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AEROBIC ENDURANCE CAPACITY AFFECTS SPATIAL MEMORY AND SIRT1 IS A POTENT MODULATOR OF 8-OXOGUANINE REPAIR

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Abstract—Regular exercise promotes brain function via a wide range of adaptive responses, including the increased expression of antioxidant and oxidative DNA damage-repairing systems. Accumulation of oxidized DNA base lesions and strand breaks is etiologically linked to for example aging processes and age-associated diseases. Here we tested whether exercise training has an impact on brain function, extent of neurogenesis, and expression of 8-oxoguanine DNA glycosylase-1 (Ogg1) and SIRT1 (silent mating-type information regulation 2 homolog). To do so, we utilized strains of rats with low- and high-running capacity (LCR and HCR) and examined learning and memory, DNA synthesis, expression, and post-translational modification of Ogg1 hippocampal cells. Our results showed that rats with higher aerobic/running capacity had better spatial memory, and expressed less Ogg1, when compared to LCR rats. Furthermore, exercise increased SIRT1 expression and decreased acetylated Ogg1 (AcOgg1) levels, a post-translational modification important for efficient repair of 8-oxo-7,8-dihydroguanine (8-oxoG). Our data on cell cultures revealed that nicotinamide, a SIRT1-specific inhibitor, caused the greatest increase in the acetylation of Ogg1, a finding further supported by our other observations that silencing SIRT1 also markedly increased the levels of AcOgg1. These findings imply that high-running capacity is associated with increased hippocampal function, and SIRT1 level/activity and inversely correlates with AcOgg1 levels and thereby the repair of genomic 8-oxoG.

Key words: DNA repair, OGG1, exercise, hippocampus.

INTRODUCTION

Regular exercise has been shown to promote brain function via a wide range of adaptive responses, including the induction of brain-derived neurotrophic factor (Gomez-Pinilla and Vaynman, 2005), neuropeptide, glutamic acid decarboxylase (GAD)65 and GAD67 (Buck et al., 2007; Murray et al., 2010; Groves-Chapman et al., 2011), vascular endothelial growth (Fabel et al., 2003), enhanced metabolism and neurogenesis (van Praag et al., 1999; Raichlen and Gordon, 2011), and up-regulation of antioxidant and oxidative damage-repair systems (Radak et al., 2007b). The latter could be particularly important, since it has been shown that an elevated level of oxidative damage led to impairment of spatial memory, as assessed by the maze test (Radak et al., 2001).

It has been reported that regular exercise is a powerful tool to attenuate age-associated increases in the levels of protein carbonyls (Radak et al., 2001). Moreover, an increase in damage to proteins and accumulation of 8-oxo-7,8-dihydroguanine (8-oxoG) in DNA in neurons has been associated with a wide range of neurodegeneration (Aguirre et al., 2005; Wang et al., 2006; Lovell and Markesbery, 2007). Indeed, it was recently reported that aging results in increased levels of 8-oxoG in the hippocampus, which was associated with decreased levels of acetylation of the 8-oxoguanine DNA glycosylase (OGG1), that excises 8-oxoG during the DNA base excision repair (BER) pathway (Radicella et al., 1997). Acetylation of OGG1 on Lys338/Lys341 by p300/CBP increases its activity in the presence of apurinic apyrimidinic endonuclease1 (APE1) by reducing its affinity for the abasic site product (Bhakat et al., 2006). OGG1 also interacts with class I histone deacetylases, which may be responsible for its deacetylation (Bhakat et al., 2006). The importance of OGG1's acetylation is underlined by data showing that exercise increases the acetylated (Ac) OGG1 levels in the muscles of young individuals (Bori et al., 2012). Efficient DNA repair has been shown to protect against

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Abbreviations: 8-oxoG, 8-oxo-7,8-dihydroguanine; Ac, acetylated; AcOgg1, acetylated 8-oxoguanine DNA glycosylase-1; AP, abasic; APE1, apurinic apyrimidinic endonuclease1; BER, base excision repair; BrdU, 5-bromo-2′-deoxyuridine; DNPH, 4-dinitrophenylhydrazine; ERK, extracellular signal-regulated kinase; GAD, glutamic acid decarboxylase; HCR, high-running capacity; HDAC, histone deacetylase; HRP, horseradish peroxidase; LCR, low-running capacity; NAD, nicotinamide adenine dinucleotide; NAM, nicotinamide; NGF, nerve growth factor; Ogg1, 8-oxoguanine DNA glycosylase-1; ssbs, single-strand breaks; TBS, tris-buffered saline; TBS-T, tris-buffered saline containing 0.1% Tween; TrHCR, trained HCR; TrLCR, trained LCR; TSA, trichostatin A.

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neurodegeneration and thus underline the significance of oxidative DNA damage repair in the brain (Yang et al., 2010; Liu et al., 2011).

