Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus

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Brain Research 1188 (2008) 182-188
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Introduction

Exercise

- Exercise may support brain health and function
- The regular physical activity has been indicated as a therapeutic approach to prevent age-related neurodegenerative disease

Experimental models have demonstrated that exercise gerbils submitted to forebrain ischemia show reduced neuronal damage and the infarct volumes were decreased (Stummer et al., 1994)

Moderate intensity exercise, 20 min/day for 2 week of training and three times a week of a 12-week treadmill training protocol, reduced damage to hippocampal slices from Wistar rats submitted to in vitro ischemia (Scopel et al., 2006; Cechetti et al., 2007)

Rat brain slices exposed to oxygen and glucose deprivation (OGD) have been used to model ischemic events and to investigate mechanisms of cell death and neuroprotection
- The release of lactic dehydrogenase (LDH) into the media is a index of cell damage or lysis
Effect of 20-min treadmill exercise

Moderate intensity exercise reduced ischemic injury, as assessed by reduced levels of LDH released after OGD and reoxygenation

(Scopel et al, 2006)

LDH: Lactic dehydrogenase; OGD: Oxygen and glucose deprivation

Introduction

Effect of exercise

The physical activity as reduce age-induced cognitive decline and it is recommended as a therapeutic strategy to prevent, or recover from, neurodegenerative disease

The exact molecular mechanisms by which physical exercise affects brain function are unclear

Introduction

Effect of exercise

The physical activity induces members of the neurotrophins family, especially BDNF, that modulate neuronal survival and plasticity, maturation and outgrowth in the developing brain (Lee and Paffenbarger, 1998)

Exercise induces BDNF mRNA in the hippocampus

(Neeper et al., 1996; Vaynman et al., 2004)

BDNF: brain-derived neurotrophic factor

Introduction

Effect of exercise

Exercise can induce free radical formation, which may be detrimental for cellular function

Regular exercise causes an adaptation of the cellular antioxidant system

The effects of exercise on oxidative damage or antioxidant brain status are also conflicting

Introduction

Effect of exercise

Suzuki (1983) reported the exercise increases lipid peroxidation in rat brain, while Radak (2001) presented the regular exercise attenuates the protein oxidative damage in aged rats

(Suzuki et al., 1983; Radák et al., 2001)

There are few studies on the effects of exercise on oxidative status in hippocampus

Aim of study

To investigate the modulation of hippocampal oxidative status and/or brain-derived neurotrophic factor (BDNF) is involved in exercise-induced neuroprotection
**Materials and Methods**

**Animals**
- Male Wistar rats aged 2-3 months
- Control dark-light cycle (12 hr/12 hr) with room temperature of 22 ± 2 °C
- Food and water *ad libitum*
- All experiments were approved by the Animal Care Committee

**Methods**

**Training**
- Measurement of oxygen uptake (VO₂) peak
- Moderate exercise training for 2 weeks

**Habituation**
- Oxygen and glucose deprivation (OGD)

**Biochemical assays**

**Slice preparation**

**Oxygen and glucose deprivation (OGD)**

**Measurement of VO₂ peak**
- Each rat ran on a treadmill at a low initial speed followed by increases in speed of 5 m/min every 3 min
- Point of exhaustion: failure of the rats to continue running, the time to failure (in min) and workload (in m/min)

**Exhaustion**

**Sedentary (SED)**
- (n=6–12 in each group)
- The SED group was maintained in the turned off treadmill for 5 min
- The intensity of exercise corresponded to 10-15 m/min

**2 weeks of daily 20 min/day treadmill running (EXE)**
Tissue preparation

Decapitation 16 h after the last running

Brain regions were dissected out

Hippocampal slices were submitted to OGD

Stored at -70 °C

Oxygen and glucose deprivation (OGD)

Pre-incubated in KHS solution for 15 min at 37°C with 95% O2/5% CO2

Modified Krebs-Henseleit solution (mM):
120 NaCl, 2 KCl, 0.5 CaCl2, 26 NaHCO3, 10 MgSO4, 1.18 KH2PO4, 11 glucose (pH7.4)

