DOI 10.1007/s10517-015-2955-0

Proliferative and Synthetic Activity of Nerve Cells after Combined or Individual Exposure to Hypoxia and Hypercapnia

P. P. Tregub, V. P. Kulikov, N. Yu. Rucheikin, E. V. Belova, and Yu. G. Motin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 159, No. 3, pp. 311-313, March, 2015
Original article submitted August 14, 2014

We compared synthetic and proliferative activity of brain cells in rats exposed hypoxia, hypercapnia, or both prior to experimental focal stroke. The mean number of nucleolus organizer regions in penumbra neurons did not change after normobaric hypoxia, but increased after permissive hypercapnia or hypercapnic hypoxia. These data attest to activation of proliferative and synthetic functions in nerve cells, which plays an important role in the neuroprotective mechanisms under conditions of combined exposure to hypoxia and hypercapnia.

Key Words: *hypoxia; hypercapnia; stroke; brain;* nucleolus *organizer region*

The neuroprotective effect of hypoxic training sessions decreases the intensity of nerve cell death under conditions of brain anoxia or ischemia [8,10,14]. Recently, new data were reported on the neuroprotective properties of permissive hypercapnia [11,13]. For instance, permissive hypercapnia exerted a pronounced therapeutic effect in experimental brain ischemia/reperfusion [15].

We have previously demonstrated that combined exposure to hypoxia and hypercapnia more effectively improved brain tolerance to ischemia/hypoxia than individual exposures to these factors [5,12]. We also showed that the important role in potentiation of the hypoxia-induced neuroprotective effect under conditions of hypercapnia is played by VEGF, HSP-70, and neurotrophic protein S-100B [3].

However, the mechanisms of the neuroprotective effects of combined exposure to hypercapnia and hypoxia are little studied. One of them can be upregulation of the synthetic and proliferative activity of penumbra neurons, which had been previously shown for normobaric hypoxia (NH) [1,2].

This work was designed to compare synthetic and proliferative activities of cells in rat brain after exposure of the animals to individual and combined action of hypoxia and hypercapnia prior to experimental focal stroke.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats (n=60) weighing 284.5±46.0 g; the animals were randomized into four equal groups. The respiratory exposures were performed in a sealed chamber [5,12]. During training sessions (20 min per day for 15 days), gas mixtures were different for different rat groups. In the control group, the partial pressures of oxygen and carbon dioxide were PO_2=150 mm Hg and PCO_2=1 mm Hg (no hypoxia and/or no hypercapnia). The corresponding values in NH group were PO_2=90 mm Hg, PCO_2= 1 mm Hg. In the groups of permissive hypercapnia (PH) and hypercapnic hypoxia (HH), the corresponding values were PO_2=150 mm Hg, PCO_2=50 mm Hg and PO_2=90 mm Hg, PCO_2=50 mm Hg. The air pressure was equalized with the atmospheric pressure by adding N_2.

On the next day after completion of respiratory conditioning, all rats were narcotized and experimental focal ischemic damage to the brain was modeled by injection of 3% rose bengal (30 mg/kg) into the femoral vein followed by 10-min illumination of the scalped parietal area of the skull with a 20-mW laser

Altai State Medical University, Ministry of Health of the Russian Federation, Barnaul, Russia. ***Address for correspondence:*** pfiza_asmu@mail.ru. P. P. Tregub

(532 nm) [6]. In 72 h after cerebral stroke, the rats were decapitated and their brains were isolated.

In thin (10 μM) cerebral sections, the nucleolus organizer regions (NORs) were impregnated with silver to reveal proteins with affinity to Ag in the region of nucleolar activity. To this end, AgNORs kit (BioOptica) was used [7]. The number NORs in the penumbra neurons were counted in at least 5 visual fields at ×400.

The data were analyzed statistically using Mann–Whitney nonparametric *U* test at $p<0.05$.

RESULTS

In all rats, typical photochemical stroke involving all layers of the sensorimotor cortex were found. In the stroke area, the sections cut in the region of ischemic damage demonstrated fibrous alterations and microvascular thrombosis typical of photochemically induced thrombosis model [6]. The morphological picture was similar in all groups, while the size of stroke area and numbers of NORs in cell nuclei varied (Fig. 1).

In NH rats, the mean number of NORs in penumbra neurons did not differ from the control values, but significantly increased in PH and HH rats, which attested to active proliferation of brain cells and up-regulation of synthetic activity (Fig. 2).

The increase in the mean number of NORs in nerve cells indicates enhancement of ribosomal RNA synthesis at nucleolus-forming chromosomal regions [7], which up-regulates the synthesis of neurotransmitters in neurons and structural proteins and neurotrophic factors in glial cells [4]. This process plays an important role in the mechanism of neuroprotection under conditions of ischemic damage to the nerve tissue, because activation of glial cell proliferation moderates the deleterious effects of trophic abnormalities, while elevated expression of neurotransmitters is of great reparative importance for neurons entering the paranecrotic and necrobiotic phase [9].

