Swimming Physical Training Promotes Cardiac Remodeling and Improves Blood Perfusion in the Cardiac Muscle of SHR Via Adenosine-Dependent Mechanism

EXERCISE AND SPORTS SCIENCES

ORIGINAL ARTICLE

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ABSTRACT

Physical training (PT) has been used as non-pharmacological therapy for hypertension treatment and swimming physical training is recognized for yielding cardiac remodeling in experiments. However, little is known on the effects of adenosine (Ado) resulting from PT as hypertension prevention and treatment. Objective: To evaluate cardiac remodeling and the role of adenosine in cardiac blood flow distribution (BF) to the myocardium after aerobic PT on SHR. Methods: 28 male SHR, babies and adults, were submitted to swimming training protocol during 10 weeks (5 times a week – 1 h a day). Colored micro spheres protocols were used to evaluate blood flow, morphological techniques were used to evaluate cardiac hypertrophy and biochemical analysis were performed to verify enzyme activity in the adenosine formation. Results: PT attenuated the evolution of hypertension in the SHR babies group (S: 145 2; T: 140 2mmHg), HR was lower in adult SHR (S: 340 4; T: 321 6bpm) and CH increased in both groups (TB: 12%; TA: 10%). At basal condition, BF was increased in trained babies (S: 4.745 ± 2.145; T: 6.970 ± 2.374mi/heart) and higher vasodilatation response was observed due to adenosine infusion (S: 18.946 ± 6.685; T: 25.045 ± 7.031mi/heart). In this group, the PT promoted a higher 5'-nucleotidase enzyme activity leading to a higher adenosine formation (S: 0.45 ± 0.09; T: 1.01 ± 0.05). Conclusion: The swimming training developed CH as well as increased adenosine formation, leading to higher coronary blood flow, and its important role in hypertension regulation was demonstrated.

Keywords: hypertrophy, coronary blood flow, colored microspheres.

INTRODUCTION

In SHR experimental animals, the effects of physical exercise on blood pressure depends on age as well as level of hypertension these animals began the training protocol with[1]. Hypertensive humans and trained SHR rats present rest BP decrease associated with decrease of peripheral sympathetic activity as well as cardiac debt[2]. In such aspect, the exacerbated normalization of cardiac sympathetic tonus observed in trained SHR rats would be associated with bradycardia at rest and consequently to reduction of cardiac debt.

Low-intensity physical training causes significant reduction of peripheral vascular resistance, determined by reduction of the vasoconstriction, improvement of endothelial function as well as structural alterations of the microcirculation. SHR submitted to low intensity aerobic training (50 to 60% VO_{2max}) present consistent decrease of the pressoric levels[3].

Besides the coronary vasodilator activity, adenosine plays an important role in the regulation of the coronary blood flow, presenting an important role in the cardiovascular system[4]. Thus, the presence of suitable levels of vascular adenosine released during exercise practice can be important in the prevention and treatment of hypertension, and can influence the blood flow to the myocardium. Thus, the aim of this investigation was to evaluate the cardiac remodeling and role of adenosine in the distribution of blood flow to the myocardium after physical training in SHR.

METHOD

Sample: 28 male SHR aged one (baby) and three (adults) months, offered by the Animal Facility of the Medicine School of USP, divided in four groups: sedentary babies (group 1); trained babies (group 2); sedentary adults (group 3) and trained adults (group 4). The rats were kept in cages in the Animal Facility of the Laboratory of Motor Activity Biochemistry, in a place with room temperature between 22° and 24°C and controlled light in inverse cycle of 12h (light-dark). Food and water *ad libitum*. The research named "Adenosine role as regulator of the blood flow in young and adult spontaneously hypertensive rats: effects of physical training" was approved by the Ethics in Research Committee of the Medicine School of USP under research protocol number 961/06. The weekly ponderal control was performed on a semi analytical precision scale (Gehaka) during the entire study period.

Physical training of the animals: performed according to protocol adapted from Medeiros[5]. Five times per week, during 10 weeks with duration of 60 minutes and gradual work overload increase (weight on tail) until 5% of body weight of the animal is reached (table 1).

