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The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain

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Abstract

We have tested the hypothesis that training with moderate- (MT), strenuous- (ST), or over- (OT) load can cause alterations in memory, lipid peroxidation, protein oxidation, DNA damage, activity of 8-oxoG-DNA glycosylase (OGG1) and brain-derived neurotrophic factor (BDNF), in rat brain. Rat memory was assessed by a passive avoidance test and the ST and OT group demonstrated improved memory. The content of BDNF was increased only in the OT group. The oxidative damage of lipids and DNA, as measured by thiobarbituric acid reactive substances (TBARS), and 8-hydroxydeoxyguanosine (8-OHdG), did not change significantly with exercise. Similarly, the activity of DNA repair enzyme, 8-oxoguanine DNA glycosylase (OGG1), was not altered with exercise training. On the other hand, the content of reactive carbonyl derivatives (RCDs) decreased in all groups and the decrease reached significance levels in the ST and OT groups. The activity of the proteasome complex increased in the brain of OT. The findings of this study imply that over-training does not induce oxidative stress in the brain and does not cause loss of memory. The improved memory was associated with enhanced BDNF content.

Keywords: Exercise; Hormesis; Free radicals; Oxidative damage; Brain function

1. Introduction

Accumulating evidence suggests that physical exercise has the capability to beneficially effect certain brain functions (Fordyce and Wehner, 1993; Cotman and Engesser-Cesar, 2002; Cotman and Berchtold, 2002; van Praag et al., 1999a,b; Radak et al., 2001a,b; Molteni et al., 2004). It has been shown that voluntary running enhances the number of new hippocampal cells (van Praag et al., 1999a,b) and brain plasticity (Cotman and Berchtold, 2002). It appears that voluntary physical exercise induces the content of trophic factors, the content of which is associated with improved memory and long-term potentiation (van Praag et al., 1999a,b). Interestingly, most of the studies used applied voluntary exercise, and the effects of enforced physical exercise, especially with different loads, are unclear.

There is little information regarding whether regular exercise above a certain intensity or duration could be harmful. It would be of interest to identify the optimum exercise loading which would enhance certain physiological functions, including brain function. According to the current understanding of adaptation to physical exercise, until an over-training syndrome appears, regular exercise has beneficial effects. However, with over-training, which is still a poorly understood process, the homeostatic balance involving a wide range of hormonal, metabolic, and immunologic changes is altered (Petibois et al., 2003). Among the over-training syndromes, a number are associated with the conditions related to the central nervous system, such as depression, impaired concentration,

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anorexia, headache, loss of appetite, or loss of coordination (Fry et al., 1991). To date, very little is known about the effect of over-training on brain function.

The generation of reactive oxygen species (ROSs) is a necessary consequence of aerobic metabolism. Interestingly, both higher oxygen demand and hypoxia are known to increase the formation of ROS (Ji and Fu, 1992; Ji, 1993; Moller et al., 2001; Banerjee et al., 2003; Aoi et al., 2004). Physical exercise, especially a single bout of exercise, because of the limited adaptation of intensity and/or duration, could generate increased levels of ROS and result in oxidative damage to macromolecules (Davies et al., 1982; Radak et al., 2001a,b). There are few studies on the effects of exercise on oxidative damage or the antioxidant status of brain (Suzuki et al., 1983; Somani et al., 1995; Radak et al., 1995) and the findings are conflicting. Suzuki et al. (1983) reported that voluntary exercise increased the lipid peroxidation in the brain. On the other hand, we have shown previously, that regular swimming attenuated the age-associated decline in memory (Radak et al., 2001a,b). However, the effect of severe and over-training on oxidative damage and brain function is not known.

Tiidus (1998) has suggested that ROS play a role and might be an associative or causative factor of over-training. Therefore, we tested the hypothesis that regular exercise with different loads, including a load, which aimed to achieve over-training, can cause oxidative damage to lipids and proteins in the brain. Moreover, we were interested in how different exercise regimes affect BDNF content and memory.

