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Research report

Acute and chronic exercise modulates the expression of MOR opioid receptors in the hippocampal formation of rats

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ABSTRACT

Exercise stimulates the release of beta-endorphin and other endogenous opioid peptides that are believed to be responsible for changes in mood, perception of pain and also performance. Although the vast majority of literature data support the role of physical exercise in increasing beta-endorphin levels, indirect measures such as increased endorphin levels in peripheral blood do not reflect opioid levels in the central nervous system. The purpose of the present study was to verify whether acute and chronic exercise using both voluntary and forced exercise procedures could modify the expression of μ-opioid receptors (MOR) in rat hippocampal formation. Immunoblotting analysis showed significantly enhanced MOR expression in the hippocampal formation in the acute (forced and voluntary) exercise groups when compared to the control group. Conversely, a significant reduction of MOR expression was noted in the chronic forced and chronic voluntary exercise groups compared to the acute forced and voluntary groups respectively. MOR expression was not significantly different in rats trained using both acute or chronic exercise. Immunohistochemistry analysis showed a higher number of MOR-positive cells for acute forced and voluntary exercise groups in the CA1, CA3, hilus and dentate gyrus regions compared to the control group. Our findings indicate that acute and chronic exercise modulates MOR expression in the hippocampal formation of rats.

1. Introduction

Physical exercise has been reported to induce several psychophysical effects, such as depression and stress reduction [46], mood elevation [51], and reduced pain perception [29]. Several hypotheses have been offered to explain these effects, including changes in various neurotransmitter systems. One commonly proposed theory, the beta-endorphin hypothesis [37], ascribes these effects to changes in central opioidergic transmission. However, the beta-endorphin hypothesis is not widely recognized because indirect measures such as increased beta-endorphin levels in peripheral blood [10,17,19] and cerebrospinal fluid [26] do not reflect the opioid levels in the central nervous system [47].

Few studies have examined central opioid activity with exercise. For instance, running has been shown to alter the levels of endogenous opioids in different regions of the rat brain [8,50]. Evidence for the role of the opioid system on the effects above is also derived from studies demonstrating that the opioid antagonist naloxone prevents elevation of the nociceptive threshold following exercise in normal subjects [15,24,29]. Thus, alterations in these receptors after physical exercise have been poorly explored. Pert and Bowie [43] demonstrated an increase in opioid receptor for enkephaline in the brain of rats after 15 min of fast exercise. Sforzo et al. [48] also verified an alteration in the opioid receptors of rats in a discrete brain area after swimming. Overall, there is a lack of information concerning the time course of opioid receptor expression and its role following physical exercise as well as information concerning the type of physical exercise that may exert a major influence in the expression of opioid receptors. Therefore, the purpose of our study was to verify whether aerobic physical exercise could alter opioid receptor expression in rat hippocampal formation. In this study MOR were analyzed after acute and chronic exercise (aerobic training program) using both voluntary and forced exercise.

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2. Materials and methods

2.1. Animals

Adult Wistar rats weighing 200–280 g at the moment of the initial physical training protocol were used. They were housed under environmentally controlled conditions (7:00–19:00 h light/dark cycle; 22–24 °C) and permitted free access to food and water throughout the experiment. The animals were divided randomly into five groups. Acute forced exercise ($n = 5$): animals submitted to running exercise in a treadmill for seven consecutive days. Acute voluntary exercise ($n = 5$): animals submitted to voluntary running in a wheel for seven consecutive days. Chronic forced exercise ($n = 5$): animals submitted to running exercise in a treadmill for 45 days. Chronic voluntary exercise ($n = 5$): animals submitted to voluntary running in a wheel for 45 consecutive days. The last group ($n = 5$) served as the control. A schematic diagram of the experimental design is presented in Fig. 1. All experimental protocols were approved by the ethics committee of the Universidade Federal de São Paulo (UNIFESP) and all efforts were made to minimize animal suffering in accordance with the proposals of the International Ethical Guidelines for Biomedical Research (CIOMS 1985).