Swimming protocol

Table 1. Swimming protocol, with training time from the 1st to the 10th week, performed from Monday to Friday.

Weeks	Monday	Tuesday	Wednesday	Thursday	Friday
1st	15min. n/o	20min. n/o	40min. n/o	60min. n/o	60min. n/o
2nd	40min. 3%bw	50min. 4%bw	60min. 5%bw	60min. 5%bw	60min. 5%bw
3-10th	40min. 5%bw	50min. 5%bw	60min. 5%bw	60min. 5%bw	60min. 5%bw

(n/o) = no overload (bw) = body weight.

Measured straight from the blood pressure and heart rate: the animals were anesthetized with sodium pentobarbital (40mg/kg, ip) and cannulated 24 hours after the last training session. Two P10 catheters filled with saline were inserted in the right femoral artery and vein. A third P50 polyethylene catheter was used for the cannulation of the left ventricle by the right carotid artery. The catheter was then inserted until the ventricle and its position determined by the observation of the characteristic of the wave of ventricular pressure and subsequently confirmed by necropsy. The cannule was connected to an electromagnetic transducer (P23 Db; Gould-Statham) and to an amplifier (General Purpose Amplifier-Stemtech, Inc.) with realtime register (SIATEMA CODAS) at sample frequency of 1,000Hz per channel.

Microspheres infusion into the left ventricle: solution containing 200,000 white spheres/180µl was infused in the left ventricle through the P50 (75cm) catheter extension, which was connected to a 1ml syringe with pre-heated saline (40ºC) containing Tween 80 (0.01%). The other cannule positioned in the abdominal aorta was connected to a pre-heparinized 1ml syringe for blood withdrawal during the infusion. Ten seconds prior to the spheres injection, the blood withdrawal was initiated with the use of a pump (Infusion and Withdrawl Pump, Harvad Apparatus, South Natick, Mass, USA) at a flow of 0.5ml/min, continuous for 75 seconds after the beginning of the infusion. On a second phase, the procedure was repeated; however, with red microspheres, anteceded by the administration of the adenosine vasodilator at the 300µg/kg/min dose, through the cannule placed in the femoral vein. Each blood sample withdrawn was weighed and identified. A total of 4ml of hemolysis reagent was added and the sample was centrifuged during 30 minutes at 2,000g. The supernatant was discarded and 2ml of sodium hydroxide (2N) was added to the tube. The samples were incubated in water bath (WB) at 70°C, following from this time on the same procedure of the remaining tissues.

Morphological study: at the end of the experimental protocol the animals were decapitated, their hearts removed from the thoracic cavity and dissected to separate the LV (free wall of the left ventricle and septum), RV (right ventricle) and atria (right and left atria). The cardiac hypertrophy was assessed from the relation between the LV cavity weight (humid weight) and the body weight of the rat in mg/g.

Tissues: processed according to technique adapted from Hakkinen et al.[6]. After the tissue removal, it was weighed and identified. After addition of 4ml of sodium hydroxide (2N), the tubes were covered and placed in water bath (WB) at 70ºC for approximately two hours. The samples were agitated (Vortex Maxi Mix II, Thermolyne, Dubuque, Iowa, USA) at every 15 minutes until tissue dilution in the digestion I and II reagents. The absorbance readings in the spectrophotometer (DU-640 Spectrophotometer, Beckmann Instruments, Inc., Fullerton, CA, USA) were performed on a 0.7ml quartz cube tray (Sigma), where 200µl of the supernatant of each centrifuged sample were added. The absorbance spectra peaks of the white and red microspheres were respectively 370 and 530nm, using light band width < 1.8nm. The minimum accepted absorbance was of 0.01AU.

