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Research report

Neurochemical alterations in frontal cortex of the rat after one week of hypobaric hypoxia

Olena V. Bogdanova[a,*], Osama Abdullah[b], Shami Kanekar[a,c], Volodymyr B. Bogdanov[d], Andrew P. Prescot[a,e], Perry F. Renshaw[a,c,f]

[a] Brain Institute, University of Utah, 383 Colorow Drive, Salt Lake City 84108, UT USA
[b] Department of Bioengineering, University of Utah, 36 S. Wasatch Drive, Salt Lake City 84112, UT USA
[c] Psychiatry Department, University of Utah, 383 Colorow Drive, Salt Lake City 84108, UT USA
[d] INRA, Nutrition et Neurobiologie Intégrée and University Bordeaux, Nutrition et Neurobiologie Intégrée, UMR 1286, 146 rue Léo-Saignat, Bordeaux Cedex 33076, France
[e] Department of Radiology, University of Utah, 30 North 1900 East, Salt Lake City 84132, UT USA
[f] VISN19 MIRECC Salt Lake City UAMC, 500 Foothill Drive, Salt Lake City 84148, UT USA

HIGHLIGHTS

- Increased depression and suicide rates are associated with life at higher altitudes.
- Rats were housed for one week at 10,000 ft of simulated altitude.
- Animals were tested with the forced swim test and magnetic resonance spectroscopy.
- Hypoxia-treated rats had augmented depression-like behavior in the forced swim test.
- Neurochemical alterations after hypobaric hypoxia were found in frontal cortex voxel.

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ABSTRACT

Residing at high altitude may lead to reduced blood oxygen saturation in the brain and altered metabolism in frontal cortical brain areas, probably due to chronic hypobaric hypoxia. These changes may underlie the increased rates of depression and suicidal behavior that have been associated with life at higher altitudes. To test the hypothesis that hypobaric hypoxia is responsible for development of mood disorders due to alterations in neurochemistry, we assessed depression-like behavior in parallel to levels of brain metabolites in rats housed at simulated altitude.

32 female Sprague Dawley rats were housed either in a hypobaric hypoxia chamber at 10,000 ft of simulated altitude for 1 week or at local conditions (4500 ft of elevation in Salt Lake City, Utah). Depression-like behavior was assessed using the forced swim test (FST) and levels of neurometabolites were estimated by *in vivo* proton magnetic resonance spectroscopy in the frontal cortex, the striatum and the hippocampus at baseline and after a week of exposure to hypobaric hypoxia.

After hypoxia exposure the animals demonstrated increased immobility behavior and shortened latency to immobility in the FST. Elevated ratios of myo-inositol, glutamate, and the sum of myo-inositol and glycine to total creatine were observed in the frontal cortex of hypoxia treated rats. A decrease in the ratio of alanine to total creatine was also noted. This study shows that hypoxia induced alterations in frontal lobe brain metabolites, aggravated depression-like behavior and might be a factor in increased rates of psychiatric disorders observed in populations living at high altitudes.

1. Introduction

With an increasing world population, regions of high elevation have become more inhabited. In 1998, more than 140 million people lived at an altitude above 8000 ft [1], and millions more visit regions of high elevation every year. Living at high altitude is accompanied by exposure to low partial pressures of oxygen, potentially leading to oxygen deficits. Exposure to high altitudes

* Corresponding author at: Brain Institute, University of Utah 383 Colorow Drive, Salt Lake City 84108, UT, USA. Tel.: +1 801 587 1477; fax: +1 801 585 5375.
E-mail address: basilevsis@yahoo.com (O.V. Bogdanova).

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has been found to detrimentally affect cardiovascular, pulmonary and nervous system function [2]. The symptoms of cerebral dysfunction associated with high altitude involve decreased physical and mental performance, including increased fatigue and impaired sleep [2]. High altitude climbers exhibit signs of focal brain damage (leukoaraiosis and/or mild cortical atrophy) along with neuropsychological deficits [3]. High altitude residents have structural modifications of the brain, including regional decrease of the grey matter and changes in the white matter [4]. Adolescents living at high altitude exhibit altered delta and beta frequencies in resting state EEG in parallel to reduced blood oxygen saturation [5]. Chronic exposure to hypobaric hypoxia in people living at high altitudes has been associated with lower resting metabolic states in the brain, particularly in frontal cortical areas [6] and altered brain metabolism in the anterior cingulate cortex [7] which may contribute to adverse mental health outcomes, including depression and suicidal behavior [8–11] as well as with increased rates of illicit drug use [12]. Despite decades of research, the impact of altitude as an environmental factor that underlies mental disorders remains incompletely understood.

Major depressive disorder (MDD) is a global medical problem due to its high prevalence and incomplete responsiveness to treatment [13]. MDD affects almost 10% of the population of the United States and national mental health surveys indicate that rates of depression are highest in the intermountain west [14]. Changes in neurometabolite concentrations in MDD patients occur within brain regions which are involved in the processing and communication of emotions, which can be monitored by proton magnetic resonance spectroscopy, 1 H MRS
[15].

