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Research report

Eating habits modulate short term memory and epigenetical regulation of brain derived neurotrophic factor in hippocampus of low- and high running capacity rats

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ABSTRACT

Exercise capacity and dietary restriction (DR) are linked to improved quality of life, including enhanced brain function and neuro-protection. Brain derived neurotrophic factor (BDNF) is one of the key proteins involved in the beneficial effects of exercise on brain. Low capacity runner (LCR) and high capacity runner (HCR) rats were subjected to DR in order to investigate the regulation of BDNF. HCR-DR rats out-performed other groups in a passive avoidance test. BDNF content increased significantly in the hippocampus of HCR-DR groups compared to control groups ($p < 0.05$). The acetylation of H3 increased significantly only in the LCR-DR group. However, chip-assay revealed that the specific binding between acetylated histone H3 and BNDF promoter was increased in both LCR-DR and HCR-DR groups. In spite of these increases in binding, at the transcriptional level only, the LCR-DR group showed an increase in BDNF mRNA content. Additionally, DR also induced the activity of cAMP response element-binding protein (CREB), while the content of SIRT1 was not altered. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) was elevated in HCR-DR groups. But, based on the levels of nuclear respiratory factor-1 and cytocrome *c* oxidase, it appears that DR did not cause mitochondrial biogenesis. The data suggest that DR-mediated induction of BDNF levels includes chromatin remodeling. Moreover, DR does not induce mitochondrial biogenesis in the hippocampus of LCR/HCR rats. DR results in different responses to a passive avoidance test, and BDNF regulation in LCR and HCR rats.

1. Introduction

It has been postulated that aerobic running could be important in the evolution of *Homo sapiens* (Bramble and Lieberman, 2004; Dumas et al., 2007; Lieberman and Bramble, 2007). A life-style associated with physical activity provides excellent condition for the regulation of brain derived neurotrophic factor (BDNF) (Mattson, 2012). Early in the evolution of humans it would have been difficult to have consistent, daily nutrition. Eating on alternate days during the hunting/gathering period would be considered normal. Therefore, genes apparently developed to store energy, based on irregular eating conditions (Chakravarthy and Booth, 2004). Alternate day eating has been used as a tool to study the effects of mild dietary restriction (DR) (Brown-Borg and Rakoczy, 2013; Pesic et al., 2010; Rodriguez-Bies et al., 2010; Terzibasi et al., 2009), and found to result in decreases in body mass and cardio protection (Garcia Ramos, 1980), and enhanced muscle performance, and increased lipid metabolism (Rodriguez-Bies et al., 2010). DR is well known to increase life expectancy (Sohal and Weindruch, 1996; Weindruch and May, 1995). Moreover, it also has been reported that DR can promote brain function (Qiu et al., 2012; Quintas et al., 2012; Singh et al., 2012) as it has been suggested that DR can mediate BDNF signaling and consequently play a role in memory neuro-protection, synaptic plasticity, and neurogenesis (Kumar et al., 2009; Radak et al., 2013a; Rich et al., 2010; Rothman et al., 2012).

The neuro-protective effects of physical exercise are well demonstrated (Barrientos et al., 2011; Radak et al., 2010, 2014;

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Stranahan and Mattson, 2011) and include up-regulation of BDNF levels as well as improved brain function as measured by the Morris maze and passive avoidance tests (Griesbach et al., 2009; Radak et al., 2006; Sarga et al., 2013; Wu et al., 2013).

An experimental model was created for the running capacity of rats (Koch and Britton, 2001). Low capacity runners (LCR) and high capacity runners (HCR) were identified. LCR rats were found to have shorter life-spans and decreased resistance to oxidative stress (Hart et al., 2013; Koch et al., 2011, 2012). LCR rats also readily develop cardiovascular disorders, and markers of metabolic syndrome, such as visceral adiposity, increased blood pressure, dyslipidemia and insulin resistance when compared to HCR rats (Koch et al., 2011). Therefore, due to the shorter life span, adiposity, and impaired brain function of LCR rats, this appears to be an excellent model to study the effects of DR. HCR rats also have been shown to outperform LCR rats in cognitive tests (Wikgren et al., 2012).

Regular exercise and nutrition could have an effect of epigenetics (Barnes and Ozanne, 2011; Milagro et al., 2011; Radak et al., 2013b; Vaquero and Reinberg, 2009). Reversible modification of lysine residues of histone proteins, such as acetylation and deacetylation, can readily alter the gene expression levels of proteins (Kaelin and McKnight, 2013; Sanders et al., 2013; Shankar et al., 2013), and these modifications can be inherited. It would be interesting to know whether DR can alter the level of histone acetylation at the promoter regions of BDNF, and hence, have a long term effect on BDNF signaling. Therefore, the present investigation was carried out to study the effects of alternate day eating on the epigenetical modulation of BDNF in hippocampus of low and high running capacity rats.

