Prevention of Experimental Stroke by Hypercapnic-Hypoxic Preconditioning

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The effectiveness of hypercapnic hypoxic training in the prevention of acute disturbances in cerebral circulation was studied under experimental conditions. Hypercapnic hypoxic training was followed by a significant decrease in the severity of neurological deficit and locomotor and coordination disorders after cerebral ischemic injury.

Key Words: *hypercapnic hypoxia; stroke; prevention*

Hypoxitherapy is widely used for the treatment and prevention of cardiovascular and respiratory disorders and blood diseases. This procedure also improves exercise performance [2,3,8]. As differentiated from hypoxia, hypercapnia is used in medical practice for evaluation of the reactivity of cerebral vessels [4,10]. The combination of hypoxia and hypercapnia has a greater adaptogenic potential than isolated hypoxia. Hypercapnic hypoxia (HCH) has a normalizing effect on blood circulation, increases organism's resistance to acute hypoxia, and improves collateral reserve of cerebral circulation and brain tolerance to ischemia [1].

Here we studied the effectiveness of training with HCH in the prevention of cerebral ischemic injury under experimental conditions.

MATERIALS AND METHODS

Experiments were performed on 28 adult male Wistar rats weighing 300±9 g and aging 9-10 months. The animals were divided into the treatment and control groups of 14 specimens each. HCH training was performed daily for 20 min. The duration of training was 30 days. For induction of HCH, the animals were maintained in sealed chambers with 3-mm gauge hole for air supply. CO_2 concentration in the inspired gas mixture (4.5±0.3%) was regulated using a Spirolit-2 gas analyzer. O_2 deficiency was 5.6±0.3%. Control animals placed in the same chambers for the same periods breathed atmospheric air.

By the end of training, experimental cerebral ischemia was induced by ligation of the right common carotid artery. The severity of neurological deficit and locomotor and coordination disorders was evaluated on day 10 of the postoperative period.

Neurological status was estimated from the maximum score (Menves scale): 0, no neurological deficit; 1 point, tonic flexion of the forelimb contralateral to common carotid artery occlusion (CCAO) in tail suspension; 2 points, paresis of the forelimb contralateral to CCAO in tail pulling; 3 points, movement of the rat to the side contralateral to CCAO in tail holding; and 4 points, spontaneous rotation of the rat to the side contralateral to CCAO [12].

The severity of locomotor and coordination disorders was estimated by the ability of animals to maintain balance on a rotating rod (diameter 70 mm, length 200 mm) positioned at a height of 800 mm from the floor [9]. During training, the animals were placed 3 times on the rotating rod for 5 min (rotation rate 7 rpm). We recorded the time that rats spent on the rod at a rotation rate of 21 rpm. The test was terminated when this time exceeded 2 min.

Quantitative parameters were studied. The mean values of 2 samples (M1 and M2) were compared

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by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

The severity of experimental ischemic injury to the brain was estimated by the Menves scale on day 10 of the postoperative period. Severe neurological deficit was revealed in control animals (Fig. 1, *a*). In rats of the treatment group with HCH training, cerebral ischemic injury was accompanied by mild neurological deficit (3 times lower than in control animals).

The difference in neurological score between control and treated animals attests to high effectiveness of HCH training as the means preventing structural and functional changes in the brain after ischemic injury.

High effectiveness of HCH in the prevention of acute disturbances in cerebral circulation was confirmed by studying the severity of locomotor and coordination disorders after cerebral ischemic injury. The degree of locomotor and coordination disorders after CCAO was much lower in animals with HCH training (Fig. 1, *b*).

Our results indicate that HCH training is followed by a significant decrease in the severity of neurological deficit and locomotor and coordination disorders due to cerebral ischemic injury.

One of the mechanisms for improvement of brain tolerance to ischemia under conditions of reduced Po_2 and CO_2 excess is stimulation of angiogenesis in the brain and increase in the number of functioning microvessels. Our previous experiments showed that the increase in the number of microvessels in brain tissue is a mechanism underlying improvement of collateral reserve of cerebral circulation in rats during HCH training [5]. Published data show that the increase in the number of capillaries is followed by a decrease in the distance between capillaries. Under these conditions, nerve cells are not subjected to hypoxia even under conditions of sharply reduced blood Po_2 [7].

Another mechanism for improvement of brain tolerance to ischemia during HCH training is a decrease in the tone of resistive cerebral vessels, elevation of volume blood flow in the brain, and increase in capillary filtration due to high-intensity production of nitric oxide (NO). We previously showed that NO concentration in the brain increases by 1.5 times during HCH training [6]. NO is a potent vasodilator. The increase in NO concentration determines the improvement of blood supply to the brain [11]. Moreover, NO decreases O_2 demands of cells [13].

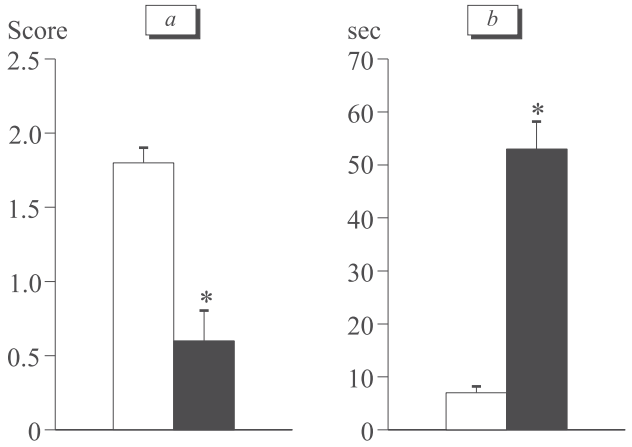

Fig. 1. Neurological status (*a*) and severity of locomotor and coordination disorders in rats after CCAO (*b*). Light bars, control; dark bars, treatment. *$p<0.05$ compared to the control.

We conclude that HCH training is followed by a significant decrease in the severity of neurological deficit and locomotor and coordination disorders after cerebral ischemic injury. Our results contribute to the development of preventive methods for cerebral circulatory disorders.

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