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The effects of voluntary wheel running on neuroinflammatory status: Role of monocyte chemoattractant protein-1

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ABSTRACT

The health benefits of exercise and physical activity (PA) have been well researched and it is widely accepted that PA is crucial for maintaining health. One of the mechanisms by which exercise and PA exert their beneficial effects is through peripheral immune system adaptations. To date, very few studies have looked at the regulation of neuroimmune reactions in response to PA. We studied the effect of voluntary wheel running (VWR) on pro- and anti-inflammatory cytokine levels, patterns of glial cell activation and expression of immune receptors in the brains of female C57BL/6 mice. By using homozygous monocyte chemoattractant protein (MCP)-1 null mice, we investigated the role of this key immunoregulatory cytokine in mediating VWR-induced neuroinflammatory responses. We demonstrated that, compared to their sedentary counterparts, C57BL/6 mice exposed for seven weeks to VWR had increased levels of pro- and anti-inflammatory cytokines, markers of glial cell activation and a trend towards increased expression of toll-like receptor (TLR) 4 in the brain. Measurements of serum cytokines revealed that the alterations in brain cytokine levels could not be explained by the effects of PA on peripheral cytokine levels. We propose that the modified neuroimmune status observed in the VWR group represents an activated immune system, as opposed to a less activated immune system in the sedentary group. Since MCP-1 knockout mice displayed differing patterns of pro- and anti-inflammatory brain cytokine expression and glial activation when compared to their wild-type counterparts, we concluded that the effects of VWR on neuroimmune reactions may be modulated by MCP-1. These identified immunomodulatory effects of PA in the brain could contribute to the observed positive relationship between physically active lifestyles and a reduced risk for a number of neurodegenerative diseases that possess a significant neuroinflammatory component.

1. Introduction

It is generally understood that physical activity (PA) is important for maintaining a healthy body. The benefits of both PA (any bodily movement involving the contraction of skeletal muscle that increases energy expenditure above the basal level) and exercise (a subcategory of PA that involves deliberate, structured and repetitive activity aimed towards enhanced muscular tone or endurance abilities) are well known (CDC, 2015). PA and exercise enhance cardiovascular endurance, increase muscular strength, boost metabolism, improve signaling of various hormones and decrease adiposity (Bergman, 2013; Egan and Zierath, 2013; Fiuza-Luces et al., 2013; Peixoto et al., 2015; Stewart et al., 2005; Stojanovic et al., 2012). Research has highlighted the overall anti-inflammatory effect that exercise and PA produces on the body when compared to a sedentary (SED) lifestyle (Gleeson et al., 2011).

Some of the key immune-modulating effects that regular exercise and PA have in the periphery include a reduction in circulating pro-inflammatory cytokines, like tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 (Bruun et al., 2006; Gleeson et

Abbreviations: ANOVA, analysis of variance; BBB, blood brain barrier; BDNF, brain-derived neurotropic factor; BSA, bovine serum albumin; CBS, calf bovine serum; CNS, central nervous system; DMEM-F12, Dulbecco's modified Eagle medium: nutrient mixture F-12 Ham; DMSO, dimethyl sulfoxide; ECL, enhanced chemiluminescent; ELISA, enzyme-linked immunosorbent assay; GDNF, glial cell line-derived neurotrophic factor; GFAP, glial fibrillary acidic protein; H_2O_2, hydrogen peroxide; HRP, horseradish peroxidase; HSP, heat shock protein; IBA-1, ionized calcium binding adaptor molecule 1; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; LSD, lest significant difference; M1, pro-inflammatory macrophage; M2, anti-inflammatory macrophage; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; NSERC, Natural Sciences and Engineering Research Council of Canada; PA, physical activity; RIPA, radioimmunoprecipitation assay; SDS, sodium dodecyl sulphate; SED, sedentary; SEM, standard error of the mean; STAT, signal transducer and activator of transcription; TBST, tris-buffered saline with 0.1% tween; TEMED, tetramethylethylenediamine; TLR, toll-like receptor; TNF, tumor necrosis factor; UBC, University of British Columbia; VEGF, vascular endothelial growth factor; VWR, voluntary wheel running; ZO, zonulae occludens.

