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Comparative proteome analysis reveals differential regulation of glycolytic and antioxidant enzymes in cortex and hippocampus exposed to short-term hypobaric hypoxia

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ABSTRACT

Hypoxia is one of the major stressors at high altitude. Exposure to hypobaric hypoxia induces several adverse consequences to the structural and functional integrity of brain. In an attempt to understand the proteome modulation, we used 2-DE coupled with MALDI-TOF/TOF for cortex and hippocampus exposed to short-term temporal (0, 3, 6, 12 and 24 h) hypobaric hypoxia. This enabled us in the identification of 88 and 73 hypoxia responsive proteins in cortex and hippocampus respectively. We further compared the proteomes of both the regions and identified 37 common proteins along with 49 and 32 specific proteins for cortex and hippocampus respectively. We observed significant up-regulation of glycolytic enzymes like Gapdh, Pgam1, Eno1 and malate-aspartate shuttle enzymes Mdh1 and Got1in cortex as compared to hippocampus deciphering efficient use of energy producing substrates. This was coupled with concomitant increase in expression of antioxidant enzymes like Sod1, Sod2 and Pebp1 in cortex to neutralize the hypoxia-induced reactive oxygen species (ROS) generation. Our comparative proteomics studies demonstrate that efficient use of energy generating pathways in conjugation with abundance of antioxidant enzymes makes cortex less vulnerable to hypoxia than hippocampus.

1. Introduction

Ascent to high altitude is associated with fall in the partial pressure of the inspired oxygen leading to reduced tissue oxygenation. This condition of hypobaric hypoxia exerts a spectrum of neuropsychological, pathophysiological changes and structural modifications in oxygen dependent brain [1,2]. The neurological clinical syndromes include acute mountain sickness, high altitude cerebral edema, neuroinflammation, brain injury, neuropsychological impairment, insomnia, dizziness, nausea, hypophagia, sleep disturbance, motor impairment, memory impairment and cognitive dysfunctions [2]. Several compensatory mechanisms like hyperventilation, tachycardia, erythropoietin-induced polycythaemia and increased cerebral blood flow can partially maintain cerebral oxygen delivery at high altitudes [1,2]. However, inadequacy of these oxygen compensatory mechanisms during hypobaric hypoxia results in adverse consequences on the structural and functional integrity of the brain [2,3]. Along with this environmental factor, several pathological conditions like pulmonary diseases, cardiac failure, orthologic hypotension, stroke and obstructive sleep apnea can also result in acute or chronic hypoxia and similar consequences [4].

Lack of oxygen promotes down-regulation of both ATP-producing and ATP-consuming processes also known as metabolic depression. Paucity of oxygen as terminal electron

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acceptor in electron transport chain surmises the hypoxic cells to undergo anaerobic glycolysis (Pasteur effect) which partially compensates for cellular energy demands. This increased glycolytic flux under transcriptional control of hypoxia-inducible factor 1 (HIF-1) and hypoxia-inducible factor 2 (HIF-2) during passively decreasing mitochondrial respiration has been considered as one of the critical metabolic adaptation to hypoxia [5]. Recent studies have demonstrated that, HIF-1 also trans-activates pyruvate dehydrogenase kinase 1 (PDK1) which subsequently phosphorylates and inhibits pyruvate dehydrogenase (PDH) enzyme complex that converts pyruvate to acetyl-coenzyme A, thereby inhibiting pyruvate metabolism via the tricarboxylic acid (TCA) cycle [5,6]. This metabolic switch shunts glucose metabolites from the mitochondria to glycolysis to maintain cellular ATP production during hypoxia [5,6].

