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Positive changes in femoral nerve morphometry in older rats following aerobic training

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ABSTRACT

The objective of the present study was to analyze alterations of the femoral nerve of aged rats subjected to aerobic training. Wistar rats (12-mo of age) were divided in to two groups: S group (sedentary) and T group (trained). The exercise protocol were 16 weeks long. The groups were sacrificed at 16 months. Ultrafine sections of the femoral nerve have been used. There was no change in the body weight between the groups. T group showed a significant increase in myelinated fiber area, axon diameter, myelin sheath thickness and myelin fiber number compared with sedentary controls. In exercised trained animals, histograms of the frequency distribution of myelinated axons according to their areas showed increased number of medium and large fibers in relation to small fibers, which decreased in number. Aerobic training animals, showed the distribution of myelinated fiber population according to their area being bimodal, with the distribution shifted to the right, indicating increased fiber area. The T group showed a percent damage of large myelinated fibers significantly lower compared to controls. No significant difference was observed between the groups for the *g*-ratio. The T group also showed a significant increase in the number of microtubules and neurofilaments in myelinated fibers, which was not observed in S group. In conclusion, aerobic training improves nerve structure without evidence of nerve damage and produces an attenuation on the modifications in femoral nerve that develop in old age.

1. Introduction

Many researchers have used the laboratory rat to study the adaptation of peripheral nerve to chronic aerobic training (Samorajski and Rolsten, 1975; Nakano et al., 1997; Shokouhi et al., 2008) and much useful information has emerged from these studies. The objective of the present work was to extend previous findings in two important ways. First, the aerobic training in this experimental model can minimize deleterious effects of aging on myelinated peripheral nerve.

Shokouhi et al. (2008) examined the effects of long-term aerobic exercise training on lipid peroxidation, Schwann cell (SC) apoptosis and ultrastructural changes in the sciatic nerve of rats during aging. Three groups of 12-week old Wistar rats ran on a treadmill for 6, 9 and 12 months according to an exercise training program targeted at a speed of 22 m/min (at 7 degrees incline), 60 min/day, 6 days/week. The trained group had significantly diminished nerve lipid peroxidation and SC apoptosis. In the trained group, nerve fibers had a thick myelin sheath with frequent folding. These findings suggest that aerobic exercise training protects peripheral nerves by attenuating oxidative reactions, and preserving SCs and myelin sheath from pathologic changes, which occur during normal aging.

Sakita et al. (2014) investigate the age-related changes in the capillary architecture of the tibial nerve in spontaneous aging and with aerobic exercise intervention in rats. Aerobic training of low intensity was performed for 10 weeks using a treadmill. The capillary diameter, cross-sectional area and the number of microvascular ramifications in this group were significantly smaller than those observed in the control group. The authors concluded that aerobic exercise training protects peripheral nerves by attenuating oxidative reactions and protecting Schwann cells and myelin sheaths from the pathologic changes that occur during normal aging.

Functionally, there is a direct relationship between aging changes

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and motor and sensory disorders (Larsson and Ansved, 1995), including gait problems in the elderly, promoting falls, which constitute an important cause of hospitalization in this age group (Rubenstein, 2006) since several muscles of the lower limb are innervated by the femoral nerve. However, there are no observations of the femoral nerve where aged aerobic trained rats were compared with aged sedentary controls. In this study, the effects of aerobic training (for 16 weeks) on morphometry of myelinated fibers of the femoral nerve were assessed by comparison of two groups of aged rats: sedentary controls (S group) of the same age, and others of the same body weight as the trained group (T group).

Second, it is well known that the microtubules and neurofilaments are essential for the function of peripheral nerves because they determine the caliber of the nerve, the pattern of growth, stabilize the axolemma and encourage basic machinery required for both anterograde and retrograde axoplasmic transport (Ouyang et al., 2013). The effect of aerobic training on nerve density of microtubules and neurofilaments, in aged rats however, has not been investigated. The femoral nerves of aged trained rats were examined for evidence of influence on the components of cytoskeleton, and the results were compared with those observed at similar time points after 4-mo without training. In this way, the purpose of this study was to analyze morphometric parameters of the femoral nerve of elderly rats subjected to an individualized program of aerobic training.

2. Methods

All experimental procedures conformed to the guiding principles of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. The Institutional Animal Care and Use Committee of the university approved the experimental protocol (protocol number 059/09). The animals used in the present study were male Wistar rats (12-month-old) purchased from the Laboratory Animal Center of São Judas Tadeu University, São Paulo, Brazil). The rats were housed in plastic cages with access to food and water ad libitum and maintained on a 12 h light/dark cycle at room temperature (23–26 °C). The food stuff consumption of each rat per day and night was measured regularly.

