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CHARACTERIZATION OF SPINAL CORD GLIAL CELLS IN A MODEL OF HINDLIMB UNLOADING IN MICE

Y. A. CHELYSHEV, [a,c] **Y. O. MUHAMEDSHINA,** [a*] **T. V. POVYSHEVA,** [c] **G. F. SHAYMARDANOVA,** [b] **A. A. RIZVANOV,** [c] **M. V. NIGMETZYANOVA,** [a] **O. V. TIAPKINA,** [b] **N. I. BONDARENKO,** [c] **E. E. NIKOLSKIY** [a,b,c] **AND R. R. ISLAMOV** [a]

[a] *Kazan State Medical University, Kazan, Russia*

[b] *Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia*

[c] *Kazan (Volga Region) Federal University, Kazan, Russia*

Abstract—Exposure to microgravity has been shown to result in damaging alterations to skeletal muscle, bones, and inner organs. In this study, we investigated the effects of microgravity by using a hindlimb unloading model (HUM) in mice. The characteristics of the lumbar spinal cords of HUM mice 30 days after hindlimb unloading were examined. Morphometric analysis showed reductions of the total area, gray matter, and white matter by 17%, 20%, and 12%, respectively. Myelinated fibers in the white matter showed prominent myelin destruction. Analysis of the number of glial fibrillary acidic protein (GFAP+)/S100 calcium-binding protein B (S100B−), GFAP+/S100B+, and GFAP−/S100B+ astrocytes in the ventral horn (VH), central channel area (CC), dorsal root entry zone (DREZ), main corticospinal tract (CST), and ventral funiculi (VF) showed that the number of GFAP+/S100B− astrocytes was increased in the DREZ and CST of HUM mice. Additionally, GFAP+/S100B+ cell numbers were significantly decreased in the VH and CST but did not differ in the CC or DREZ of HUM mice, as compared with the control. The numbers of GFAP−/S100B+ cells were significantly reduced only in the VH of HUM mice. Moreover, the number of ionized calcium-binding adaptor molecule 1 (Iba1+) microglia cells was significantly increased in the CC and DREZ of HUM mice. In control mice, homeobox protein HoxB8 (HoxB8+) cells were found only in the CC; in contrast, HoxB8+ cells were observed in all studied areas in HUM mice, with the greatest number found in the CC. Genome-wide transcriptome analysis of the lumbar spinal cords of HUM mice showed decreased expression of genes encoding myelin, extracellular matrix, cytoskeleton, and cell adhesion proteins. Real-time polymerase chain reaction (PCR) confirmed reductions in the expression of *mpz*, *pmp2*, *pmp22*, and *prx* genes, which are involved in myelination, as well as decreases in the levels of genes encoding extracellular matrix molecules, including glycoproteins (matrix gla protein (MGP), osteoglycin (OGN), microfibrillar associated protein 5 (MFAP), and collagen, type IV, alpha 1 (COL4A)), proteoglycans (perlecan (heparan sulfate proteoglycan) (HSPG)), and metalloproteinases (lysyl oxidase (LOX)). Thus, our results showed that hindlimb unloading caused decreases in gray and white matter areas, changes in gene expression, alterations in myelination, and phenotypic modifications in glial cells in the lumbar spinal cords of mice.

Key words: hindlimb unloading model, spinal cord, glia, myelin.

INTRODUCTION

The negative effects of long-term microgravity have been well documented, and many organs and limb skeletal muscles have been shown to be particularly vulnerable. Even during the first day of exposure to microgravity, the skeletal muscles responsible for postural control display alterations typical of microgravity locomotion syndrome (MLS) (Caiozzo et al., 1996; Ohira et al., 2002; Kozlovskaya et al., 2007; Fitts et al., 2010). Therefore, elucidation of the mechanisms mediating these effects of microgravity is essential.

Changes in spinal cord neural muscle control have been hypothesized to be responsible for the deleterious influence of microgravity during space trips or in ground-based animal models. In a ground-based model of microgravity (the hindlimb unloading model [HUM]), we previously showed that motor neurons display decreased choline acetyltransferase (ChAT) levels, undergo mild stress changes (upregulation of heat-shock protein [Hsp] 25 and 70), and do not exhibit signs

*Corresponding author. Address: Kazan State Medical University, Butlerov Street 49, Kazan 420012, Russia. Tel: +7-9274307511; fax: +7-843-236-0393.

E-mail address: yanakazmedhist1@rambler.ru (Y. O. Muhamedshina).

Abbreviations: ANOVA, analysis of variance; ANXA3, annexin A3; CC, central canal; COL4A1, collagen, type IV, alpha 1; CST, main corticospinal tract; DHH, desert hedgehog; DREZ, dorsal root entry zone; EM, electron microscopy; GFAP, glial fibrillary acidic protein; HoxB8, homeobox protein HoxB8; HSPG2, perlecan (heparan sulfate proteoglycan 2); HUM, hindlimb unloading model; Iba1, ionized calcium-binding adaptor molecule 1; LOX, lysyl oxidase; MFAP5, Microfibrillar-associated protein 5; MGLAP, matrix Gla protein; MGP, matrix gla protein; MLS, microgravity locomotion syndrome; MPZ, myelin protein zero; OGN, osteoglycin; Olig2, oligodendrocyte transcription factor 2; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PI3K, phosphoinositol 3-kinase; PLEKHA4, pleckstrin homology domain-containing, family A (phosphoinositide binding-specific) member 4; PMP2, peripheral myelin protein 2; PMP22, peripheral myelin protein 22; PRX, periaxin; RT, room temperature; S100A11, S100 calcium-binding protein A11 (calgizzarin); S100B, S100 calcium-binding protein B; UACA, uveal autoantigen with coiled-coil domains and ankyrin repeats; VF, ventral funiculi; VH, ventral horn.

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