2. Materials and methods

2.1. Animals and exercise

The protocol of the study was reviewed and approved by the local ethics committee. Twenty eight-, twenty-month old Wistar rats were assigned to the following groups: control (C); moderately trained (MT) (1 h swimming/day, 5 times/week, for 8 weeks); strenuously trained (ST) (the swimming duration increased by 30 min each week until it reached 4.5 h for the last week); over-trained (OT) (1 h swimming/day, 5 times/week, for 6 weeks, when the duration was abruptly increased to 4.5 h for the remaining 2 weeks). The swimming was performed in a plastic container (1.5 m × 1.5 m × 1.5 m) and continuously supervised. The water temperature was set to 26 °C. According to our current understanding, over-training generally occurs after a long term of strenuous exercise (ST) or when the duration increases abruptly (Fry et al., 1991; Petibois et al., 2003). In order to avoid the effect of the last exercise session and be able to measure the adaptive or maladaptive changes the rats were killed 1 day after the last training session. The brain of each animal was excised, washed and frozen in liquid nitrogen and stored at −80 °C until analyses.

2.2. Retention of passive avoidance test

The passive avoidance behavior was investigated in a one-trial step-through paradigm (Ader et al., 1972). The measurement was done as described earlier (Radak et al., 2001a,b). In brief, the assessment of the passive avoidance response was done by the registration of latency in entering the dark compartment, one day following an electric shock to the feet. Avoidance to enter the dark compartment during a three min period was set to 100% and a time-related percentage was given when the animals entered (i.e. a 50% rating was given to the animal if it entered the chamber after 1.5 min).

2.3. Biochemical assays

The whole brain was selected for the biochemical assays, in order to measure all parameters from the same region, since small regions, such as the hippocampus, would not be large enough to permit all of the measurements to be carried out. The determination of 8-OHdG was done as previously described (Kaneko et al., 1997). Isolation of nuclear DNA and the measurement of 8-OHdG were carried out as described by Kaneko et al. (1997). In brief, after the isolation of DNA, the aqueous solution containing 50 μg DNA was adjusted to 45 μl, and 5 μl of 200 mM sodium acetate buffer (pH 4.8) and 5 μg of nuclease P1 were added. After a purge with a nitrogen stream, the mixtures were incubated at 37 C for 1 h to digest the DNA to nucleotides. Then, 5 μl of 1 M Tris–HCl (pH 7.4) and 0.65 units of alkaline phosphatase were added and the mixture was incubated at 37 °C for 1 h to hydrolyze the nucleotides to nucleosides. Nucleosides in samples were analysed by a HPLC/ECD system that consisted of a Pegasil ODS column connected to a Shimadzu LC-10 pump (Tokyo, Japan) coupled to an ECD (ESA Coulechem II 5200; Bedford, MA). The solvent system used was a mixture of 6% methanol, 12.5 mM citric acid, 30 mM sodium hydroxide, 25 mM sodium acetate, and 10 mM acetic acid. The flow rate was 1.4 ml/min. The contents of dG and 8-OHdG were determined from the absorbance at 260 nm using an UV detector and simultaneously by ECD, respectively. The amount of 8-OhdG in the sample was expressed relative to the concentration of dG.

Excision assay: the assay was carried out according to the protocol described by Cardozo-Pelaez et al. (2000). In brief, 20 pmol of synthetic probe containing 8-OHdG (Trevigen, Gaithersburg, MD, USA) were labeled with P^{32} at the 5′-end using polynucleotide T4 kinase (Boeringer Mannheim, Germany). For the nicking reaction, protein extract (2–4 μg) was mixed with 20 μg of a reaction mixture containing 0.5 M of *N*-[2-hydroxyethel]piperazine-*N*′-[ethanesulfonic acid], 0.1 M EDTA, 5 mM of dithiolthreitol, 400 mM KCl, purified BSA and labeled probe (approximately 2000 cpm). The reaction was carried out at 30 C for 5–15 min and stopped by placing the solution in ice. Then, 30 μl chloroform were added and samples were centrifuged and

15 μl taken and mixed with loading buffer containing 90% formamide, 10 mM NaOH, and blue-orange dye. After three min heating at 95 °C, samples were chilled and loaded into polyacrylamide gel (20%) with 7 M urea and one time and run at 400 mV for 2 h. Gels were quantified using BAS 2000 Bioimaging Analyzer (Fuji Film Co., Japan). Radioactive signal densities were determined using the software designed for this system. The activity to repair 8-oxodG was determined and expressed as a percentage of substrate cleaved (Cardozo-Pelaez et al., 2000).