Determination of the microspheres number: solutions with a known number of microspheres were processed. The absorbance mean of these samples allows that a spectrophotometer reading constant for the red and white microspheres is determined. Calculation of the number of microspheres in the tissues:

$$\text{Number of spheres in the sample} = \frac{\text{AU sample} \times \text{Number of standard spheres}}{\text{AU standard}}$$

(AU sample = tissue absorbance in the tissue; Number of standard spheres = number of spheres in the standard solution; AU standard = mean of the standard solutions absorbance).

Preparation of the blood serum fraction: in order to obtain the blood serum fraction, the animals were sacrificed by decapitation and the blood collected and centrifuged at 3,000rpm for 15 minutes at 4ºC. The supernatant obtained from each centrifuged sample represents the fraction of the blood serum. This fraction was stored in Eppendorf tubes (1.5ml), identified and kept at –20ºC until their utilization.

ATP-diphosphohydrolase activity: incubation containing Tris-HCl 112.5mM (pH 8.0), with the protein at a 37ºC temperature for 10min in a final volume of 190ul for both through the use of serum, as described by Oses et al.[7]. The reaction started by the addition of nucleotide (ATP, ADP) for a final concentration of ATP 3mM and ADP 2mM. The incubation time and the amount of protein added to the reaction medium were chosen in order to guarantee the linearity of the product formation.

5'-nucleotidase activity: the protein which was pre-incubated for 10 minutes at 37ºC is added to a medium containing Tris-HCl 100mM, pH 8.9. The reaction was initiated by the addition of AMP 2mM. The enzymatic reaction was interrupted by the addition of 200ul of trichloroacetic acid (TCA) final concentration of 10%. The samples were kept on ice, for at least 10 minutes, centrifuged (5,000rpm, 4°C, 15min) and, subsequently, aliquots of 100ul were removed for inorganic phosphate determination by the method by Lanzetta et al.[8]. The non-enzymatic hydrolysis was corrected by controls, in which the enzymatic material was added to the tube after the reaction had been interrupted with TCA. The samples were read in a spectrophotometer (630nm) and the specific activity was expressed in phosphate nmol released per minute and per mg of protein (nmol Pi/min/mg). The protein was determined by the Bradford method[9], using bovine serum albumin as standard (BSA, 1mg/ml).

Statistical analysis

Two-way ANOVA was used for analysis of the differences between groups and Tukey post hoc was used when statistical significances were observed for all the experiments. Student's t test was used to verify the distribution of the microspheres before and after the adenosine injection. The significance level adopted in all experiments was of $p \leq 0.05$.

RESULTS

Swimming physical training (PT) was efficient in reducing blood pressure of adult SHR (SA: 161 ± 2; TA: 149 ± 2mmHg) and attenuated the blood hypertension evolution (HBP) in baby SHR (SB: 145 ± 2; TB: 140 ± 2mmHg) – figure 1. The hemodynamic alteration demonstrated by the BP decrease was followed by statistically significant bradycardia at rest in adult animals (SA: 371 ± 10; TA: 312 ± 21mmHg).

The results presented in figure 2 demonstrated the cardiac hypertrophy indices (weight of left ventricle + right ventricle/ body weight) obtained after the protocol: 12% and 10% for the baby and adult groups, respectively.

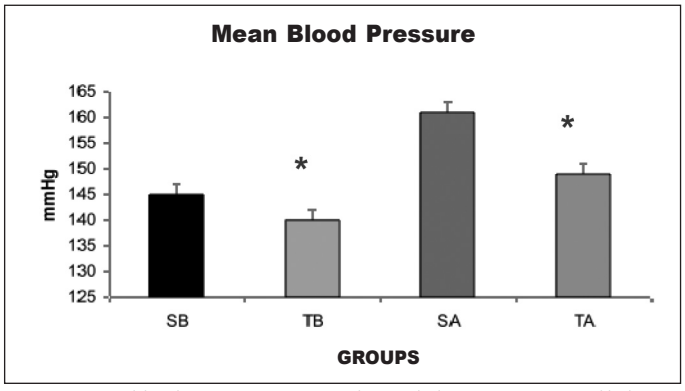

Figure 1. Mean blood pressure (mmHg). Sedentary baby (SB, n = 7), trained baby (TB, n = 7), sedentary adult (SA, n = 7) and trained adult (TA, n = 7). Values are expressed in mean ± standard deviation. (*) p < 0.05.

