mm Hg for 3 h, which corresponded to an altitude of 11,000 m above sea level. Group 2 animals were exposed to repeated moderate preconditioning hypoxia (three 2-h sessions at

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24-h intervals) and then to SHH (24 h after the last session). Pressure was maintained at 160-180 mm Hg for 2 h, which corresponded to an altitude of 5000 m above sea level. Intact rats served as the control.

Series II was performed *in vitro* at various stages of the recovery after SHH. The rats of both groups were decapitated, and slices of the olfactory cortex were prepared. Slices were examined 3, 6, 24, and 72 h after SHH (subgroups 1, 2, 3, and 4, respectively). We analyzed 7-9 slices from rats of each group. Slices (400 μ) were prepared in a vibrotome (EMS-4000, Electron Microscopy Sciences) and placed in a flow thermostat with incubation medium. Changes in the content of intracellular bound Ca^{2+} (Ca-b) in slices were determined spectrophotometrically on a LY-uMAM-KF fluorescence microscope (LOMO). Chlortetracycline served as an indicator of Ca-b concentration. The procedures of incubation and spectrophotometry of slices were described elsewhere [6]. Glutamate (final concentration 50 μM) was 2-fold applied to slices for 2 min at 30-min intervals. Changes in Ca-b content in slices after repeated treatment with glutamate (primary and secondary calcium responses) were determined by fluorescence signals recorded at 5-min intervals over 30 min after application. The results were analyzed by Dunnett test (ANOVA).

RESULTS

Ca-b content in slices from control animals increased by 4.8% 30 min after the first application of glutamate. After the 2nd application of glutamate Ca-b content increased only by 1.5% (Fig. 1). The secondary calcium response to glutamate was less pronounced, which probably reflects desensitization of glutamate receptors. These changes are realized via intracellular regulatory mechanisms directly or indirectly initiated by the increase in intracellular Ca^{2+} content [11,13,14]. Calcium responses were not detected in slices from non-preconditioned rats 3 and 6 h after SHH. Ca-b content did not increase after repeated application of glutamate (Fig. 1). Blockade of calcium responses during the first few hours after SHH is probably realized via the mechanisms that mediate *in vivo* and *in vitro* suppression of the reactions to exogenous glutamate for the first tens minutes after anoxia. It is probably related to changes in intracellular Ca^{2+} content initiated by hyperactivation of NMDA receptors during severe hypoxia and early stages of reoxygenation [1, 7,8]. The calcium response was pronounced 24 h after SHH, which reflects activation of Ca^{2+}-mediated glutamatergic signal transduction. At this term the secondary response was similar to the primary reaction. Moreover, the secondary response in slices from animals subjected to SHH markedly surpassed that in control slices. Therefore, glutamate receptors in rats of the SHH group were not desensitized. It can be hypothesized that these changes characterize the late stage of hyperactivation in NMDA and/or AMPA receptors, which underlies the excitotoxic mechanism of serious posthypoxic damage to neurons during calcium overload [10,12,14]. By the 72nd hour after SHH calcium responses were below the level observed 24 h after treatment and in control animals (Fig. 1, *a*, *b*).

The effects of hypoxic preconditioning were evaluated 3 and 24 h after SHH. In these periods slices from non-preconditioned rats were characterized by most pronounced changes in the reaction to glutamate.