Increased ATP production may not be sufficient for hypoxic adaptation since hypoxia paradoxically causes oxidative stress from uncontrolled generation of mitochondrial reactive oxygen species (ROS) that may be critical for cell survival [5]. The ROS produced during aerobic energy production is neutralized by catalase, peroxiredoxins and superoxide dismutase. Under hypoxic conditions, leakage of electrons from respiratory chain results in an increased ROS which could further damage cellular macromolecules. The high rate of oxygen utilization, higher content of polyunsaturated fatty acids and transition metals like copper and iron along with a deficient antioxidant defence makes brain vulnerable to hypoxia-induced oxidative stress. However, hypoxia adaptive responses like metabolic reprogramming and ROS neutralization depends majorly on the intrinsic resistance of a certain brain region and even cell type [4]. Both biochemical and morphological studies have revealed that brain regions like hippocampus and cortex are highly vulnerable to hypoxic stress, each possessing specific dynamic response of protective and harmful effects [3,4,7,8]. Hence, understanding of molecular events elicited in hippocampus and cortex during short-term hypobaric hypoxia exposure can greatly help in the management of altitude-induced neuropathologies as well as several other pathological conditions.

The classical proteomic approach, two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS) is a powerful tool to capture dynamics of global proteomic changes by simultaneous resolution of large number of cellular proteins. The methodology is particularly advantageous for identification of stress induced proteins along with their post-translational modifications and to correlate altered protein abundance/modifications with physiological function(s) [9,10]. Several groups have investigated the proteomic changes in brain and brain regions during hypobaric hypoxia, normobaric hypoxia, intermittent hypoxia, and hyperoxia [4,11–15]. These studies have either focused on whole brain, or only hypoxia sensitive, and cortex regions [4,11–14]. Since the final outcome of high altitude exposure majorly depends on the duration of stay, it is important to decipher the temporal proteomic changes in brain regions like cortex and hippocampus for a better understanding of sequential cascade of molecular events regulated by hypoxia. Moreover, comparison of proteome profiles will provide a molecular insight into differential hypoxia response of both the aforementioned brain regions.

In this study, we conducted a comprehensive temporal (0, 3, 6, 12 and 24 h) proteomic analysis of rodent hippocampus and cortex regions exposed to hypobaric hypoxia. Using 2-DE and MALDI-TOF/TOF analysis we could be able to identify 161 differentially expressed proteins. To enhance the comprehensiveness of the analysis, we compared the protein expression of both hippocampus and cortex at each exposure time point. We have also used computational analysis to identify the biological processes and regulatory networks in both the studied tissues for each time point. Our temporal studies decipher that efficient utilization of energy producing metabolic pathways in cortex as compared to hippocampus. Moreover, cortex also possesses a better antioxidant defence system and lover levels of protein carbonyls making it less vulnerable to hypoxia than hippocampus.

2. Materials and Methods

2.1. *Experimental animals and hypobaric hypoxia exposure*

Male adult Sprague–Dawley rats (220±10 g) were maintained under 12 h light/dark cycles and were provided with food and water at *ad libitum*. All experimental protocols were approved by the Animal Use and Care Committee of Defence Institute of Physiology and Allied Sciences and were in accordance with the principles and guidelines of the American Physiological Society's "Guiding Principles in the Care and Use of Animals". To study the effect of acute hypobaric hypoxia exposure, 105 male Sprague–Dawley rats (220±10 g) were randomly divided into five groups (I to V; $n=21$). Group I served as normoxia group and groups II to V served as hypoxia groups where the rats were exposed to simulated hypobaric hypoxia for 3, 6, 12 and 24 h, respectively at 25,000 ft (7620 m, 282 mm Hg). The temperature and humidity were maintained at 28±2 °C and 60±5% respectively. The rate of ascent to altitude was maintained at the rate of 300 m/min and it took a period of 20–25 min to reach the desired altitude. The behaviour, food and water intake of the exposed animals were closely observed.

2.2. *Sample preparation*

After hypoxic exposure, both control (normoxic) and hypoxia exposed rats were sacrificed by cervical dislocation, the brain regions were collected and used fresh or stored in liquid nitrogen for further use. For protein isolation, 100 mg (wet weight) of hippocampus and cortex was homogenized on ice with a tissue tearer in 500 μl lysis buffer [(40 mM Tris (pH 7.5)], 8 M urea, 2.5 M thiourea, 3% 3-[(3- holamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 10 mM dithiothreitol (DTT), 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF)], and protease inhibitor cocktail (Sigma, St. Louis, USA). The homogenate was sonicated, vortexed, and centrifuged at 15,000 ×*g* at 4 °C for 45 min. The supernatant was collected and protein concentration was estimated using Bradford reagent (Sigma, USA).