It has been previously shown that NH up-regulates the synthetic and proliferative activity of cells [1,2]. However, that study used more severe hypoxia (57-71 mm Hg) and far longer exposure (1-6 h) in comparison with our protocol. The milder respiratory stimulation in NH group can be responsible for the lack of positive effect on cellular activity in our study. However, we employed milder hypoxia, because in combination with PH, it demonstrated a high efficacy in preventing the experimental ischemia in rats [5].

The present study demonstrated the dominant effect of hypercapnia on up-regulation of the synthetic and proliferative activity in the nerve cells after the focal cerebral ischemic lesions. It is indicated by the fact that the significant differences in comparison with the control rats were observed only in PH and HH groups, while there were such differences between PH and HH rats.

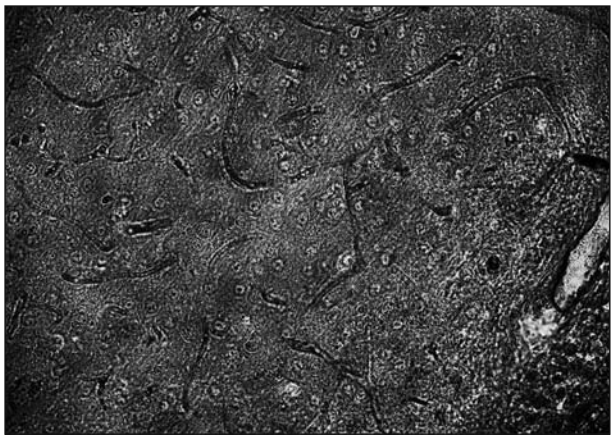

Fig. 1. Nerve cells with NORs on a thin section cut across the penumbra in the rat brain. Silver staining, ×400.

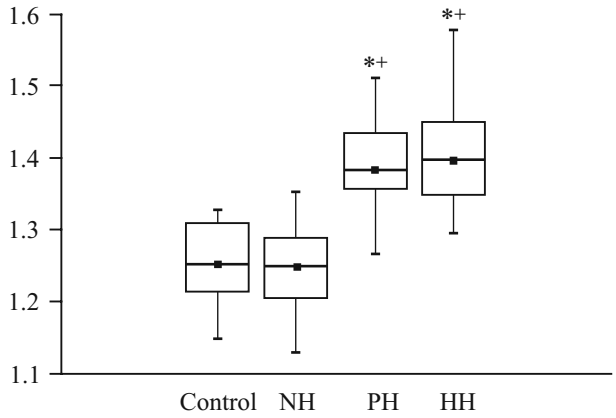

Fig. 2. Boxplots of NORs scores in the nerve cells of the penumbra. The boxes show upper quartile, median and lower quartile from top to bottom, respectively. The whiskers indicate the 10th and 90th percentile. $p<0.001$ in comparison with *control and +NH groups.

Thus, the important role in neuroprotection observed during the combined action of hypoxia and hypercapnia is played by up-regulation of the synthetic and proliferative function in the nerve cells, and the major part in this action is given to the hypercapnic component.

REFERENCES

1. O. A. Antonova, S. A. Loktionova, N. V. Golubeva, *et al*., *Bull. Exp. Biol. Med*., **144**, No. 4, 504-506 (2007).
2. L. V. Antonova, V. G. Matveeva, M. N. Chernova, *et al*., *Tsitologiya*, **56**, No. 1, 67-76 (2014).
3. A. G. Bespalov, P. P. Tregub, V. P. Kulikov, *et al*., *Patol. Fiziol. Eksp. Ter.*, No. 2, 24-27 (2014).
4. V. A. Zuev, I. V. Viktorov, and N. P. Borodina, *Bull. Exp. Biol. Med*., **129**, No. 3, 271-274 (2000).
5. P. P. Tregub, V. P. Kulikov, A. G. Bespalov, *et al*., *Bull. Exp. Biol. Med*., **155**, No. 3, 327-329 (2013).

6. F. Chen, Y. Suzuki, N. Nagai, *et al*., *MAGMA*, **17**, Nos. 3-6, 103-108 (2004).
7. N. Husain, M. Bagchi, M. Husain, and B. Tiwari, *Indian J. Pathol. Microbiol*., **40**, No. 4, 503-509 (1997).
8. J. Neckár, E. Papousek, O. Nováková, *et al*., *Basic Res. Cardiol*., **97**, No. 2, 161-167 (2002).
9. T. P. Obrenovitch, *Physiol. Rev*., **88**, No. 1, 211-247 (2008).
10. E. Rybnikova, T. Gluschenko, E. Tulkova, *et al*., *J. Neurochem*., **106**, No. 3, 1450-1458 (2008).
11. T. Tao, Y. Liu, J. Zhang, *et al*., *Brain Res*., **1533**, 52-62 (2013).
12. P. Tregub, V. Kulikov, and A. Bespalov, *Pathophysiology*, **20**, No. 3, 165-170 (2013).
13. R.C. Vannucci, J. Towfighi, D. F. Heitjan, and R. M. Brucklacher, *Pediatrics*, **95**, No. 6, 868-874 (1995).
14. L. Zhan, T. Wang, W. Li, *et al*., *J. Neurochem.*, **114**, No. 3, 897-908 (2010).
15. Q. Zhou, B. Cao, L. Niu, *et al*., *Anesthesiology*, **112**, No. 2, 288-297 (2010).