2.1. Physical training

Rats of approximately the same age (12-mo-old) were randomly distributed in two groups: the age-sedentary controls (S group) (n = 8) and the trained group (T group) (n = 8). The animals of the T group were trained to perform physical training, voluntarily, running on a treadmill, five times a week. The program of physical training lasted 4 months and was divided into periods of 4 weeks. A test of maximum effort (MET) was carried out at the beginning of the experiment on an electric treadmill with a speed of 0.3 km/h. Every 4 min the speed on the treadmill was increased in the same proportion (0.3 km/h) (Fontinele et al., 2013). The METs were performed every 4 weeks for the T group. After the first MET, the animals of the T group were submitted to 4 months of training on the treadmill, 5 days a week, with progressive speed up to 60% of that achieved in the effort test. In the first week, after the test, the animals ran for 30 min, increasing this time by 10 min a week, until reaching 60 min in the fourth week. At the end of this period, another MET was performed to adjust the intensity of the training for the next 4 weeks. Rats in the S group were placed on the stationary treadmill daily for 10 min (Brooks and White, 1978). The rats of the two age groups were sacrificed at 16 months. The data obtained for the MET speed for the two groups of animals were tabulated, the averages calculated and statistically compared.

2.2. Measurement of body weight and systolic blood pressure

The body weight (BW) and systolic blood pressure (SBP) of each rat were measured at the beginning and at the end of the experiment. The SBP was verified by using the method of the tail cuff plethysmography.

2.3. Tissue preparation

Under pentobarbital anesthesia (40–50 mg: kg; i.p.), a small slice of the right femoral nerve (0.5 cm) was removed in its passage by the psoas major muscle in the pelvis and fixed in 2.5% glutaraldehyde. The slices were later osmicated in 1% OsO4 and included in Araldite resin. Semithin cross sections were cut at about 1 mm thickness and stained with toluidine blue after trimming under the light microscope. Ultrathin sections (40–60 nm) were cut perpendicularly to the axis of nerve fibers and after staining with uranyl acetate and lead citrate, were observed in a transmission electron microscope and microphotographs were taken under the electron microscope (JEOL Ltd., Japan). For light microscopy, the blocks were sectioned at 2 μm with every tenth section being saved and mounted on glass slides. Each set of semithin sections was stained with 1% toluidine blue.

2.4. Morphometric evaluation

Photomicrographs of the 2 μm cross sections were printed to make montages of the entire nerve at a final magnification of ×220. Five montages were made of each two age groups. The cross-sectional areas of the entire sections of the femoral nerve of both groups were measured using an image-processing system (Axio Vision, Zeiss). Numbers of myelinated fibers were counted manually.

For electron microscopy, electron micrographs of five areas from each femoral nerve – a total of 40 areas from each two groups (sampled by standardized random protocol designed to give every part of the nerve an equal chance of being sampled) – were taken at ×1500 and printed at a final magnification of ×5250. This represented an area of 2465 μm^2. The total number of myelinated fibers was counted in each micrograph. The myelinated fibers overlying the upper and left margins were included in the counts whereas those overlying the lower and right margins were excluded.

Myelinated fiber/axon diameters, were determined by measurement of their smallest diameters. Myelin sheath thickness was measured at 4 equidistant points of the fiber and averaged. Axonal diameters were calculated by subtracting ×2 the mean myelin sheath thickness from the measured fiber diameter. The ratio of axon diameter to the total fiber diameter (*g*-ratio) was also calculated for the two groups). Five electron photomicrographs from myelinated fibers per animal were taken (×50 000) and microtubules and neurofilaments were counted over a representative unit rectangular sampling area (3.2 μm^2).

2.5. Statistical analysis

We analyzed comparisons between groups along the time periods using a two-way ANOVA with repeated measures, followed by Bonferroni's post-hoc test. Statistical analyses were performed with GraphPad Prism software (version 4.0, San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effects of training on body weight, systolic blood pressure and maximum effort test

No differences ($p > 0.05$) were found on body weight and systolic blood pressure as showed on Table 1. The results obtained for the MET performed at the end of the experiment showed that animals in the 16-month-old T group showed no significant difference in maximum speed compared to the initial test ($p > 0.05$). For the animals in the 16-month-old S group, there was a significant decrease of the maximum speed at the end of the study, compared to the initial test (Table 1).