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TRAINING ALTERS CARDIAC NEURON SIZES IN WISTAR RATS

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Abstract. The action of the parasympathetic nerves on the heart is made through a group of neurons located on the surface of the atria. This study evaluated the effect of a chronic training protocol on the number and sizes of the cardiac neurons of Wistar rats. Whole mount preparations of the atria of 12-month old male sedentary and trained rats (40 weeks of running on a treadmill 3 times a week, 16 m/min) were assessed for number and size (maximal cellular profile area) of the cardiac neurons. The cardiac neurons were ascertained by using the NADH-diaphorase technique that stains the cell bodies of the neurons in dark blue. The number of cardiac neurons in the trained rats ($P>0.05$) did not change significantly. In the sedentary group there were small, medium sized and large neurons. However there was a notable increase in the percentage of small neurons in the rats submitted to the training compared to the sedentary group ($P<0.05$). Previous studies have shown that electrophysiologically, the small neurons are more easily excitable than the large neurons. It is possible that the results of the present work reflect an adaptation mechanism of the cardiac neurons presumably with the objective of increasing the excitability of the neurons for the vagal action and resulting facilitation of the sinusal bradycardia observed at rest and in the exercise. We concluded that the training affects significantly the size of the cardiac neurons in Wistar rats. ***(Biol.Sport 26:245-254, 2009)***

Key words: Cardiac neurons - Exercise - Morphometry - Rats

Introduction

The action of the autonomic nervous system in the control of the cardiac activity at rest and in the exercise is well documented. The parasympathetic tonus predominates at rest, whereas in the exercise, there is a rapid reduction of the vagal tonus and activation of the sympathetic division of the nervous system. Adversely,

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when the physical activity stops, the vagus nerve is once more activated and the action of the sympathetic nerves diminishes [11].

The vagal action on the sinuatrial node is made through a group of neurons located in the atrial subepicardium [6,19]. These neurons innervate the sinuatrial and atrio-ventricular nodes. In the adult rat, there are approximately 1100 cardiac neurons, whose size (maximal cellular profile area) varies between 100 and 1800 μm^2 [6]. Both the number and size of neurons may be modified by several factors. In a range of studies the neuron number and sizes have appeared as significant indexes displaying the age-related alterations in the intrinsic neurons of the trachea [3], small intestine [9,12] and heart [1]. Furthermore, counting of neurons and determination of neuron sizes have played an important role in studies that were aimed to analise the effects of pre-natal undernutrition on the rat intestines [2,25] as well as the effect of the Chagasic infection on the rat heart [(7].

There were many studies on the effects of physical activity in tissues and organs. However, the influence of the exercise on the size and number of cardiac neurons has not been studied so far. This work is trying to reply to the question: Does the aerobic exercise change the number and size of cardiac neurons? Based on the results, their possible relations with the vagal action in rest and in exercise, will be discussed.

Materials and Methods

Animals: Twenty 3-week old male Wistar rats (Rattus norvegicus) were used to carry out this study. These were divided at random in two groups: Group 1 (G1) – Sedentary control group, comprising ten 3-month old animals that were sacrificed at the age of 13 months; Group 2 (G2) Running rats, comprising ten 3-month old animals that were submitted to running on a treadmill for 10 months, sacrificed at the age of 13 months. The animals of both groups were kept in environmental conditions with controlled temperature (22º C) and lightness (cycle of 12 hours light/12 hours dark). A commercial special food for rats (Nuvital®) and water ad libitum were provided for the two groups.

Training program: Before training all rats were familiarized with the treadmill by walking/running for 10 min/day for 10 days. The training group commenced training at 10 m/min for 50 min/day.This was progressively increased throughout two weeks. Then, the rats were running continuously at 60 min/day, 3 days/wk. This training intensity and duration were maintained for 40 weeks. These speed/grade combinations have previously been shown to do not produce left ventricular hypertrophy [5]. This study has been conducted in conformance with

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the policy statement on research with experimental animals. Sedentary rats walked/ran on the treadmill 10 min daily to ensure similar handling.