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al., 2011; McFarlin et al., 2006; Michigan et al., 2011; Stewart et al., 2005), with an increase in anti-inflammatory cytokines including interleukin (IL)-10 (Gleeson et al., 2011; McFarlin et al., 2006; Starkie et al., 2003; Steensberg et al., 2003). These cytokines and chemokines are capable of crossing the blood brain barrier (BBB), and can serve as a means of communication between the peripheral and central nervous system (CNS) immune system (Banks and Erickson, 2010; Banks et al., 1995). There are a number of additional ways in which the CNS and peripheral immune system communicate, including the recently discovered (and still somewhat debated) CNS lymphatic system, extravasation of immune cells across the BBB, and secretion of cytokines and chemokines by brain endothelial cells (Aspelund et al., 2015; Coisne et al., 2013; Louveau et al., 2015a; Louveau et al., 2015b; Shoemaker et al., 2014; Verma et al., 2006). PA and exercise may alter some or all of the above mechanisms involved in the peripheral to CNS immune system communication.

Exercise can also transform adipose tissue resident macrophages from a pro-inflammatory (M1) to an anti-inflammatory (M2) state (Bruun et al., 2006). The mechanism behind these phenomena is not fully understood, but is thought to involve the downregulated expression of toll-like receptor (TLR) 4 on several cell types, including monocytes, adipocytes, myocytes and hepatocytes (Gleeson et al., 2011; McFarlin et al., 2006), leading to a significant reduction of inflammation. Overall, while much is known about the effects of PA and exercise on peripheral immunity, the effects of PA on the brain immune system are relatively unknown.

PA and exercise can have several beneficial effects on functions of the CNS, such as improved mood and mental health, as well as enhanced memory and cognitive function (Moore et al., 2014; Roig et al., 2013). Exercise is known to enhance neuronal release of the neurotransmitters serotonin and dopamine (Melancon et al., 2014; Monteiro-Junior et al., 2015). Exercise has also been associated with enhanced long-term potentiation and neurogenesis (Yu et al., 2013), as well as higher expression of CNS cell survival signals, like brain-derived neurotropic factor (BDNF), vascular endothelial growth factor (VEGF) and glial cell line-derived neurotrophic factor (GDNF) (Gyorkos et al., 2014; Uysal et al., 2015). However, only limited information is available regarding the effects of exercise and PA on glia, the immune and helper cells of the brain.

In this study, we used voluntary wheel running (VWR) mice to examine the effects of PA on brain cytokine production and glial cell activation. We also studied the role that MCP-1 plays in these responses, since previous research has shown that MCP-1 mediates cross talk between the peripheral immune system and the immune system of the brain. MCP-1 is an extracellularly secreted chemokine, which is capable of crossing the BBB (Erickson and Banks, 2011). It is produced by many peripheral and CNS cell types, including smooth muscle cells, fibroblasts, monocytes, astrocytes and microglia (Deshmane et al., 2009).

This immune cytokine is upregulated during pathological events in both the periphery and the CNS, including arthritis, cardiovascular disease, multiple sclerosis and Alzheimer's disease (Deshmane et al., 2009). More specifically, MCP-1 has been shown to increase permeability of the BBB (Paul et al., 2014) by downregulating the expression of the tight junction proteins zonulae occludens (ZO)-1 and occludin by brain endothelial cells (Song and Pachter, 2004). This leads to increased extravasation of immune cells across the BBB (Carrillo-de Sauvage et al., 2012; Sewell et al., 2004) and facilitates increased crosstalk between the peripheral and CNS immune systems.

Our results demonstrate that VWR upregulates expression of several pro- and anti-inflammatory cytokines in the brain, activates glial cells and induces changes in CNS TLR4 expression. We also demonstrate that the neuroimmune-modulatory effects of VWR in the CNS are at least partially mediated by MCP-1. Furthermore, we demonstrate that the changes in brain cytokine levels in response to VWR appear to be independent of changes in circulating peripheral cytokine levels, indicating that PA may have unique and brain-specific immune-modulatory roles.