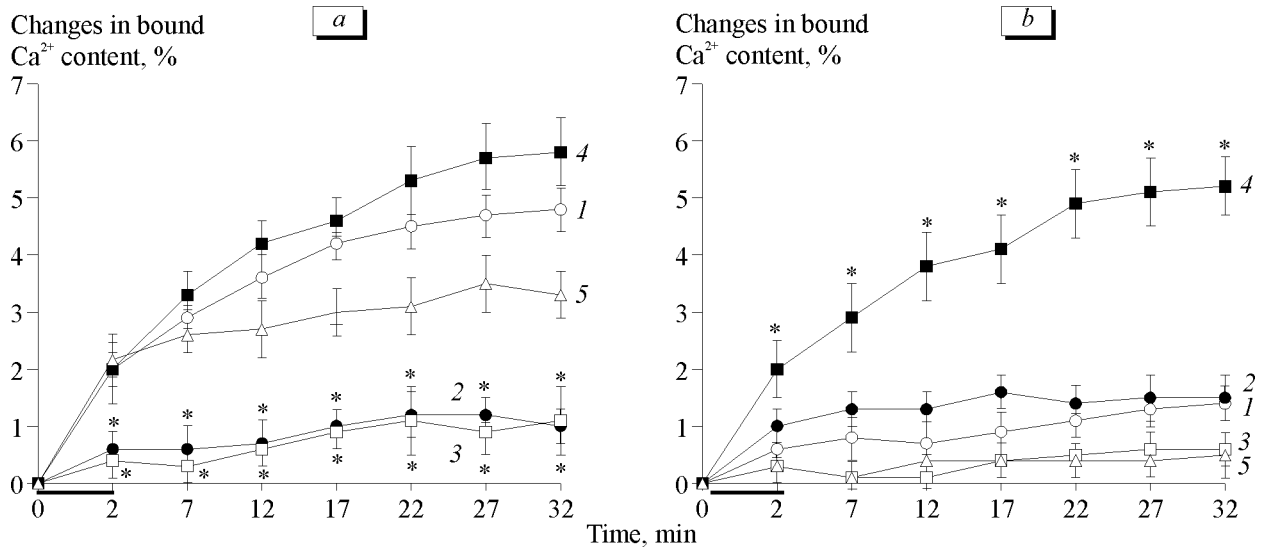

Fig. 1. Primary (*a*) and secondary calcium responses (*b*) to glutamate application in slices obtained from animals at various stages after severe hypobaric hypoxia (SHH, *M*±*m*): control (*1*) and 3 (*2*), 6 (*3*), 24 (*4*), and 72 h after SHH (*5*). Horizontal segments: application of glutamate. Here and in Fig. 2: **p*<0.05 compared to the control.

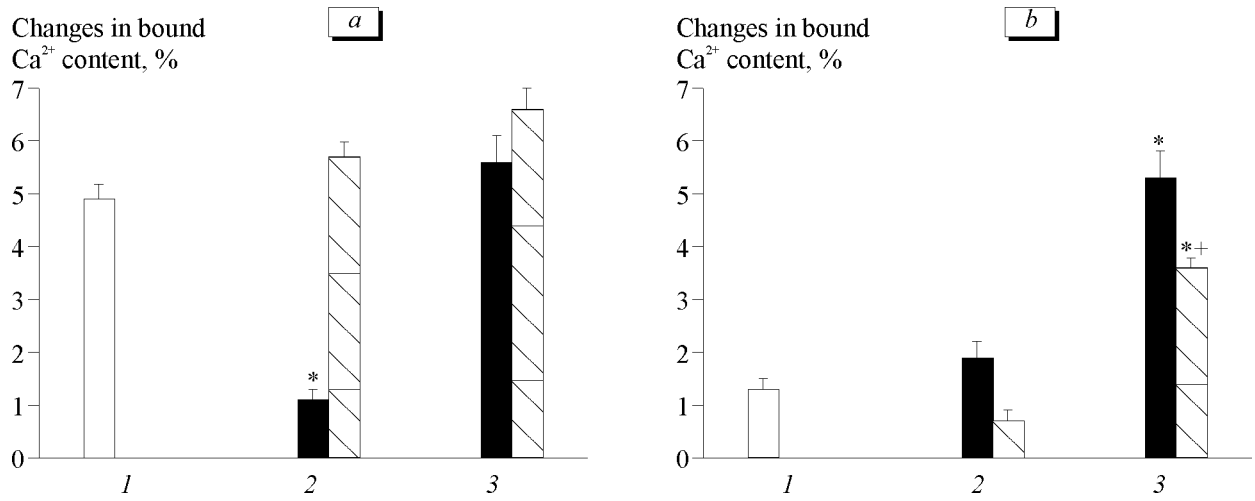

Fig. 2. Effect of preconditioning on primary (*a*) and secondary calcium responses (*b*) in slices 3 and 24 h after SHH. Light bars: maximum calcium responses in slices from control animals 30 min after application of glutamate. Shaded bars: SHH and preconditioning. Dark bars: SHH without preconditioning. Slices from control rats (*1*) and 3 (*2*) and 24 h after SHH (*3*). $^{+}p<0.05$ compared to non-preconditioned animals.

Three hours after SHH calcium responses in slices from preconditioned animals did not differ from the control (Fig. 2, *a*, *b*). The primary response to glutamate 24 h after SHH approached the control level. It should be emphasized that under these conditions the secondary response was less pronounced than in slices from non-preconditioned rats. Therefore, desensitizing properties of glutamate receptors partially recovered 1 day after SHH.

Our findings suggest that calcium responses of brain slices to glutamate were suppressed 6 h after SHH, but considerably increased on day 1 after treatment. These changes were accompanied by blockade of the mechanisms underlying receptor desensitization. Hypoxic preconditioning prevented the impairment of Ca^{2+}-mediated glutamatergic signal transduction over the first day after SHH.

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