Staining of the cardiac neurons: The NADH staining technique [8] was used to show the cardiac neurons. After the intraperitonial injection with Pentobarbital sodium (0.1 ml/100g body wt) to anesthetize the animals, these were perfused with Krebs solution. Once the thoracic cavity was opened, the blocks heart-lung were isolated and, subsequently, the atria separated from the ventricles and by careful dissection with stereoscopic microscope, the subepicardium fatty tissue was removed. The atria were left in a Krebs solution for 30 minutes and afterwards placed in Triton-X solution (0.3% in Krebs solution) for 10 minutes. They became slightly agitated and were then washed four times for 3 minutes in the Krebs solution. Then the parts were kept in the solution for incubation (25 parts of stock solution 0.5mg/ml of Nitroblue Tetrazolium in distilled H_2O, 25 parts of 0.1M phosphate buffer pH 7.3, 50 parts of distilled water and 0.5 mg/ml of b-NADH diaphorase [8] for 45 minutes, with the reaction monitored through a stereoscopic microscope. Once this time was over, the reaction was interrupted by the fixation of the material in buffered neutral 10% formalin solution (0.1 M sodium phosphate buffer, pH 7.3). The pieces were reduced to whole mount preparations through dissection under stereoscopic microscope and immediately after, washed in distilled water, and mounted in buffered glycerin jelly (0.2 M sodium phosphate buffer, pH 7.3) at 70º for light microscope examination.

Morphometry: The total number of neurons from each heart was counted directly in whole-mount preparations stained as described above, at a magnification of 120x. The results obtained from each animal in the groups were tabulated and the means and standard deviations calculated.

The size of the neuron bodies (maximum cell profile area) was determined by using an AxioVision (Zeiss) image analysis program, linked to a light microscope (Zeiss), increased by 40x. The profiles of 50 randomly chosen nerve cell pericarya were determined for each animal. Thus, 1000 nerve cell pericarya were outlined and measured using the image analysis program. All morphometric data were collected blindly, and the code was broken at the end of the work.

Statistical analysis: The averages of the number and size of the neurons were compared using the Student t test not paired ($P<0.05$). The ranges of cardiac neurons size groups (control and trained) were plotted on frequency histograms which were compared by the χ^2 test.

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Results

Morphology of cardiac neurons: The cardiac neurons were readily identified in the whole mount preparations of the atria stained for the dehydrogenase histochemical reaction. Most of the cardiac neurons were packed in ganglia, but some isolated neurons were also observed. There was a small variation in the intensity of stained cardiac neurons. The nerve cell profiles were rounded or pear shaped. There were no neurons within the atrial wall. A great variability in the size of the neurons was found in G1 (Fig. 1A, B). In the running rats (G2) the cardiac neurons were smaller than those in the group G1 (Fig. 1C, D).

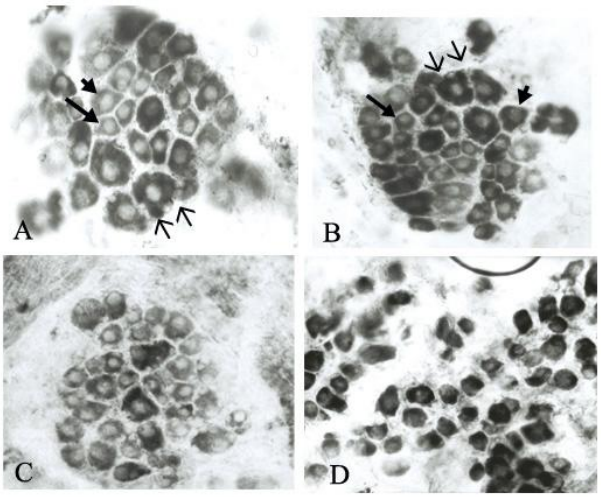

Figura 1

Fig. 1
NADH-stained cardiac neurons of sedentary (A, B) and trained (C, D) rats. Nerve cell bodies are intensely stained, except for the nuclei. In A and B, microscopic fields showing groups of large (double arrow), medium sized (arrowhead) and small neurons (arrow). In C and D, a great number of small neurons can be seen among medium sized and large neurons. (x256)

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Neuron counting and size: The number of neurons in the cardiac ganglia for the 2 groups studied were: 1071±98 (mean ±SD) neurons in G1, and 1104±205 in G2. No significant difference was observed in the number of neurons, between the 2 groups (P>0.05).