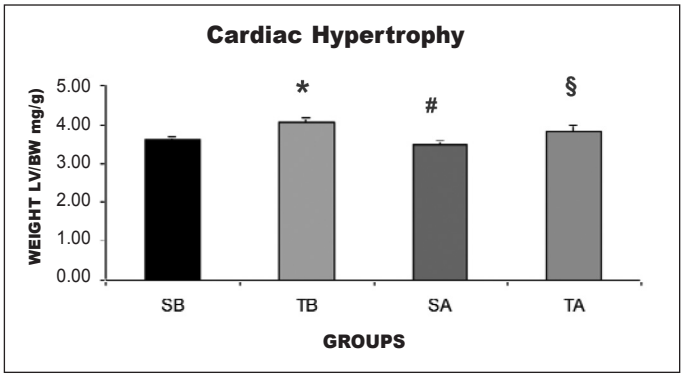

Figure 2. Cardiac hypertrophy (mg/g). Sedentary baby (SB, n = 7), trained baby (TB, n = 7), sedentary adult (SA, n = 7) and trained adult (TA, n = 7). Results presented in mean ± standard error, (*) p < 0.05. * P = 0.002 (SB vs.TB); # P < 0.001 (TB vs. SA); § P = 0.029 (SA vs.TA).

The distribution of the blood flow at rest (basal) and after infusion of the adenosine vasodilator to the myocardium is demonstrated in figures 3 and 4, respectively. Figure 3 presents the increase in the number of spheres after adenosine infusion, both in the sedentary baby group and trained baby group. The number of spheres in the basal trained baby group is 1.92 times higher than in its basal control, with no adenosine infusion.

Increase in the number of spheres after adenosine infusion was observed only in the sedentary adult group, as demonstrated in figure 4. In the trained adult group post-adenosine infusion, there was a tendency to vasodilatation, but without statistical significance. It was observed that the basal number of spheres in the trained adult group is 1.83 times higher than in its basal control group, without adenosine infusion.

No statistical differences were observed between groups in the ATP and ADP hydrolysis in the fraction of blood serum; however, it was observed in the AMP hydrolysis that the trained baby rats presented higher enzymatic activity of the 5´-nucleotidase, as seen in figure 5.

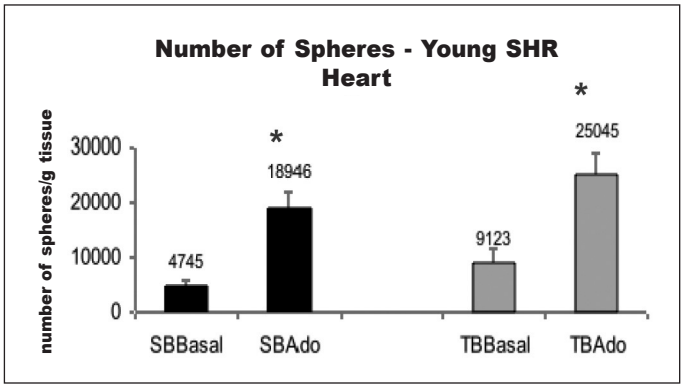

Figure 3. Basal coronary blood flow (number of spheres/tissue weight). Sedentary baby (SB, n = 5), trained baby (TB, n = 5), sedentary adult (SA, n = 5) and trained adult (TA, n = 5). Results presented in mean ± standard error, p < 0.05.

