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HYPOBARIC HYPOXIA CAUSING HISTOMORPHOLOGICAL ALTERATIONS IN DIFFERENT PARTS OF THE RAT'S BRAIN

R. A. SHUSHANYAN [*], A. V. GRIGORYAN [**], A. F. KARAPETYAN [***]

Chair of Human and Animal Physiology, YSU, Armenia

Hypobaric hypoxia causes degenerative changes in different parts of the brain, which leads to the formation of cerebral edema. This study aimed to investigate the morphohistological changes in the brain of rats exposed to hypobaric hypoxia. In particular, the hippocampus, cerebral cortex, and striatum were studied. Animals were exposed to hypobaric hypoxia for 10 days. Histomorphological examination showed degenerative changes in the hippocampus, dentate gyrus, and striatum, accompanied by pyknosis of neuronal nuclei, cytoplasmic vacuolization, and neuronal shrinkage, while the cortex of the large hemispheres remained almost intact. Histological studies showed that different parts of the brain respond to hypoxic conditions with different manifestations.

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Introduction. High-altitude environments can cause altitude sickness, and with the increasing number of people working, traveling, and living in high-altitude environments, the incidence of high-altitude illness is growing [1]. Acute hypobaric hypoxia is one of the main causes of acute mountain sickness (AMS), and high-altitude cerebral edema (HACE) [2]. HACE is uncommon, but sometimes fatal and constitutes about 0.5–1.0% at 4000–5000 *m* and 3.4% of those who suffer from AMS. The lowest altitude at which a case of HACE was reported was 2100 *m* [3]. Both HACE and AMS are typically associated with unacclimatized individuals, who rapidly ascend above 2500 *m* [4]. Current high-altitude human research demonstrates increased cerebral blood flow after a single hypoxic hypobaric exposure to 7620 *m* for occupational training, which persists at 72 *h* causing mild cerebral edema [5, 6].

Although all these conditions may contribute to the development and progression of chronic and acute high-altitude illnesses, the reduced partial pressure of oxygen (hypobaric hypoxia) is considered [7].

Generally, HACE is characterized by increased intracranial pressure secondary to cytotoxic and vasogenic edema. As such, the pathophysiology of HACE is poorly understood [8].

[*] E-mail: ruzanna.shushanyan@ysu.am
[**] E-mail: annakarapetyan@ysu.am
[***] E-mail: annagrigysu@gmail.com

Repeated episodes of hypoxia give result in periods of intermittent hypoxia characterized by free radical production, and alterations to neurophysiological and cognitive functions that can persist even for a while after they return to sea level [9]. Short-term memory decreased following exposure of human volunteers to acute, mild, or moderate hypoxia for 1 *h* at 4400 *m*, and these effects were exacerbated at increasing altitudes causing learning, and memory impairments [10], cardiovascular overload, O_2 deficit, neuroendocrine alterations, and energy deficit [11].

Neurons are inherently susceptible to oxidative damage, because of high respiratory turnover, dependency on oxidative phosphorylation reactions, high concentrations of catalytic iron, and low levels of antioxidant defense enzymes [12, 13]. Free radicals (oxidative toxins) have been implicated in the destruction of cells via lipid peroxidative damage to cell membranes [14]. The extent of the injury is usually expressed as the percentage of dead neurons of the total neurons in the defined regions examined [15].

It has been known that pathologic insults such as ischemia, hypoglycemia, oxidative stress, and anoxia, can cause structural damage to the hippocampus [16], including the dentate gyrus (DG) region [17]. Histologic studies have shown that the hippocampus is especially sensitive to hypoxic damage. Moreover, pyramidal cells, mainly in the CAl region of the hippocampus, are particularly vulnerable to ischemically induced damage [18].

The main purpose of this study was to investigate the histomorphological changes in the rat's brain under hypobaric hypoxia (henceforth HH) to reveal the possible progress of cerebral edema caused by high altitude in various parts of the brain. Consequently, the hippocampus, dental gyrus, cortex, and striatum were examined in the frames of this study along with some behavioral patterns.

Materials and Methods.