2. Methods

2.1. Materials

Ammonium persulfate, dimethyl sulfoxide (DMSO), ExtrAvidin alkaline phosphatase, phosphatase substrate tablets, protease inhibitor cocktail, sodium deoxycholate, tetramethylethylenediamine (TEMED) and Triton X-100 were obtained from Sigma Aldrich (Oakville, ON, Canada). Bromophenol blue was obtained from Van Waters and Rogers International (Mississauga, ON, Canada). Murine enzyme-linked immunosorbent assay (ELISA) development kits for MCP-1, IL-4, IL-10, TNF-α and interferon (IFN)-γ were purchased from PeproTech (Rocky Hill, NJ, USA). Acrylamide/bis (29:1, 30% solution), bovine serum albumin (BSA), calf bovine serum (CBS), Dulbecco's modified Eagle medium: nutrient mixture F-12 Ham (DMEM-F12), diethanolamine, glycine, hydrochloric acid, penicillin/streptomycin, Pierce BCA protein assay kit, sodium dodecyl sulphate (SDS) and SuperSignal West Pico enhanced chemiluminescent (ECL) substrate were purchased from ThermoFisher Scientific (Ottawa, ON, Canada). Precision Plus Protein Kaleidoscope ladder was purchased from Bio-Rad (Mississauga, ON, Canada). DAKO rabbit anti-glial fibrillary acidic protein (GFAP) antibodies were purchased through Cedarlane (Burlington, ON, Canada). Rabbit anti-ionized calcium binding adaptor molecule 1 (IBA-1) antibodies were purchased from WAKO (Irving, CA, USA). Rabbit anti-actin antibody, anti-TLR4 and anti-signal transducer and activator of transcription (STAT) 1 antibodies were purchased from SantaCruz Biotechnology (San Jose, CA, USA). Horseradish peroxidase (HRP) labelled goat anti-rabbit antibody was purchased from Cell Signaling (Orange County, CA, USA).

2.2. Mice

Female C57BL/6 mice (termed MCP-$1^{+/+}$) and female MCP-1 knockout (MCP-$1^{-/-}$) mice on a C57BL/6 background were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). Mating pairs were set up to breed in house at the Modified Barrier Facility at the University of British Columbia (UBC). All procedures involving the care and handling of the mice were approved by the UBC Committee on Animal Care, under the guidelines of the Canadian Council on the Use of Laboratory Animals. Mice were housed individually in “shoebox” style cages (Columbus Instruments) in a temperature-controlled room (22 °C) on a 12 h light/dark cycle with access to food and water *ad libitum*. At the age of 6 weeks, mice were randomly divided into two groups: 1) VWR (with access to free running wheel (wheel diameter of 10.16 cm, interior diameter of 9.2 cm, wheel width of 5.1 cm, Columbus Instruments), as a model for PA and 2) SED (with no access to free running wheel). All mice were placed in their respective cages for 3 days prior to ‘day 1’ of the experiment to acclimate to the wheels and solitude. Mice were housed in these conditions for seven weeks. There were four experimental groups in total: MCP-$1^{+/+}$ SED ($N = 8$), MCP-$1^{+/+}$ VWR ($N = 8$), MCP-$1^{-/-}$ SED ($N = 3$) and MCP-$1^{-/-}$ VWR ($N = 7$). Only three animals were available for the MCP-$1^{-/-}$ SED group.

It is important to note that previous research determined that housing mice individually can have an impact on certain aspects of neuroinflammation (Karelina et al., 2009); however, it was necessary to house mice in solitude to preclude competition for access to the free running wheel, and to prevent fighting associated with social ranking, which can produce a considerable stress response (Kinsey et al., 2008). To check that the mice were, in fact, performing PA, sample wheel rotation numbers were collected at 1 h intervals for the first two weeks of the seven-week experimental period. Wheel rotations were counted by magnetic switches interfaced to a computer using windows software. SED mice were intentionally housed in absence of a locked free running wheel, since studies have demonstrated that rodents climb and play on locked wheels (Koteja et al., 1999), providing a form of PA. Following the seven-week period, mice were anesthetized with isoflurane, blood was collected *via* cardiac puncture, and mice were sacrificed by cervical