Washed with KHS medium without glucose

Incubated for 60 min at 37°C in anaerobic chamber (OGD period)

Methods

Tissue preparation

Ice-cold phosphate buffer (0.1 M, pH 7.4): 140 mM KCl and 1 mM EDTA

Teflon-glass homogenizer

Biochemical assays

Supernatant were collected

Centrifugation at 960 x g for 10 min

Methods

Free radical levels

2,7-dichlorofluorescein diacetate (DCFH-DA) at 37 °C for 30 min

Stop reaction in ice

Fluorescence spectrophotometer

DCFH-DA: 2,7-dichlorofluorescein diacetate

DCF: dichlorofluorescein

Methods

Thiobarbituric acid reactive substances (TBARS)

10% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA)

Heated for 30 min

n-nutanol

Fluorescence spectrophotometer

TBARS+ TBA → A fluorescent product

Fluorescence spectrophotometer

Exctiation: 280 nm

Emission: 345 nm

Methods

Oxidation of protein tryptophan residues

0.1 % sodium dodecyl sulfate (SDS)

Fluorescence spectrophotometer

Exciitation: 280 nm

Emission: 345 nm
Oxidative of protein tyrosine residues

Methods

0.1% sodium dodecyl sulfate (SDS)

Fluorescence spectrophotometer
Excitation: 277 nm
Emission: 320 nm

Total reactive antioxidant potential (TRAP) assay

Methods

+10 mM 2,2-azobis (2-amidinopropane) dihydrochloride (ABAP)
+4 mM luminol
+glycine buffer (0.1 M, pH 8.6)

Sample

Scintillation counter

Induction time

5.0 ml sample

200 mM Trolox

Standard

Methods

Total antioxidant reactivity (TAR) assay

• 2 mM ABAP
• 6 mM luminol
• glycine buffer (0.1 M, pH 8.6)

Standard

20 mM Trolox

Scintillation counter

Induction time

Sample

½ µl sample

Scintillation counter

Total antioxidant reactivity (TAR) assay

TAR values were determined by assessing the initial decrease of luminescence calculated as the ratio “Io/I”

Io = The CL in the absence of additives
I = The CL after addition of the 20 nM Trolox, or the samples (1 µl)

Methods

Analysis of BDNF concentration

Four brain regions (hippocampus, frontal cortex, striata and cerebellum) were individually homogenized in lysis buffer

137 NaCl, 20 Tris-HCl (pH 8.0), Igepal (1%), glycerol (10%), 1 PMSF, 0.5 sodium vanadate, 0.1EDTA and 0.1 EGTA

Supernatant was incubated on a 96-well flat-bottom plates previously coated with anti- BDNF monoclonal antibody

Plates were incubated with polyclonal anti-human antibody for 2 h and horseradish peroxidase for 1 h

Color reaction with tetramethyl benzidine was quantified in a plate reader at 450 nm

Data analysis

Two-tailed Student’s t-test

Two-way ANOVA followed by Tukey’s test

All data are presented as mean (± SEM)
Results and Discussion

Results
Effects to treadmill daily exercise during 20-min on free radical levels and lipoperoxidation in hippocampus

Results
Effects to treadmill daily exercise during 20-min on protein damage through tryptophan and tyrosine residues content in hippocampus

Results
Total reactive antioxidant potential (TRAP) and total antioxidant reactivity (TAR) in hippocampus

Results
Quantification of BDNF levels in brain areas from sedentary (SED) and exercised (EXE) rats

Discussion
Effect of exercise on BDNF

- Epidemiological studies have shown neuroprotective effects of moderate physical activity for stroke events
- The BDNF content and oxidative stress have been suggested to participate in the mechanisms of action of exercise

The neuroprotective effect of exercise may not be dependent on BDNF expression nor on the oxidative status
**Effect of exercise on BDNF**

- No significant changes in the BDNF content were detected in the hippocampus of rats submitted to daily moderate intensity exercise.
- The previous studies showed that physical activity, running and swimming, increases the expression of BDNF in rodent brain (Oliff et al., 1998; Gomez-Pinilla et al., 2002; Johnson et al., 2003; Neeper et al., 1996; Radák et al., 2006).