Experimental Design of Hypobaric Hypoxia Exposure. 24 *Wistar* rats weighing 150–200 *g* were used in this study. The animals were randomly divided into control (n=6) and HH groups (n=18).

For acclimatization purposes, all animals were kept in constant 12 h light/dark cycled special rooms, where the temperature was maintained at 22–25°C, and the humidity was 45–55%. Animals were fed with a standard *pellet* diet and tap water *ab libitum*. The rats from the control group were kept in normoxic conditions. All experiments with animals were carried out by the rules of the National Center for Bioethics of Armenia and the directive 2010 (2010/63/EU) [19] for the care of laboratory animals. The animals of the experimental group were exposed to 10 days of HH exposure for 30 *min* each day. Rats were acclimatized in the hypobaric chambers in room air for 30 *min* before experiments. The desired altitude was selected according to the literature [20–22] with minor modifications. The 6000 *m* altitude was received (approximately 20 000 feet, 9.7% O_2) at a velocity of 300 *m*/*min*.

Histomorphological Examination and Assessment of the Brain Weight. Histomorphological examination and assessment of the brain weight. On the last day of the experiment, the animals were removed from the chamber into normobaric normoxic conditions and were euthanized with chloroform. The samples of the brain were removed from the skull without reperfusion and fixed in 10% formalin buffer.

Brain weights were recorded before and after the experiments and assessed with an electronic balance. The brain samples were fixed in formalin for about 24–48 *h* and were exposed to dehydration with different solutions of ethanol and xylol according to protocols ("Sigma-Aldrich", hematoxylin stains, Procedure No. GHS) [23]. About 5 μm thick serial sections were processed after embedding the samples. Hematoxylin-eosin staining was used for dyeing the histological sections. Samples were viewed with a trinocular microscope, (BM-180/SP model) and the pictures were recorded with Scopelmage 9.0.exe software.

Statistical Analysis. All experimental data are presented as mean ± standard deviation, using MS. Excel. The Student's *t*-test was used to compare the differences between the two groups, and $p<0.05$ was considered significant.

Results and Discussion.

Behavioral Observations. Although we did not use any specific behavioral tests, however, some behavioral changes were observed during the experimental period. Before the hypobaric hypoxia exposure, the animals had normal behavior. In parallel with raising the altitude during HH, the behavioral alterations became obvious. Mainly, the increased respiratory rate with shortness of breath, hypokinesia, dizziness, lethargy, and restlessness of the muscles was indicated among almost all animals. Some animals even maintained their body twitched after the exposure. The normal behavior was gradually recovered only after the removal of rats from the decompression chamber for 1 *h*. Such disturbances in the rat's behavior were aggravated especially during the last days of the experiments, which indicated the stress response of the animals toward hypobaric hypoxic conditions as a physiological stressor.

Assessment of Brain Weights. Because changes in environmental oxygen availability are encountered in normal physiology, e.g., by changes in altitude of habitation, these adaptations are part of the normal acclimatization mechanisms within the brain and thus reveal useful insight into normal and pathological brain function. Brain tissue oxygen tension falls almost immediately when the partial pressure of inspired oxygen is reduced even moderately. Although the systemic mechanisms elicited by continuous hypoxic exposure differ by species, the rat responses appear to be very similar to those of the humans adapting to high altitudes. As in humans, rats exposed to long-term hypoxia fail to gain weight [24].

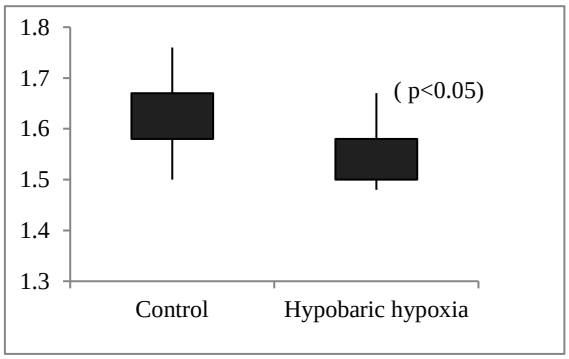

Fig. 1. Brain weight changes after hypobaric hypoxia exposure.

After HH exposure, the animals indicated a loss in brain weight (p=0.03), which is shown in Fig. 1, therefore, affirms the degenerative changes of neuronal cells that coincided with brain weight decline.