2. Materials and methods

2.1. Animals

Selectively bred rat strains differing in intrinsic aerobic capacity – low capacity runners (LCR) and high capacity runners (HCR) – were used in this study (Koch and Britton, 2001). Endurance running capacity was assessed on a treadmill and the total distance run during a speed-ramped exercise test was used as a measure of maximal aerobic capacity. Rats with the highest running capacity were bred to produce the HCR strain and rats with poor running capacity were bred to generate LCR rats. A subgroup of male rats from generation 22 was phenotyped for intrinsic treadmill running capacity when11 weeks old, at the University of Michigan (Ann Arbor, USA) and then shipped via air freight to Semmelweis University (Budapest, Hungary) for further study. Investigations were carried out according to the requirements of The Guiding Principles for Care and Use of Animals, EU, and approved by The Ethics Committee of Semmelweis University.

2.2. Animal setting and dietary restriction (DR)

LCR and HCR male rats, aged 13 months, were assigned to control LCR (LCR-C), dietary restricted LCR (LCR-DR), control HCR (HCR-C) and dietary restricted HCR (HCR-DR) groups (n = six rats per group). Dietary restriction was performed by feeding the animal every other day for 16 weeks (Fig. 1).

2.3. Tissue samples

After decapitation, brains were rapidly removed, hippocampi from both hemispheres were dissected and flash frozen in liquid nitrogen. All samples were kept at −80 °C until homogenized. Samples thawed on ice were quickly pre-homogenized for 10 s at medium speed (IKA's Ultra-Turrax homogenizer) in two volume of PBS (pH 7.4) to gain a crude homogenate. Crude bilateral hippocampal homogenates were divided into four aliquots for ChIP, qRT-PCR, Western blot, and ELISA assays. Aliquots for ChIP assay were processed immediately, whereas the others were stored at −80 °C awaiting further processing.

2.4. Passive avoidance test

The test was performed according to the step-through method as described earlier (Sarga et al., 2013). The apparatus consists of a two-compartment acrylic box with a lighted chamber connected to a darkened chamber 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 ten days later in the retention test.

2.5. Assays

2.5.1. AcH3-ChIP

This assay was based on the Acetyl-Histone H3 Immunoprecipitation Assay Kit (Cat# 17-245Upstate/Millipore). In brief, DNA formaldehyde was added directly to 100 μl aliquots of total prehomogenate (preH) representing about 30 mg of hippocampus in

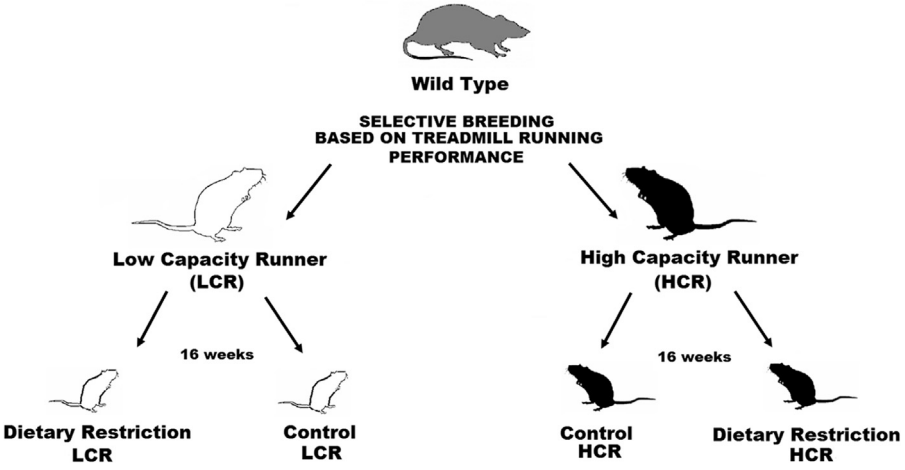

Fig. 1. Experimental setting. Low capacity runner (LCR) and high capacity runner (HCR) male rats, aged 13 months, were assigned to control LCR (LCR-C), dietary restricted LCR (LCR-DR), control HCR (HCR-C) and dietary restricted HCR (HCR-DR) groups (n = six rats per group). Dietary restriction was performed by feeding the animal every other day for 16 weeks.