2.2. Physical exercise procedure

Animals allotted to the exercise group were familiarized with the apparatus for three days by placing them on a treadmill (Columbus instruments) for 10 min/day at a speed of 12 m/min at a 0% degree incline. To provide a measure of trainability, we rated each animal's treadmill performance on a scale of 1–5 according to the following anchors 1 = refused to run, 2 = below average runner (sporadic, stop and go, wrong direction), 3 = average runner, 4 = above average runner (consistent runner, occasionally fell towards the back on the treadmill), 5 = good runner (consistently stayed at the front of the treadmill) [2,14]. Animals with a mean rating of 3 or higher were included in the exercise groups. This procedure was used to exclude possible different levels of stress between animals. Subsequently they were submitted to an aerobic exercise program of 45 sessions on a treadmill, 5 days per week. The intensity of exercise (60% VO_{2max}) was determined for each animal according to an established protocol [1,3]. Each training session started with a 5-min warm-up at 12–15 m/min. Running time and speed gradually increased from 30 min at 18 m/min during the first 3 days to 60 min at 18–22 m/min during the subsequent days. Exercise intensity was similar for all animals. Animals submitted to the voluntary exercise were placed in a wheel in which they could run voluntarily (Panlab Harvard Apparatus), with free access to food and water.

2.3. Immunohistochemistry

Immunohistochemistry was performed to analyze the expression of MOR in the hippocampal formation. Animals from both exercise and control groups (five from each group) were deeply anesthetized (chloral hydrate, 0.3 mg-kg, i.p.) and perfused transcardially with 0.01 M phosphate-buffered saline (PBS), followed by 4% formaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brains were removed, briefly post-fixed in 4% paraformaldehyde in PBS, and cut coronally with a cryostate in 40-μm thick sections. A sequence of three sections per animal (Bregma, −1.6, −2.6, −3.6 mm [40]) was selected for the immunocytochemistry process. The immunoperoxidase procedure was performed on free-floating sections using antibodies against MOR (polyclonal, 1:1000, Oncogene, USA). Paired slices of each group were processed in the same vial in order to minimize the differences during the immunohistochemical procedure. The sections were pre-treated with 0.1% H_2O_2 for 30 min to block endogenous peroxidase activity, rinsed in Tris–HCl, pre-incubated for 90 min in 0.1% normal serum in PBS with 0.1%, and then incubated in primary antibodies at 4 °C overnight. Sections were then rinsed in PBS, incubated in biotinylated anti-rabbit IgG (Vector) at a dilution of 1:500 in PBS (90 min at room temperature), rinsed in PBS, incubated in avidin–biotin peroxidase complex (ABC; Vector) for 90 min, washed several times in PBS, and then incubated in 0.06% diaminobenzidine in 0.001% H_2O_2. Tissue sections were washed in PBS, mounted on gelatin-coated slides, dehydrated, coverslipped with Entellam (Merck) and analyzed with a microscope NIKON HD, SONY-USA (NIH) under bright-field illumination. Cellular counts were quantified in subfields CA1, CA3 and dentate gyrus of the dorsal hippocampus. For each animal, the average number of neuronal cell bodies in a given region was obtained from the bilateral counts of three frames of each subfield (CA1, CA3, DG, and hilus) from each slice. Counts of MOR-positive cells were performed using the magnification of 200× and values were expressed as mean ± S.D. Briefly, image acquisition was performed using a system of digital camera Sony coupled to a light microscope, a Nikon Eclipse E600. Counts of MOR-immunopositive cells were performed using the image analysis software Image Tool-UTHSCSA.

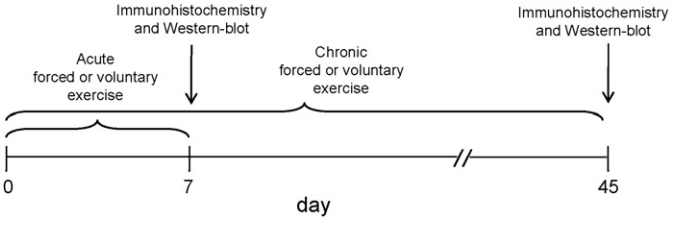

Fig. 1. Experimental design of physical exercise protocol and immunohistochemistry and Western-blot analysis for MOR expression in the hippocampal formation.