Histomorphological Observations. A major factor for neuronal cell death in inflammation is oxidative stress. Oxidative stress is characterized by a relative increase of reactive oxygen species (ROS) causing DNA damage and protein modification and eventually leading to cell apoptosis [25]. After 10 days of exposure to hypobaric hypoxia, we observed histomorphological changes in the hippocampus, mainly in the CA1 region, where the degenerated neurons with pyknotic nuclei and vacuolation were indicated in comparison to the control group. The shrunken pyramidal neurons (arrows) of CA1, CA2, and CA3 regions are presented in Fig. 2. From the results it can be considered that the karyopyknosis in neuronal cells with shrunken cytoplasm are distributed in three regions of the hippocampus, moreover, the vacuolation (edema) had been indicated particularly in the CA1 region that was accompanied by eosinophilia of the neuropil (asterisk).

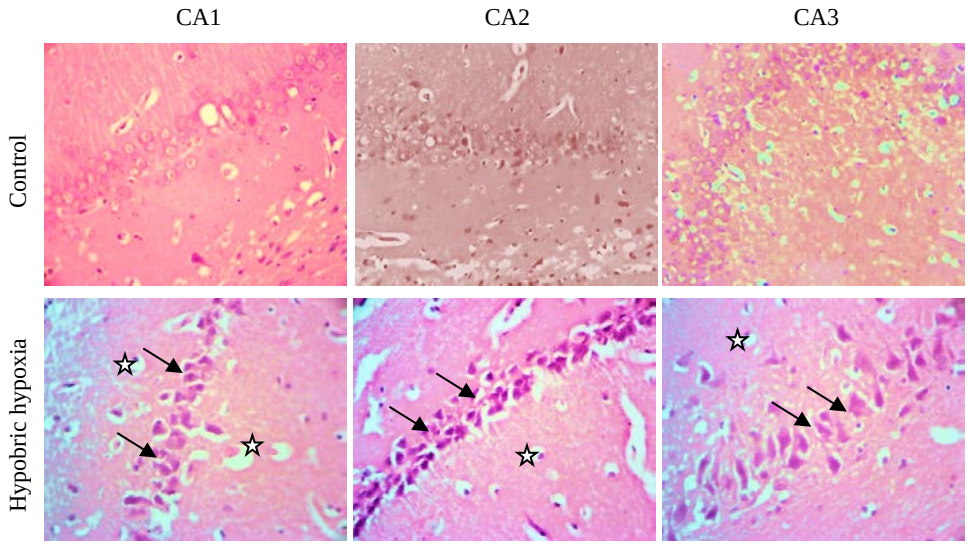

Fig. 2. Histom orphological changes in rat's hippocampus after hypobaric hypoxia exposure (H&E staining, magnification = ×400, scale bar = 50 *um*).

The histological alterations of the cerebral cortex, striatum, and DG of the hippocampus were shown in Fig. 3. The DG considers the most vulnerable area of the brain, where neurogenesis mostly occurs.

Additionally, GABAergic neurons (parvalbumin (PV), calretinin, somatostatin, and neuropeptide Y-immunoreactive) in the polymorphic layer of the DG are among the most vulnerable populations of hippocampal neuronal cells [26]. In our study, we observed intense vacuolation of the cytoplasm and a decline of the neurons in DG. The severe deteriorative changes were indicated in the striatum, mostly in the thalamus with necrotic foci and white matter vacuolation (Fig. 3, arrow). The cortex was accompanied by relatively mild changes, which are presented in Fig. 3.

The morphohistological assessment reveals eosinophilia of the neuropil and pyknosis of the Purkinje cells in the cerebral cortex.