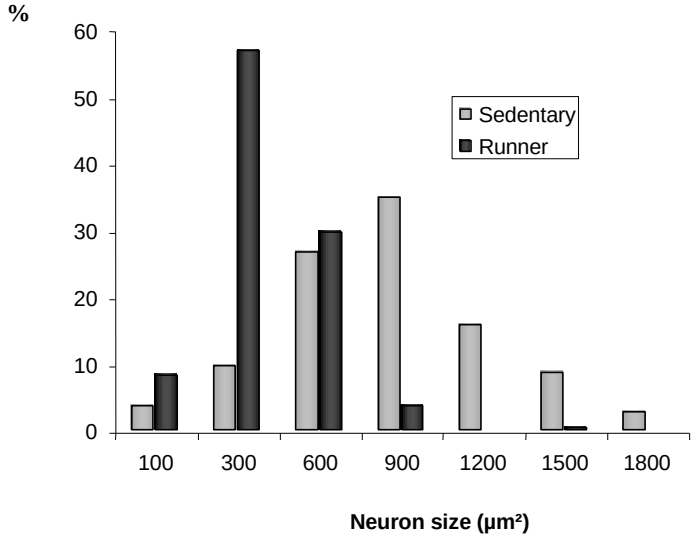

Fig. 2
Effect of training on distribution of the sizes of cardiac neurons in sedentary and running rats (24 weeks of running on a treadmill 3 times a week). Data are reported as a histogram of neuronal cell profile area for 1000 neurons measured in cardiac tissue from ten rats in each group. Chi-square test (P<0.05)

The sizes of the neurons (area of maximal cell profile) ranged from about 201 to 1583 µm² in G1 (mean ±SD=547±233), and 100 to 854 µm² (389±170) in the running rats (G2). The spread of neuron sizes is different in sedentary and running rats (Fig. 2). While in sedentary rats about three quarters of the neurons measure between 300 and 800 µm², with a peak at 500 µm² (maximal value=1583 µm²), in running rats, the same proportion of neurons spreads between 200 and 600 µm² with a peak at 300 µm² (maximal value=854 µm²). The histogram from the running rats shows a large shift to the left, and the average neuronal cell size decreases from 547 µm² to 389 µm². There was a significant difference between G1 and G2 (P<0.05). Based on the results obtained, we can arbitrarily divide the neurons in

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small (200-500 μm²), medium (501-800 μm²) and large neurons (801-1583 μm²) (Fig. 3). The data show that the percentage of small neurons practically duplicated and the percentage of the medium and large neurons significantly reduced in the running rats.

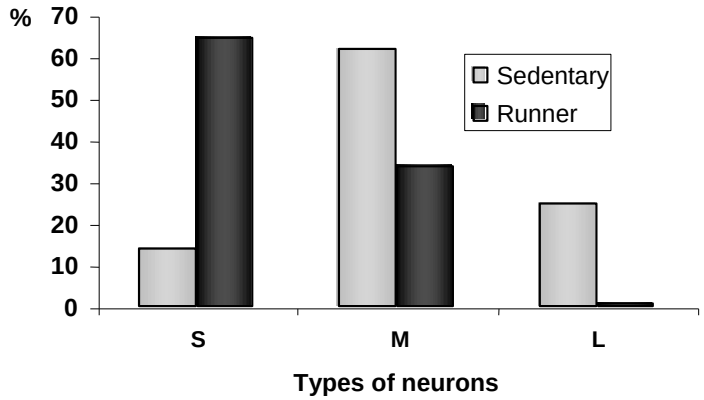

Fig. 3
Distribution of small, medium and large sized cardiac neurons in sedentary and running rats. Data are reported as a histogram of neuronal cell profile area for 1,000 neurons measured in cardiac tissue from ten rats in each group. Chi-square test ($P<0.05$)