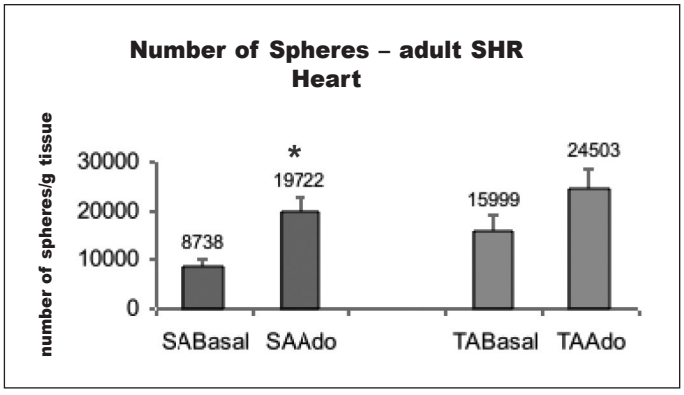

Figure 4. Coronary blood flow after adenosine (number of spheres/weight tissue). Sedentary baby (SB, n = 5), trained baby (TB, n = 4), sedentary adult (SA, n = 4) and trained adult (TA, n = 4). Results presented in mean ± standard error, p < 0.05.

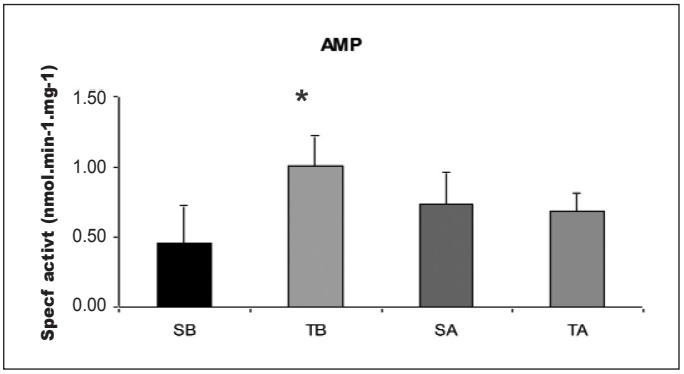

Figure 5. Measurement of the enzymatic activity of 5'-nucleotidase in the fraction of blood serum through AMP hydrolisis (nmol Pi/min/mg protein). Sedentary baby (SB, n = 7), trained baby (TB, n = 6), sedentary adult (SA, n = 10) and trained adult (TA, n = 7).

DISCUSSION

In the present study, the 10-week training protocol by swimming was efficient in reducing the MBP values of the trained adult animals and attenuated the hypertension evolution in trained baby rats. This reduction in the pressoric levels is in concordance

with previous studies conducted with hypertensive individuals, as well as in young SHR rats with PT on treadmill[10,11] and swimming[1]. A suggestion to evidence the mechanisms involved in the BP attenuation induced by exercise would be to reduce the cardiac debt verified in trained animals[12], since the HR reduction could justify the effect of the aerobic exercise decreasing the cardiac debt and consequently blood pressure. Another factor responsible for the decrease of the pressoric values of trained SHR could be the attenuation of total peripheral vascular resistance by the increase of the vascular complacence, mainly arterial, induced by exercise. Amaral et al.[13] verified that SHR submitted to training on treadmill present increase in venule density.

HR at the end of swimming training of the TA group showed significant attenuation after the protocol. Although a tendency to bradycardia at rest has been observed also in group TB, this alteration was not significant. Bradycardia at rest is an adaptation characteristic of the organisms to the aerobic training and it is considered a marker of physical training. Similar results were described in previous experiments conducted by Zamo et al.[1] in which the young trained SHR presented bradycardia at rest after exercise protocol and that this bradycardia was kept from the fourth training week on[5,12]. Medeiros et al. (2000) reports that bradycardia at rest resulting from swimming PT does not occur by the decrease in sympathetic activity or by the decrease of the rate intrinsic to the heart[12], but due to the increase of vagal tonus.