Carbonyl content: the oxidative modification of amino acid residues was measured by the accumulation of reactive carbonyl derivatives (RCDs) as described by Radak et al. (1999).

Lipid peroxidation: the level of lipid peroxidation (LIPOX) was assessed by the determination of thiobarbituric acid reactive substance (TBARS) concentration (Mihara and Uchiyamam, 1978).

Proteasome activity: the chymotrypsin-like activity of proteasome complex from nucleus was determined fluorometrically by measuring the release of 7-amino-4-methylcoumarin from the peptides succinyl-Leu-Leu-Val-Tyr-MCA (SUC-LLVY-MCA) at 380 nm excitation and 440 nm emission, respectively (Hayashi and Goto, 1998).

BDNF content: the BDNF content was measured using the Emax ImmunoAssay System (Promega, Madison, USA) according to the company manual. In brief, acid-treated samples were added to a coated plate and incubated as is suggested in the technical bulletin supplied by the manufacturer. After washing, anti-human BDNF antibodies were added. Following incubation and washing, 100 μL anti-IgY HRP conjugate were injected into each well. After adding the TMB solution and 1 N hydrochloric acid, the absorbance was measured at 450 nm and the content was expressed in pg/ml.

2.4. Statistical analysis

The Whitney *U* test was used to evaluate the data obtained in the passive avoidance test. For the other tests, the statistical significance was assessed using ANOVA, followed by Scheffe's post hoc test. The significance level was set at $p < 0.05$.

3. Results

The body weights of control and MT rats (521.4 ± 23.6 and 512.8 ± 23.9 g) were significantly higher than those of the ST and OT rats (447.86 ± 11.5 and 432.8 ± 14.7 g).

Over-training has previously been associated with a loss in body mass (Fry et al., 1991).

The results of the passive avoidance test indicate that severe exercise loading and over-training resulted in improved memory as assessed by this test (Fig. 1). The content of BDNF increased in OT group compared to other groups (Fig. 2). The level of DNA damage did not change significantly (Fig. 3). The activity of OGG1, the enzyme that repairs 8-OHdG, did not change as a result of exercise training (Fig. 4). On the other hand, the content of reactive carbonyl derivatives (RCDs) decreased in all groups (Fig. 5). The proteasome activity increased significantly in the brain of OT rats, suggesting a possible link between the activity of this enzyme complex and RCD concentration (Fig. 6). The training did not modify the levels of lipid peroxidation (Fig. 7).

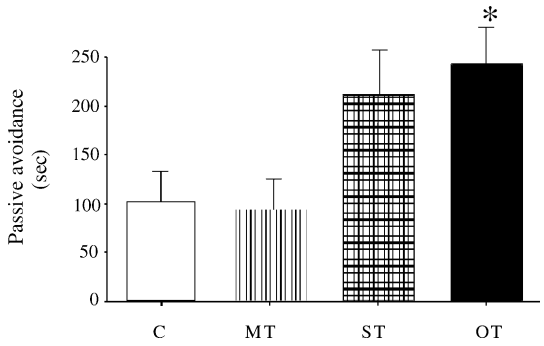

Fig. 1. On the second day of the passive avoidance test, electric shock was delivered to the feet of the rats when they entered the dark chamber. Panel shows the time of entrance in seconds. Values are mean ± S.D. for six rats per group. Significantly different from control ($^{*}P < 0.05$).