2.4. Western-blot analysis

For Western-blot analysis, rat hippocampi from the exercise group and the control group (five from each group) were homogenized in lysis buffer Tris–HCl (10 mM, pH 7.6) containing: 0.1 M NaCl, 10% glycerol, 1% NP-40, 0.001 M EDTA and protease inhibitor cocktail (Sigma, P-8340, −20 °C) containing: 10 μM PMSF, 1 mM sodium metavanadate, 2 nM okadaic acid, 2 g of NaF, 10 mg/ml aprotinin and 20 μM leupeptin. Samples were sonicated and protein concentration was determined by the Bradford method [9]. A standard curve was done to determine the linear rate of the method. In this line, 20 μg of protein was separated by SDS/polyacrylamide gel electrophoresis (10 cm × 10 cm; 10% separating gel; 4% stacking gel). The gels were blotted on nitrocellulose sheets (GE Healthcare Bio-Sciences, NJ, USA) in 25 mM Tris, 192 mM glycine, 20% (by vol.) methanol, pH 8.3. The blots were incubated at 4 °C overnight with a monoclonal anti-MOR opioid antibody (1:2000, Abcam). Anti-mouse peroxidase-conjugated secondary antibody (Vectastain, USA) was used for visualization with enhanced luminescence (ECL kit, GE) after exposure to X-ray film (Hyperfilm and ECL kitm Healthcare Bio-Sciences). The reprobing of membranes was required for incubation with monoclonal anti-β-actin immunoglobulins (Sigma–Aldrich, 1:1000). The blots were stripped by incubating with 0.1 M NaOH solution for 5 min at room temperature.

The molecular weights of MOR and β-actin (46 kDa, and 42–45 kDa, respectively) were determined by running a pre-stained protein ladder (Rainbow, Amersham-Pharmacia Biotech). Relative quantification of protein concentrations corresponding to the immunoreactive band was done by densitometric analysis using the image analysis system Densirag (Biocom, France). Protein expression of β-actin was determined to be an internal control whose expression is supposed not to change under experimental conditions.

2.5. Statistical analyses

Comparisons of the mean values for protein expression of MOR between exercise regimes, as analyzed by Western-blot and immunohistochemistry, were performed using one-way ANOVA followed by Tukey's test. For comparison of the running distance between groups, the Student's-t test was used. In all cases, the statistical significance was set at $p < 0.05$.

3. Results

Animals from the forced exercise program presented similar performance during the exercise protocol. Statistical analysis did not demonstrate significant changes in running distance between groups ($p > 0.05$). With the voluntary exercise, no significant differences among animals in running distance were found ($p > 0.05$). In addition, when running distance was computed for voluntary and forced running, no significant changes were observed among groups ($p > 0.05$) (Fig. 2).

Quantitative immunoblotting analysis showed a significant increase of MOR expression in the hippocampal formation of

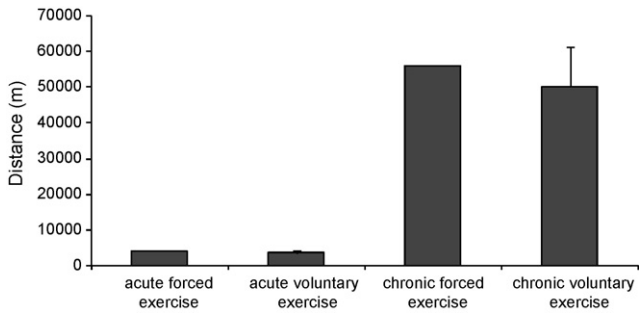

Fig. 2. Total running distance (mean ± S.D.) from animals submitted to voluntary (wheel) and forced (treadmill) exercise. Animals from acute forced and voluntary exercise were submitted to seven consecutive days of running. Animals from chronic forced exercise were submitted to 45 sessions of running, 5 days/week. Animals from chronic voluntary exercise were submitted to 45 consecutive days of running. No significant changes in running distance were noted between groups ($p > 0.05$).