Concerning the developed cardiac hypertrophy, the data described in the literature demonstrate left ventricle/body weight ratio (LV/BW) indices at about 7% of left ventricular hypertrophy in adult trained SHR with swimming[14]. In this study, indices of 12% were observed in baby trained rats and of 10% in adult trained rats, above the indices found in the literature. Similar results were observed by Redondo (2007), who observed cardiac hypertrophy of 16% in normotensive rats trained in swimming. Evangelista et al. (2003)[16] describe that the ventricular hypertrophy index is dependent on the characteristics of the physical training. The cardiac hypertrophy in this experimental model can be evaluated by direct measurement of the diameter of the cardiac myocytes. Increase of the cardiomyocytes diameter can be observed as a response to the swimming aerobic physical training, consequence of volume overload by the preload increase[17,18]. Data from our laboratory corroborate these results, in which increase in the diameter of cardiomyocytes of 32% was observed in young trained SHR and of 38% in trained SHR trained with swimming protocol, when compared to their controls, and this increase was not followed by alterations n the interstitial collagen volume. As increase in collagen deposition was not verified, the LVH was considered physiological.

Coronary blood flow was determined through the number of spheres attached to the tissue in ml/min, and, although they demonstrate similar results, statistical differences have not been observed either. Such fact is due to the fact of the lower number of animals which compose the sample when the blood flow is determined in ml/min, and this smaller sample is a result from limitations which occurred during the experiment.

In our study higher blood flow was observed in the basal period of the rats trained with swimming protocol, demonstrated through the number of spheres found in the tissue. This higher blood flow can be a result of greater ventricular filling which occurs during the cardiac diastole in response to physical training. Thus, bradycardia at rest observed as response to physical training in this study, is a possible explanation to the increase in coronary blood flow observed in the trained rats.

Rocha et al. (2007)[19], in a study conducted in our laboratory and with the same physical training protocol, promoted improvement in the cardiac positive inotropic status observed by the increase of the contractility indices (+dP/dt) and improvement of the diastolic function through reduction of initial diastolic pressure of the left ventricle of the rats. In SHR submitted to the swimming protocol, the training also promoted improvement in the derivate left ventricle contraction, but with no statistical difference in the final diastolic pressure of the left ventricle[1]. Thus, physical training promoted cardiac hypertrophy and proportional increase in the coronary blood flow associated with the maintenance of the cardiac function. Muders and Elsner[20], in 2000, demonstrated preservation of diastolic function of SHR during their first year of life.

When the vasodilator response to adenosine is considered, it can observed that in the SB, TB and SA groups increase in the coronary blood flow occurred due to the presence of a higher number of spheres in the cardiac tissue. The number of spheres of the TA group after adenosine infusion was not statistically significant, despite a tendency to increase of blood flow in this sample. Since the adult trained rats started from a basal condition with greater vasodilation they did not present higher response to the vasodilation by the drug, as they already possess mechanisms of self-regulation in response to the established hypertension.

In this study, the adenosine was evaluated through the activity of enzymes involved in the hydrolysis cascade of the adenine extracellular nucleotides, which have as final product the adenosine formation. The activity of the ATP-diphosphohydrolase enzymes which hydrolises ATP and ADP in AMP, and 5'-nucleotidase which hydrolises AMP in adenosine were measured in the blood serum fraction which represents the formation of systemic adenosine. The increase of the adenosine production observed in the TB group in our study corroborates the data by Pierce et al. (1989), who demonstrated that swimming training in rats promotes increase of the activity of the 5'-nucleotidase enzyme as well as increase in the adenosine release in the myocardium, leading to greater vasodilation and influencing on the blood flow to the myocardium. Moreover, the vasodilator effect depends on the variety of the used drug and adenosine does not require endothelial participation in relaxation.

Higher AMP hydrolisis was also observed in a study conducted by Redondo (2007), where the normotensive rats group submitted to training with swimming protocol also presented higher adenosine formation, indicating once again the participation of the way of this nucleotide in the hypertension prevention and treatment during exercise.

CONCLUSION

Physical training attenuates the pressoric levels of SHR regardless of the age at which the experimental protocol begins, promotes bradycardia at rest and leads to a cardiac remodeling by the increase of the left ventricular hypertrophy. These factors, associated to the increase in the coronary blood flow, probably mediated by higher formation of circulating adenosine in trained baby rats, plays hence an important role in the prevention of HBP and better blood perfusion in the cardiac muscle.

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All authors have declared there is not any potential conflict of interests concerning this article.

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