4. Discussion

Regular exercise is carried out in order to evoke adaptations, which include a wide range of beneficial effects, such as maintained brain health, improved cognitive processes and increased resistance to brain injury (Radak et al., 2001a,b; Griesbach et al., 2004; van Praag et al., 1999a,b; Mattson, 2000; Mattson et al., 2002). The obtained data suggest that over-training, which in humans is associated with a number of impaired functions of the central nervous system (Fry et al., 1991), does not result in impaired memory in rats. The improved memory was not associated with altered oxidative damage of lipids, protein or

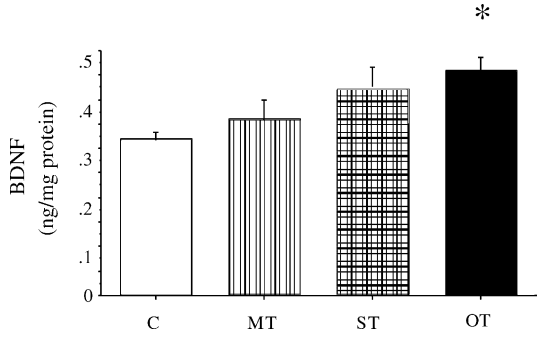

Fig. 2. The BDNF content, measured by ELISA method, increased significantly in the brain of OT animals. Values are mean ± S.D. for six rats per group. Significantly different from control ($^{*}P < 0.05$).

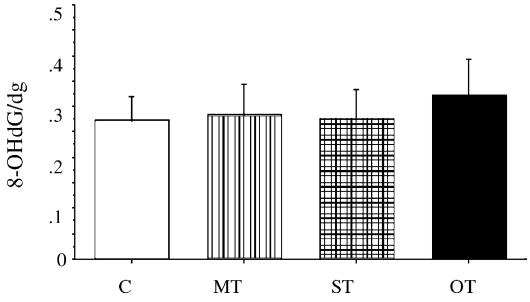

Fig. 3. The oxidative damage of nuclear DNA was measured by 8-OHdG. No significant differences were observed. Values are mean ± S.D. for six rats per group.

DNA. However, the level of RCD of OT rats was less, although not significantly, than control animals. Similarly, the activity of proteasome, the enzyme that is primarily responsible for the repair of oxidatively modified proteins (Grune et al., 1997), increased in the brain of OT rats. Our laboratory and others have reported a possible link between the accumulation of RCD and certain brain functions (Carney et al., 1991; Forster et al., 1996; Radak et al., 2001a,b), and pointed out the critical role of protein breakdown. In the current study, the activity of proteasome complex was significantly elevated in the brain of OT rats, and tended to be elevated in other exercise groups. The exercise-induced increase in protein breakdown could have an important clinical consequence, since it has been suggested that decreased degradation of β-amyloid peptide is one of the causative factors of Alzheimer's disease (Celsi et al., 2004). Slower β-amyloid degradation is thought to result in increased production of a long form of amyloid β-peptide which self-aggregates and forms insoluble plaques in the brain, resulting in increased ROS production and oxidative damage (Mattson et al., 2002).

This is one of the first studies to measure the content of BDNF with different exercise loads. BDNF is one of the most abundant neurotrophins, and is widely distributed in the central nervous system and associated with memory, some learning processes, and brain-insults, including a variety of stress types (Marmigere et al., 2003). In addition, it appears that the expression of BDNF mRNA and protein are dramatically up-regulated in models of inflammatory pain (Pezet et al., 2002). BDNF increases in sensory neurones innervating the inflamed paw, so neurones become BDNF immunoreactive (Cho et al., 1997). Interestingly,

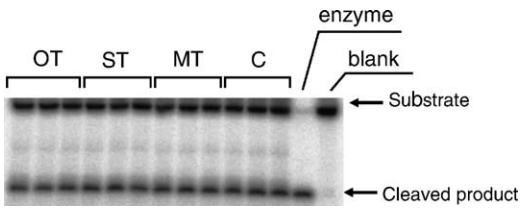

Fig. 4. The repair of OHdG was measured by the activity of OGG1. The graph shows representative data of three animals per group. No significant differences were observed. Values are mean ± S.D. for six rats per group.