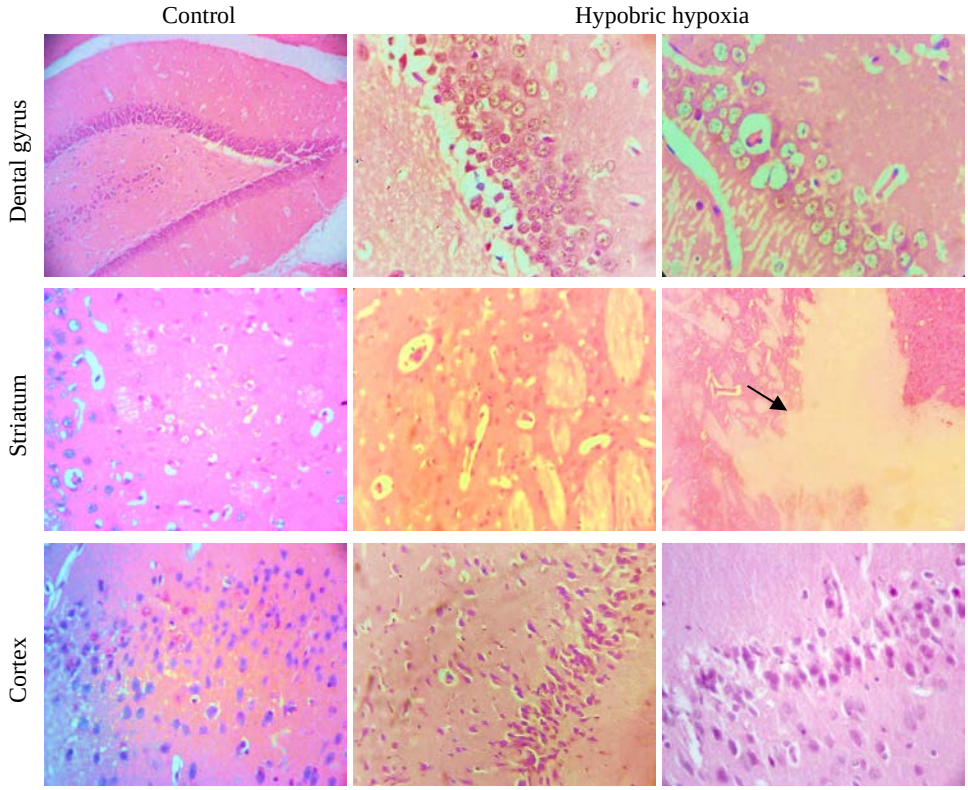

Fig. 3. Histomorphological changes in rats DG (magnification= ×100) of the hippocampus, striatum (thalamus) and and cerebral cortex after hypobaric hypoxia exposure (H&E staining, magnification = ×100, ×400, scale bar = 50 *um*).

The obtained results indicated that the hippocampus and DG were mostly affected by the hypobaric hypoxic exposure accompanied by swelling of the pyramidal cells, pyknosis with hyperchromatic nuclei, and vacuolar degeneration. The cells distributed in the striatum underwent retrograde changes and white matter vacuolation with necrosis and intense apoptosis. While, in the cerebral cortex the Purkinje cells were maintained almost intact, and only some pyknotic cells were observed. However, the hypobaric hypoxia-induced the reduction of the cells especially in the CA1 region of the hippocampus, whereas, the other regions and the cerebral cortex were more resistant and, therefore, retained normal histomorphological architecture after HH exposure. Such changes demonstrate the progress of hypoxia-ischemia that causes oxidative stress in the brain inducing the formation of ROS. The oxidative stress provokes lipid peroxidation, DNA damage, and cell apoptosis, therefore, weakening the antioxidative enzymes in the brain. HH exposure causes cognitive function impairment including learning and memory function, glutamate excito-toxicity, high influx of calcium-mediated apoptosis cascade [27], accumulation of intracellular calcium, and induction of cell membrane injury with neuronal death

[28]. In addition to oxidative stress, apoptosis is an important consequence of reperfusion, thus hypoxia is associated with the production of ROS under high oxygen concentrations, which participate in the induction of apoptosis [29].

Clarifying the molecular mechanism underlying the pathophysiology of neuronal degeneration and death is very important in the quest for effective therapy not only for hypoxia/ischemia conditions but also for high-altitude cerebral edema. Although more profound studies should be performed [21].

Currently, the study of pathophysiological responses at altitude is a model to investigate the mechanisms of response to hypoxia in any condition, also in critical illnesses [30].