SIRT1 is a redox-sensitive deacetylase, targets acetyl groups on DNA repair proteins, such as APE1, and a number of regulatory proteins, including forkhead homeobox-type O protein, nuclear factor kappa B (NF-κB), p53, peroxisome proliferator-activated receptor gamma coactivator-1 alpha, and hypoxia inducible factor 1 alpha (Herranz and Serrano, 2010; Kelly, 2010). Due to silent mating-type information regulation 2 homolog 1's (or NAD^+-dependent deacetylase sirtuin-1, SIRT1) multiple roles in cellular physiology, the activation of SIRT1 has been shown to retard the aging process (Agarwal and Baur, 2011), as well as to increase the resistance against oxidative stress (Csiszar et al., 2009), and attenuate neurodegeneration (Chao et al., 2008).

To examine the incidence and development of life-style-related diseases, Koch and Britton (Koch and Britton, 2001) used artificial selection for intrinsic aerobic endurance running capacity to develop a heterogeneous N:NIH strain of rats [low-running capacity (LCR) and high-running capacity (HCR)]. These rat models allowed the study of the effects of exercise on a variety of factors, including the incidence and development of life-style related diseases (Wisloff et al., 2005; Schwarzer et al., 2010). Wisloff and co-workers have found that LCR rats develop mitochondrial dysfunction in the heart and metabolic syndromes earlier than do the HCR ones (Wisloff et al., 2005). Moreover, it has also been shown that LCR rats have increased insulin resistance, visceral obesity, dyslipidemia, and decreased life-span compared to HCR rats (Bowman et al., 2010; Koch et al., 2011).

By utilizing LCR and HCR rat models, we showed that HCR rats had higher cognitive abilities and running capacity and expressed significantly less Ogg1. Further, exercise via SIRT1-mediated deacetylation transiently decreased the levels of AcOgg1 levels when compared to those of the LCR. These unexpected results imply that the Ogg1-initiated base excision repair of 8-oxoG during exercise may have a complex effect on the brain's function in terms of endurance and raises the possibility that a delay in the repair of oxidized guanine lesions could be advantageous for hippocampal function during exercise.

EXPERIMENTAL PROCEDURES

Animals

Artificial selective breeding, starting with a founder population of 186 genetically heterogeneous rats (N:NIH stock), was used to obtain rat strains differing in inherent aerobic capacity. The procedure was described in detail previously (Koch and Britton, 2001). Briefly, endurance running capacity was assessed on a treadmill, and the total distance run during the test was used as a measure for maximal aerobic exercise capacity. Rats with the highest running capacity from each generation were bred to produce the HCR strain, and rats with the lowest capacity were bred with each other to produce the LCR strain. A subgroup of male rats (24 HCR and 24 LCR) from generation 22 was used in the present investigation, carried out according to the requirements of The Guiding Principles for Care and Use of Animals, EU, and approved by the local ethics committee.

Exercise protocol

Twenty-four LCR and HCR male, 13-month-old rats were assigned to groups as follows: control LCR, trained LCR (TrLCR), control HCR, and trained HCR (TrHCR). Exercised rats (six animals per group) were introduced to treadmill running for three days, and, then, for the next 2 weeks, the running speed was set to 10 m/min on a 5% incline for 30 min. Next, the maximal oxygen uptake (VO_{2max}) was measured on the treadmill (Columbus Inst., Columbus, OH, USA) with a gradually increasing intensity. VO_{2max} was measured for each animal by using three criteria: (i) no change in VO_2 when speed is increased, (ii) rats could no longer keep their position on the treadmill, and (iii) respiratory quotient ($RQ = VCO_2/VO_2$) > 1. Then, based on the level of VO_{2max}, the speed corresponding to the 60% VO_{2max} was determined and used for daily training for 1 h five times a week. The VO_{2max} was measured every second week, and the running speed was adjusted. The training period lasted for 12 weeks. In order to monitor new cell formation, 5-bromo-2′-deoxyuridine (BrdU) was injected into each animal for the last four weeks of the program. The animals were sacrificed two days after the last exercise session to avoid the metabolic effects of the final run, part of the hippocampus was excised and frozen, and the other portion used for histochemistry.

Passive avoidance test

The test was performed according to the step-through method described by Jarvik and Kopp (1967). The apparatus consists of a two-compartment acrylic box with a lighted chamber connected to a darkened one by a guillotine door. As soon as the rats entered the dark chamber, they received an electrical shock (0.5 mA, 1 s). The latency times for entering the dark chamber were measured in the training test, after 24 h and 10 days in the retention test.

Cell culture

Rat medullary pheochromocytoma (PC12) cells (obtained from the American Type Culture Collection (ATCC), Manassas, VA, USA) were maintained in DMEM/F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12). PC12 (rat adrenal gland phaeochromocytoma) cells were terminally differentiated into neuronal cells by nerve growth factor (NGF: 50 ng/mL) and characterized as we described previously (Bacsi et al., 2005). HCT116 cells (ATCC No.: CCL-247, human colorectal carcinoma cells) were maintained in Ham's F12 (GIBCO-BRL, Grand Island, NY, USA). All media were supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals,