Neurochemical Research 2008 ; 33 : 2335-2341

Proteomic analysis of mouse hypothalamus under simulated microgravity

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Background

The space environment provides is an extreme living and working conditions to which humans are not naturally suited and which demands complex process of physiological and psychological adaptation. Space travel has many deleterious side effects on the flight crew due to the '0' g environment. Changes in gravity have detrimental effects on cardiovascular, respiratory and gastrointestinal functions. The vulnerability of the brain to microgravity stress has been of concern as it relates to the fluidic nature of the gray and white matter. Space missions affect the neurophysiological functions of the astronauts which include perpetual, cognitive and psychomotor changes, orthostatic intolerance and vestibular-related changes. Hence, studies carried out under altered gravitational environment might represent a useful tool to investigate the neurobiological and behavioral responses to stressors and may provide insights into the mechanisms underlying development and plasticity of the nervous system. At the molecular level, the extreme conditions of space flight induce lasting changes in growth, cellular structure, and cell-to-cell interactions including down regulation of genes regulating cell proliferation, growth factor cascades, cell cycle, and signal transduction proteins. Induction of oxidative stress by microgravity has been detected both in simulated microgravity as well as in space. Oxidative stress is considered as one of the major factors that can modulate cellular signaling.

The central control stations of the stress system are located in the hypothalamus. The hypothalamic-pituitary-adrenal (HPA) axis is the key player in stress responses; it is well established that both external and internal stressors activate the HPA axis. There is also an increasing amount of experimental evidence that oxidative stress is a causal, or at least an ancillary factor in the neuropathology of several adult neurodegenerative disorders, as well as in stroke, trauma, and seizures. Gravitational stress induced augmentation of lipid peroxidation along with activation of nuclear transcription factor κ B (NF-κB) has also been reported in the hippocampus of male mice. In the present study, an attempt has been made to identify some of the changes in protein expression using proteomics approach in the hypothalamic region of mouse brain which underwent simulated microgravity environment and to understand the effect of stress on the proteome.

Study Design

The aim of this study was to explore the effect of microgravity on the hypothalamus of the mouse brain. The Ground based models for studying microgravity are Head-down bed rest and the Tail Suspension Model that cause a pooling of fluids in the upper body similar to that experienced by astronauts in space. In this study, Poonam et al., have employed the tail suspension model which simulated the microgravity exposure to mice. Of the 2 groups, control mice (n = 6) were maintained in normal cages with free access to drinking water and diet, and the test group mice (n = 6) were suspended by their tail in the center of the cage but allowed to touch the floor with their front paws and left for 7 days with free access to drinking water and food. The brain was dissected out at the end of the experiment. As previous studies have demonstrated that oxidative stress and lipid per oxidation were increased in different brain regions of mouse exposed to simulated microgravity therefore, glutathione (GSH) levels were estimated in the hypothalamus to elucidate the localized oxidant status. Decrease in GSH was detected in the present study which shows disruption in oxidant capacity of the hypothalamus that indirectly indicates generation of oxidative stress. However, in the present study,

simulated microgravity induced no change in lipid per oxidation levels, possibily due to decreased protein synthesis.

Further proteomic analysis showed differential expression of redox sensitive proteins. To assess the effect of microgravity on the protein expression profile in hypothalamus of mouse brain, the proteins in hypothalamus homogenate were analyzed by 2DE following which the 2DE protein patterns were examined visually. The authors identified seven spots in the protein patterns which were significantly different between the control and microgravity exposed hypothalamus. Peptide mass fingerprinting analysis was performed for all the seven spots using MALDI-TOF-MS following which the peptide masses were compared with the theoretical peptide masses of all available proteins from all species. Malate dehydrogenase (MDH) and stress-related protein ubiquitin carboxy-terminal hydrolase L1 (UCLH-1) were found to be increased in the hypothalamus of mice exposed to microgravity. UCLH-1 regulates hydrolysis of larger ubiquitin conjugates after stress stimuli. The ubiquitin protects cells under stress conditions. MDH catalyzes the interconversion of oxaloacetate and malate linked to the oxidation/reduction of dinucleotide coenzymes. Oxaloacetate plays a crucial role in many metabolic pathways including operation of the TCA cycle, glyoxylate bypass, amino acid synthesis, gluconeogenesis, maintenance of oxidation/ reduction balance, and facilitation of the exchange of metabolites between cytoplasm and sub cellular organelles. MDH is also proposed to have increased to counteract the altered redox status in hypothalamus. Peroxiredoxin (PRX), an antioxidant protein that plays a critical role in protecting against endogenously produced peroxides in both prokaryotes and eukaryotes was found to be abundant in hypothalamus of microgravity-treated brain as compared to controls. On the other hand, glutathione-S-transferase (GST) and superoxide dismutase-2 (SOD-2) were significantly decreased and the expression of these 2 proteins has been demonstrated to be critical in modulating the response of neurons to various kinds of stress. This supports the occurrence of oxidative assault in the hypothalamus.

To corroborate the changes in amount of SOD-2 in the hypothalamus tissue, MALDI-TOF-MS spectrum identifying SOD-2 was analyzed. Immunoblot analysis with SOD-2 specific antibody was also performed to validate the protein that showed loss of SOD-2 protein in hypothalamus exposed to simulated microgravity. Hence the changes in the proteome under simulated microgravity indicates the loss of oxidative stress related proteins and it can be interpreted as a result of failure of antioxidant defense systems in the brains.

In summary, the study shows the differential expression of redox-sensitive proteins in the hypothalamus of the mouse brains subjected to simulated microgravity. In conclusion, the analysis warrants safety concerns regarding long duration human space flight and design counter-strategies against the effects of microgravity.

Implications

This study focuses on basic research which contributes to understanding how the microgravity environment affects the different regions of brain, particularly in relation to protein expression of hypothalamus and the effects of space exploration on brain. This work could give investigators insight into how biomolecules respond to microgravity environment.

doi : 10.5214/ans.0972.7531.2009.160305