Neuroprotective Effects of Individual or Combined Exposure to Hypoxia and Hypercapnia in the Experiment

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We studied the effects of hypoxic, hypercapnic, and hypercapnic-hypoxic exposures on brain tolerance to ischemia. All respiratory training modes had a neuroprotective effect, but the most pronounced effect was observed after exposure to hypercapnic hypoxia. Experimental stroke in rats preliminary exposed to hypercapnic hypoxia was associated with minimal neurological deficit and motor coordination disturbances in comparison with training modes.

Key Words: *hypoxia; hypercapnia; adaptation; stroke; brain*

The neuroprotective effects of hypoxic exposures is well established; it is associated with a decrease in neuronal loss after brain hypoxia and ischemia [9,14]. In 2004, a significant increase in brain tolerance to ischemia was shown during combined exposure to hypoxia and hypercapnia [1]. It was also found that individual exposure to hypercapnia has significant therapeutic effects during experimental ischemia reperfusion injury of brain [15]. All these data suggest that combined exposure to hypercapnia and hypoxia can potentiate the neuroprotective effects of hypoxia. However, little is known about the comparative efficacy of exposures to hypoxia, hypercapnia, and hypercapnic hypoxia in improving brain tolerance to ischemia. Here we compared these modes of respiratory training.

MATERIALS AND METHODS

The experiments were performed on mature albino male Wistar rats (n=60) weighting 284±46 g. The animals had free access to food (standard ration for laboratory rats) and water. All rats were randomized using the method of random numbers table and divided into 4 groups. Group 1 was exposed to normobaric hypoxia (NH); groups 2 and 3 were exposed to individual hypercapnia (IH) and hypercapnic hypoxia (HH), respectively. Group 4 served as the control.

For modeling of respiratory states, a special flow-type chamber (vital volume was 4 litters per animal) was used, in which the certain gas mixture was supplied by a compressor (15 liter/min). The chamber had a discharge hole connected via a pipe with a tank with water. This construction provided excessive pressure expel. The gas mixture containing 13% O_2 or 7% CO_2 was used for modeling of NH and IH, respectively. HH was modeled using a gas mixture containing 13% O_2 and 7% CO_2. The controls were also put in the chamber, but breathed atmospheric air delivered with a compressor instead of gas mixture. Gas analysis was performed using Mikon gas analyzer (Laspek).

The animals breathed the specified gas mixtures daily for 20 min (15 days). On the next day after exposures, experimental brain ischemia was modeled in all animals under thiopental narcosis (50 mg/kg, i.p.) by the ligation of right carotid artery.

Neurological deficit was scored at the end of 10-day postoperational period using Katz 100-point scale [5]. Motor coordination disturbances were evaluated using rotarod test (cylinder with a diameter of 70 mm and length 200 mm was positioned at the height of 800 mm) [3].

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The data were processed using SPSS 11.5 software. All parameters were presented as median (*Me*) and lower and upper quartiles (25%; 75%). The data distribution was estimated by Shapiro–Wilk test. The experimental data in all study groups did not correspond to the normal distribution law. The significance of differences was evaluated using the nonparametric Mann–Whitney *U* test. The values were significant at $p<0.05$.

RESULTS

Experimental ischemic damage of the brain was followed by severe neurological deficit in control animals (Fig. 1). In trained animals, the neurological deficit after brain ischemia was less pronounced. Neurological deficit in animals of HH group was 3-fold lower than in controls. Neurological deficit after experimental brain ischemia was similar in rats trained under the conditions of HH and IH and was lower in HH group than in NH group (by 45%).

Motor coordination disturbances were most pronounced in the control group (Fig. 2). HH, NH, and IH groups demonstrated better rotarod performance in comparison with control animals (by 3, 2, and 2.5 times, respectively). No significant differences in motor coordination disturbances were observed between the treatment groups.

Therefore, our experiments show that all types of exposures have significant neuroprotective effects during experimental brain ischemia. The key mechanisms of neuroprotective effects of HH exposure are probably related to modulation of cerebrovascular system reactivity and increase in cerebral perfusion [6], activation of the antioxidant system [13], activation of ATP-dependent K^+-channels on the plasma membrane and inner mitochondrial membranes [7], activation of anti-apoptotic mechanisms via inhibition of caspase-3 [15], inhibition of MPT-pore (mitochondrial permeability pore) [4], activation of synthesis of protective intracellular proteins (HSP) [11], activation of DNA reparation, and inhibition of inflammatory processes [8], and genome reprogramming [10].

Published data primarily describe the efficiency of hypoxic and ischemic preconditioning. Neuroprotective effects of exposure to IH were studied during preconditioning [15]. Our study showed the formation of delayed adaptation to HH, which is associated with a significant increase in brain tolerance to ischemia [1]. Comparative studies of respiratory trainings mostly revealed the efficiency of various hypoxic exposures [2]. In our study the comparison of hypoxic, hypercapnic, and hypercapnic-hypoxic exposures shows that exposure to HH has the most pronounced neuroprotective effect. The decrease in neurological deficit after experimental brain ischemia due to individual exposures to hypercapnia and hypoxia suggests that hypercapnia and hypoxia potentiate the neuroprotective effects of each other.

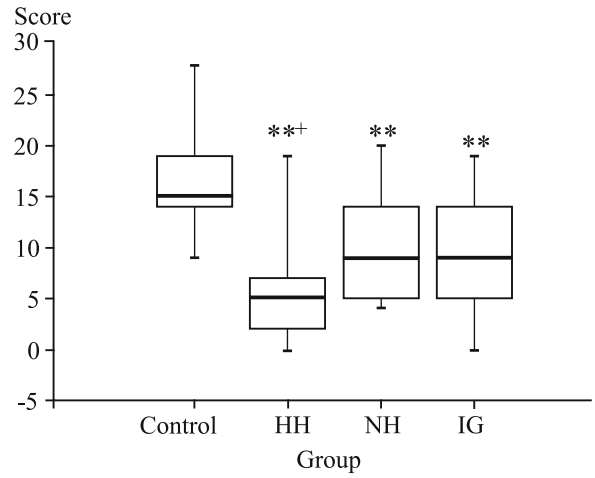

Fig. 1. Neurological deficit according to Katz scale. $^{**}p<0.01$ in comparison with the control; $^{+}p<0.05$ in comparison with exposure to NH.

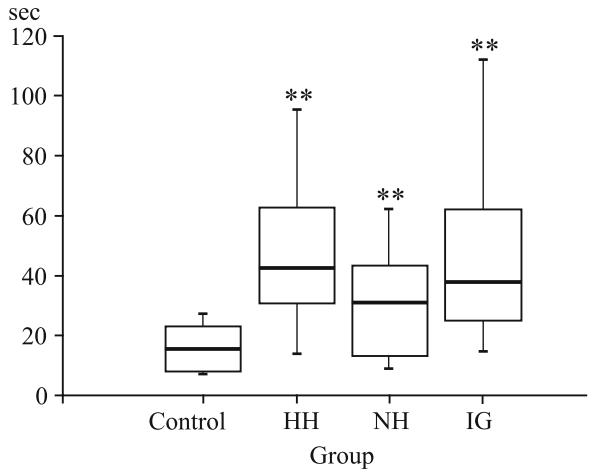

Fig. 2. Time spent on a rotating rod. $^{**}p<0.01$ in comparison with the control.

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