**Discussion**

- The increase of BDNF after exercise do not mention the interval between the end of training and the sacrifice of animals.
- They did not assess the markers immediately after exercise; the animals were sacrificed at least 16 h after the last treadmill running.

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**Effect of exercise on oxidative stress**

- There are just a few works on the effects of exercise on oxidative status in the hippocampus and findings reported on oxidative stress parameters are conflicting.
- The protocol here used did not alter the free radical content in hippocampus.

**Discussion**

- Their results showed that it did not modify TBARS levels and antioxidant enzyme activities in the rat hippocampus.
- This exercise protocol also did not change levels of DNA damage and protein oxidation in the hippocampus (Radák et al., 2006).

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**Effect of exercise on oxidative stress**

- In previous studies reported that voluntary exercise increased the lipid peroxidation in the brain (Suzuki et al., 1983).
- On the other hand, swimming did not change significantly the oxidative damage of lipids and DNA, measured by TBARS and 8-hydroxydeoxyguanosine (Radák et al., 2001; Čgorovszky et al., 2005).

**Discussion**

- This exercise protocol did not cause any change on total antioxidant reactivity (TAR) nor on potential (TRAP) indexes in hippocampus.
- In previous study suggest that exercise training in treadmill results in a better redox ratio in cerebral cortex (Somani et al., 1995).
**Discussion**

Effect of exercise on oxidative stress

- This exercise protocol might have not caused an oxidative stress strong enough to alter free radical levels and to cause damage to macromolecules.
- The exercise through its continuous free radical generating effect, can induce the oxidative stress and then an adaptation of the cellular antioxidant system.

**Conclusion**

The neuroprotective moderate intensity treadmill exercise does not alter neither oxidative stress markers nor BDNF levels, which might indicate that these biochemical mechanisms are not directly involved on its neuroprotective effect to hippocampal slices receiving in vitro ischemia.

**Thiobarbiturate reactive substances (TBARS)**

- Thiobarbiturate reactive substances (TBARS) are the low-molecular-weight end products, whose main component is malondialdehyde, that are formed during the decomposition of lipid peroxidation products. These compounds react with thiobarbituric acid to form a fluorescent red adduct which can be measured spectrometrically.

**2′,7′-Dichlorodihydrofluorescin (DCFH)**

- 2′,7′-Dichlorodihydrofluorescin (DCFH) is widely used to measure oxidative stress in cells. The diacetate ester form of DCFH, dichlorodihydrofluorescin diacetate (DCFH-DA), is relatively resistant to oxidation, but is readily taken up by cells, deacetylated to form DCFH, and thus available to be oxidized to the highly fluorescent compound dichlorofluorescein (DCF), in a reaction in which the oxidizing species is liberated. The oxidation of DCFH to the fluorescent DCF was considered to occur as a result of the generation of reactive oxygen species (ROS) in cells.
- (Tomoko Ohashi. *Analytical Biochemistry*, 308(2); September 2002, Pages 392-395)

- chronic (total 7 weeks) and acute treadmill exercise (an initial speed of 15 m/min gradually increased to 35 m min⁻¹ with 0°, 20-25 min per day duration)
- High intensity = 35 m/min (speed)
- Low intensity (50% VO2 max)
- High intensity (75% VO2 max)

*Phelan et al. (1997)*
Target Heart Rate

- Beginner or low fitness level . . 50% - 60%
- Average fitness level . . . . . .60% - 70%
- High fitness level . . . . . . . . . .75% - 85%

- 220-age= Maximum heart rate
  (the fastest your heart can beat)
  
  \(220-\text{age}=\text{Maximum heart rate}\)

  60%-80% of this max is your THR zone:
  - 60% of max: 180 x .6 = 108 beats per min,
  - 80% of max: 180 x .8 = 144 beats per min.

  Therefore the TARGET HEART RATE range for a 40-year old, working at 60-80% (up to 85% for very fit people) of his/her max heart rate is 108-144 beats/min.