Conclusion. Our study reveals the histomorphological changes in the different parts of the brain and shows that the CA1 region of the hippocampus, dental gyrus, and striatum were mostly affected by the hypobaric hypoxic exposure accompanied by various pathological changes such as pyknosis, eosinophilia of the cytoplasm, shrinkage, and swelling of the pyramidal cells and vacuolation of the neuropil (edema). While the Purkinje cells of the cerebral cortex showed more resistance and maintained mostly intact. The examination indicates the neurodegenerative changes of the brain after short-term HH exposure, which juxtaposed with a reduction of the brain weight and behavioral modifications. The obtained results show the progression of cerebral edema in the brain after hypobaric hypoxia and, therefore, support our understanding of the adaptive changes in the brain under hypoxic conditions, which are manifested by various pathohistological modifications in the particular areas of the rat's brain.

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Ռ. Ա. ՇՈՒՇԱՆՅԱՆ, Ա. Վ. ԳՐԻԳՈՐՅԱՆ, Ա. Ֆ. ԿԱՐԱՊԵՏՅԱՆ

ԱՌՆԵՏԻ ՈՒՂԵՂԻ ՏԱՐԲԵՐ ԲԱԺԻՆՆԵՐՈՒՄ ՀԻՍՏՈՄՈՐՖՈԼՈԳԻԱԿԱՆ ՓՈՓՈԽՈՒԹՅՈՒՆՆԵՐ ԱՌԱՋԱՑՆՈՂ ՀԻՊՈԲԱՐԻԿ ՀԻՊՈՔՍԻԱՆ

Հիպոբարիկ հիպոքսիան գլխուղեղի տարբեր բաժիններում առաջացնում է հետադիմական փոփոխություններ, ինչը հանգեցնում է գլխուղեղի այտույցի ձևավորմանը: Այս հետազոտության նպատակն է եղել՝ ուսումնասիրել առնետների գլխղուղեղի մորֆոհյուսվածաբանական փոփոխությունները հիպոբարիկ հիպօքսիայի ազդեցության պայմաններում: Մասնավորապես ուսումնասիրվել են՝ հիպոկամպը, գլխուղեղի կեղևը և զոլավոր մարմինը: Փորձակենդանիները ենթարկվել են հիպոբարիկ հիպօքսիայի՝ 10 օր շարունակ: Հիստոմորֆոլոգիական ուսումնասիրությամբ դիտվել են հետադիմական փոփոխություններ՝ հիպոկամպում, ատամնավոր գալարում և զոլավոր մարմնում, որոնք ուղեկցվել են նեյրոնների կորիզների պիկնոզով, ցիտոպլազմայի վակուոլիզացիայով և նեյրոնների կնճռատմամբ, մինչդեռ մեծ կիսագնդերի կեղևը գրեթե մնացել է ինտակտ: Հյուսվածաբանական ուսումնասիրությունները ցույց տվեցին, որ գլխուղեղի տարբեր բաժիններ հիպոքսիկ պայմաններին արձագանքում են տարբեր դրսևորումներով:

Р. А. ШУШАНЯН, А. В. ГРИГОРЯН, А. Ф. КАРАПЕТЯН

ГИПОБАРИЧЕСКАЯ ГИПОКСИЯ, ВЫЗЫВАЮЩАЯ ГИСТОМОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ В РАЗЛИЧНЫХ ОТДЕЛАХ ГОЛОВНОГО МОЗГА КРЫС

Гипобарическая гипоксия вызывает дегенеративные изменения в различных отделах головного мозга, что приводит к формированию отека головного мозга. Целью настоящего исследования явилось изучение морфогистологических изменений в головном мозге крыс, подвергшихся гипобарической гипоксии. В частности исследовались гиппокамп, кора головного мозга и полосатое тело. Животных подвергали гипобарической гипоксии в течение 10 дней. При гистоморфологическом исследовании выявлены дегенеративные изменения в гиппокампе, зубчатой извилине и стриатуме, сопровождающиеся пикнозом ядер нейронов, цитоплазматической вакуолизацией и сморщиванием нейронов, при этом кора больших полушарий оставалась практически интактной. Гистологические исследования показали, что разные отделы головного мозга по-разному реагируют на гипоксические состояния.