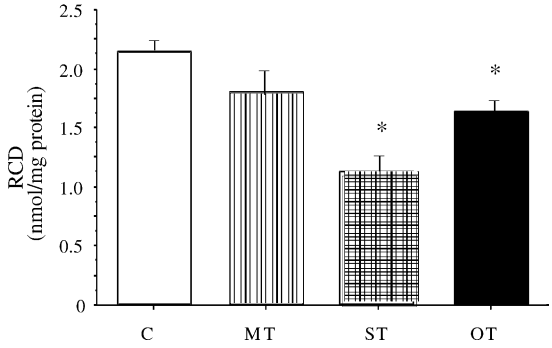

Fig. 5. The reactive carbonyl derivatives, markers of oxidative protein damage, decreased in all exercising groups, and significant difference appeared between control and moderately trained and over-trained groups. Values are mean ± S.D. for six rats per group. Significantly different from control ($^{*}P < 0.05$).

over-training is suggested to be a result of chronic inflammation (Smith, 2000). We have found increased BDNF content in OT brain, and this finding suggests accordance with this hypothesis.

It is possible that the given exercise load was not enough to cause over-training. However, it resulted in increased DNA damage in the liver (Ogonovoszky et al., in press). In addition, the changes in body, thymus, adrenal mass and adrenocorticotrophin, corticosterone levels suggest that the animals were exposed to a significant physical stress (Ogonovoszky et al., in press).

It has been shown earlier that voluntary exercise increases BDNF content and memory (Garza et al., 2004; van Praag et al., 1999a,b). The findings of the current study support this observation. Moreover, our data suggest a significant (causative?) relationship between the BDNF content and memory, judging from the data of the ST group. It is well substantiated that a steady state load does not always result in adaptation in the long term; so the load must be increased in order to cause adaptation (the overload principle). The positive relationship between maximal oxygen uptake and memory, and other brain functions in aged individuals, is in accordance with our data (Chodzko-Zajko and Moore, 1994). It should be pointed out that the effects of a regular exercise regimen (chronic) are very different from the effects

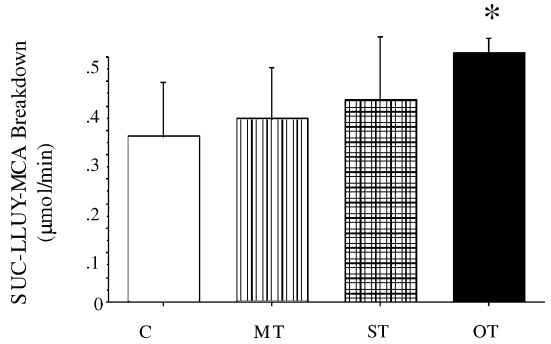

Fig. 6. The peptidase-like activity of proteasome complex is increased in the brain of OT animals. Values are mean ± S.D. for six rats per group. Significantly different from control ($^{*}P < 0.05$).

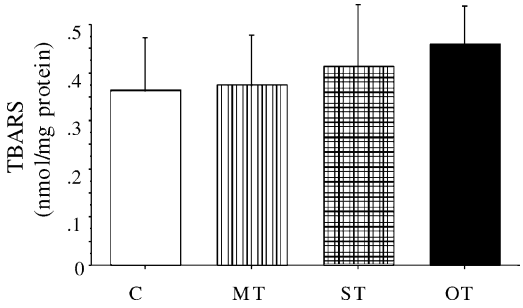

Fig. 7. The level of lipid peroxidation, measured by TBARS concentration, did not change as a result of training. Values are mean ± S.D. for six rats per group.

of a single bout of exercise (acute). Indeed, acute exercise can produce the opposite effects of chronic exercise, since regular exercise-induced adaptations are dependent upon stimuli provided by single bouts of exercise (Radak et al., 2001a,b, 2002, 2003, 2004). Hence, acute exercise challenges the redox state in the brain, and when performed regularly, can induce increased levels of antioxidant enzymes, increased resistance against oxidative stress, and enhanced physiological function (Radak et al., in press).

In summary, it appears, that physical exercise training even with a significant load, which causes over-training, does not result in impaired brain function and oxidative damage to the brain. On the other hand, the data suggest a possible relationship between low levels of oxidative protein damage, increased proteasome activity, BDNF content and certain brain functions, as a result of exercise training.

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