Discussion

As far as we know, this is the first work to report on cardiac neurons resulting from exercises. These neurons, located on the outer surface of the atria, underneath the epicardium, make up a total of approximately 1000 neurons in rats. The cardiac neurons receive synapses from parasympathetic preganglionar vagal fibers, whose cell bodies are found in the nuclei of the brain stem [10]. Some of cardiac neurons send fibers to the sinuatrial node cells [19] for control of the cardiac frequency while the others send fibers to other neurons of the heart itself, acting as association neurons [28]. The cardiac neurons are located at least in 4 distinct groups, all above the atrioventricular groove [6]. Retrogradely transported

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fluorescent tracers injected into the left or right ventricles demonstrated that different groups of ganglion cells projected to discrete or selective regions of the heart [19]. All cardiac neurons are cholinergic and some are also nitrergic [28]

The number of cardiac neurons may be decreased through several factors such as aging [1], diabetes [14,23] or infectious diseases such as the Chagas disease [24]. As cardiac neurons are part of the cholinergic system of the heart, a decrease in their number induce severe increase in chronotropic response to vagal stimulation [15,21,22,29].

The present work revealed that the number of heart neurons was not changed by the exercise. The data assessed used the total number of neurons present in the atria. The neurons were stained by a technique that successfully shows up the total number of cardiac neurons [6]. Moreover, by using this technique, measurements of cell somata are not conditioned by chemical fixation, dehydration, embedding and sectioning [20].

With regard to the autonomic cardiac activity, it is known that the exercise leads to a reduction of the sympathetic action and to an increase of the action of the parasympathetic nerves [4,18,13,16,31]. Although the bradycardia induced by the exercise has been studied by several authors, the possible participation of the cardiac neurons in this process has not been considered. Previous studies have shown that there is a relationship between the size of the neuron and its excitability threshold: small neurons are more easily excitable than large ones [30]. The results of the present work show that in untrained rats, cardiac neurons are of several sizes: small, medium and large neurons, with predominance of small and medium over large ones. As there was no change in the number of neurons, we can state that in the group of trained animals, there was a significant increase in the number of small neurons, with a decrease in the number of medium and large ones. Thus, this increase can only have taken place through the reduction in size of the medium and large neurons. Therefore, the training leads to a size reduction of the cardiac neurons. This reduction may be an adaptation mechanism possibly to make the neurons more easily excitable, facilitating the vagal action both at rest and in the exercise. This reasoning is in agreement with some authors [26,27], when they state that the rest bradycardia promoted by the training is due to an increase in the vagal activity. However, others [18] state that the rest bradycardia observed in trained animals is due to the change in sinuatrial node cells. Nevertheless, this hypothesis was not proved. Possibly, the two hypotheses may take place: alterations in the cells of the sinuatrial node and reduction in the size of the neurons discussed in this work.

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Moderate physical exercise, increasing the vagal tonus and diminishing the action of the sympathetic nerve, protects the heart. On the contrary, heart disease is often characterized by a lessening of the vagal activity and an increased sympathetic nerve tonus [11].

Size reduction of the neurons, suggesting a retraction of the cell body, was also observed in other conditions, such as the Chagas disease [24], perhaps with the same purpose: to increase the excitability of the neurons but, in this case, perhaps due to the destruction of many neurons by the infection. The mechanisms through which the neurons diminish in size are unknown, but the results of this work show another interesting side of the neuronal plasticity. Other studies in this area are necessary to explain the functional implications of the change in size of the cardiac neurons induced by the training, observed in this work.

In conclusion, the aerobic training (running on a treadmill), maintained for 6 months, significantly diminishes the size of cardiac neurons in rats, which may be related to the vagal activity in the heart of athletes.

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