In vivo 1H MRS is a unique tool providing information about metabolic changes in pathological conditions affecting the brain. Some of the neurometabolites that 1H MRS can quantify are: *N*-acetylaspartate, NAA (a major component of neuronal mitochondria, that decreases with any neurodegenerative condition); glutamate/glutamine, Glu + Gln (the major excitatory neurotransmitter that also plays a key role in synapse formation, dendrite pruning, cell migration, differentiation and death); choline/phosphocholine, Cho + PCho (a metabolic marker of membrane density and integrity, elevated in increased cellular growth/turnover); creatine/phosphocreatine, Cr + PCr (regulates energy homeostasis in the cell); lactate, Lac (increased during anaerobic glycolysis, in ischemia and hypoxic conditions), myo-inositol, Ins (regulates neuronal osmolarity and membrane biosynthesis, also is a marker of glial proliferation, that increases with inflammatory processes), γ-aminobutyric acid, GABA (inhibitory neurotransmitter), taurine, Tau (a non-proteinogenic amino acid contributing to neurotransmission and neuromodulation in the CNS, supporting detoxification, antioxidation, and osmotic regulation) and others. A wide diversity of 1H MRS methods have been applied and brain regions studied (prefrontal, parieto-occipital, cingulate cortex, hippocampus, amygdala and others), resulting in significant changes in metabolite levels assessed before and after antidepressant treatment, as reviewed by Caverzasi and co-workers [16].

The majority of hypoxia MRS studies have evaluated the impact of acute, severe hypoxia/anoxia on the levels of brain metabolites [17]. Some studies have examined the effects of hypoxia by analyzing the neurochemistry of the brain in high altitude climbers [18]. It should be noted that effect of hypoxia depends on the length of exposure (minutes, hours or days) and the type of exposure (continuous or intermittent), as well as on the intensity of exposure. For example, exposure to severe hypoxia (more than 18,000 ft of simulated altitude) resulted in prominent macroorganismic changes e.g., lost of appetite, decline in body weight [19], and symptoms of severe disturbances in cerebral function [20]. However, mild hypoxia exposure (up to 11,000 ft) does not appear to influence body weight but may have other detrimental consequences [21–23].

In this study we aimed to assess the neurobiological basis of increased MDD at high altitude by simulation of hypobaric hypoxia using a rat model. Rodents are widely used in translational research studies of depression [24]. Rodent models can be used to simulate several symptoms of MDD and to show resolution of these symptoms with antidepressant treatment. One of the rodent behavioral tests in depression research, the forced swim test (FST), was developed in 1978 by Porsolt and co-workers [25] as a model for predicting the clinical efficacy of antidepressant drugs. The FST is also one of the most commonly used tests to assess depression-like behavior in rodents. The basic FST involves two sessions with animals placed in a cylinder containing 25 °C water, from which they cannot escape. The first session is a 15 min pretest that is followed 24 h later by a 5 min test session. The pretest is a stressor which is thought to induce a state of behavioral despair [26] or passive stress coping strategy [27], since the animals become more immobile as the test session progresses. The typical posture of immobility is characterized by floating in the water with only movements necessary to keep the nose above the surface. The immobility time and also the latency to the initial immobility period [28] are the primary dependent measures [29].

Objective To test the hypothesis that hypobaric hypoxia alters the neurochemistry of the brain in parallel to behavioral changes, we aimed to assess the behavior of rats in the FST and to estimate changes in neurometabolites in the brain (within frontal lobe, striatum and hippocampus voxels) by *in vivo* proton MRS after one week of continuous exposure to mild hypobaric hypoxia at 10,000 ft of simulated altitude. In our preliminary studies we found that FST behaviors of female rats are more susceptible to hypobaric hypoxia treatment than in males, therefore we used female animals in this study.

2. Materials and methods

2.1. Animals and exposure to hypobaric hypoxia

Thirty-two female Sprague Dawley rats (Charles River, USA) (150–200 g body weight, $n = 8$–12 per group) were either housed in a hypobaric hypoxia chamber at 10,000 ft of simulated altitude (partial oxygen pressure 15%) for 1 week or were housed under local conditions (4500 ft of elevation in Salt Lake City, Utah). Body weight was measured before and after hypoxia treatment. Animals were housed separately in standard rodent cages with food and water in controlled room conditions. Separate sets of animals were used for the behavioral measurements and *in vivo* imaging to avoid the possible impact of the procedures on each other. All experimental procedures were performed according to University of Utah Institutional Animal Care and Use Committee guidelines.

2.2. The forced swim test

The behavior of 8 rats exposed to hypoxia and 12 control rats housed at local conditions for a week was assessed using the FST. The FST consists of placing the rat into a transparent tank containing 38 cm deep water at 25 °C temperature for 15 min during a pretest session and for 5 m during the test session, 24 h later according [30]. The pretest FST was scheduled after 6 days in the chamber for hypoxia treated rats or after 6 days at local conditions for the control group. After the pretest session, animals were removed from water, dried with paper towels and returned to their home cages at the appropriate altitude condition. The test session was performed 24 h later. Behavior in the test session of the FST was videotaped,