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EFFECTS OF CHRONIC SWIMMING ON BLOOD PRESSURE AND SODIUM PUMP OF HYPERTENSIVE RATS

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ABSTRACT

Osiecki R, Malfatti CRM, Royes LFF, Sampedro RMF, Mello CF. Effects of chronic swimming on blood pressure and sodium pump of hypertensive rats. *JEPonline* 2008;11(5):35-41. In this study we investigated whether chronic exercise alters erythrocyte Na⁺,K⁺-ATPase activity and hemodynamical parameters in adult male spontaneously hypertensive rats (SHR). Animals were randomly assigned to trained (n= 8) and untrained (n= 8) groups. Training was conducted 6 days/week for 12 weeks, alternating 60 and 90 min swimming sessions daily. At the end of the training program, animals underwent a 90 min swimming challenge and blood lactate was measured. Twenty hours after the challenge, a cannula was introduced into the right carotid artery for direct recording of the blood pressure and heart rate. After obtaining hemodynamical measures, blood was collected for erythrocyte Na⁺,K⁺-ATPase activity. Training significantly reduced systolic (-9.2 %; p<0.01), diastolic (-13.3 %; p<0.01), mean blood pressure (-11.3 %; p<0.001), resting heart rate (-14.5 %; p<0.001), plasma lactate levels (-44.8%; p<0.05) and a higher erythrocyte Na⁺,K⁺-ATPase activity (41.5 %; p<0.05). It is suggested that the exercise-induced increase of erythrocyte Na⁺,K⁺-ATPase activity may contribute to decreased blood pressure.

Key Words: Na⁺,K⁺-ATPase, Exercise, Hypertensive.

INTRODUCTION

The pathogenesis of essential hypertension is poorly understood, although accumulating evidence suggests that genetic and environmental factors are of important etiological relevance [1]. One of the factors involved in the development of essential hypertension is the alteration of cellular sodium metabolism. It has been suggested that biochemical and biophysical abnormalities of cell membranes [2] may actively participate in the pathogenesis of hypertension [3], and that such abnormalities seem to be involved not only in vascular smooth muscle cells, but also in circulating blood cells [4]. In fact, it has been reported that viscosity and rigidity of erythrocyte membranes are increased in spontaneously hypertensive rats (SHR) and in patients with essential hypertension [5], and that erythrocyte membrane fluidity depends on Na^+, K^+ -ATPase activity [3]. Interestingly, erythrocyte Na^+, K^+ -ATPase activity is diminished in hypertensive patients, and enzyme activity is restored to normal by a calcium channel blocker [6]. These findings reinforce the view that alterations in erythrocyte Na^+, K^+ -ATPase activity are linked to hypertension.

Numerous investigators have reported, based on the results of experimental, clinical and epidemiological studies, that exercise reduces blood pressure and prevents the occurrence of hypertension in animals and humans [7,8]. In fact, blood pressure of SHR is reduced by forced running on a motor-driven treadmill [9], or by swimming [8]. Moreover, the development of hypertension in Dahl salt-sensitive hypertensive rats is prevented by exercise [10]. Blood pressure is reduced by various forms of exercise in young and elderly hypertensive and normotensive men and women [8]. While physical exercise ameliorates hypertension and its complications [8], it is not clear whether physical exercise alters Na^+, K^+ -ATPase activity, as well. Therefore, the present study was undertaken to investigate the effects of chronic low intensity swimming and its hemodynamic influences on resting heart rate, blood pressure and on the erythrocyte Na^+, K^+ -ATPase activity of SHR.

METHODS

Subjects

Adult male spontaneously hypertensive rats (12 week old) were obtained from the SHR colony of the Federal University of São Paulo, São Paulo, Brazil. The rats were housed four per cage (40 x 25 x 18 cm) and maintained on a 12:12 h light/dark cycle, with free access to tap water and standard lab chow (Guabi, Santa Maria, RS, Brazil). The animal utilization protocols followed the Official Government Ethics guidelines and were approved by the University Ethics Committee.

Procedures

All reagents were purchased from Sigma (St. Louis, MO, USA) and all solutions were prepared with type I ultra pure water. The rats were randomly assigned to nontrained ($n= 8$) and trained ($n= 8$) groups. Training procedure was adapted from the procedure of Gobatto et al. (11), as follows: four rats swam together in a round swimming pool (75 cm in diameter, 40 cm deep) that had its temperature kept at 32 ± 1 °C throughout the experiments. The dimensions of the pool allowed the animals to swim freely, and not float passively, as it was deep enough so that they could not rest upon an extended tail. The training program was conducted 6 times a week. Monday, Wednesday and Friday it consisted of 90 minutes of swimming without load. Tuesday, Thursday and Saturday the animals were subjected to a 30 minutes swimming trial with working load (8% of body weight), which was followed immediately by a 30 minutes trial without load. The control group (nontrained) was handled for 3 min, 6 times a week for 12 weeks, in order to habituate to the experimental protocol. Twenty-four hours after the last swimming session all the animals (control and trained) were subjected to a single 90 min swimming challenge, without load, and had their tail blood lactate

measured by using a portable lactate analyzer (Accusport).

Twenty-four hours after the 90 min swimming challenge, the rats were cannulated. Under halothane anesthesia, a siliconized polyethylene cannula (PE-50) was introduced into the right carotid artery for direct recording of the blood pressure. The distal end of the cannula was exteriorized on the animal's back and sutured in the skin. The cannula was flushed every 12 hours with heparinized physiological saline (20 U/ml) to keep it patent. Twenty four hours after artery cannulation, hemodynamical measures were carried out in a sound-proof quiet room. Animals were kept alone, unrestrained, in small cages that allowed limited movements. The cannula was connected to a strain-gauge transducer (P23 Db; Gould-Statham), and the signal was fed into an amplifier (GPA 4-channel, model 2; Stemtech) and then to a PC-type computer for direct arterial pressure measurements. Systolic (SBP), diastolic (DBP), mean blood pressure (MBP) and heart rate (HR) were recorded on a beat-to-beat basis (AT/CODAS) at a frequency of 1000 Hz for 30 min. Twenty-four hours after blood pressure measurement, the rats' blood was collected into a tube containing sodium heparin as anticoagulant (1000 U), and erythrocyte ghosts were prepared as described by Niggli et al. [12]. The protein content was measured by method of Bradford [13]. The total Na^+, K^+ -ATPase activity was assayed at 37 °C in an incubation mixture containing: 30 mmol/L Tris-HCl, pH 7.4, 0.1 mmol/L EDTA, 50 mmol/L NaCl, 5 mmol/L KCl, 6 mmol/L MgCl_2 , 1 mmol/L ATP in the presence or absence of ouabain (0.5 mM), as described by Reinila et al. [14]. Briefly, after preincubating the isolated membranes (50 μg) for 10 min at 37 °C, the reaction was started by the addition of ATP and stopped with 50 μl of TCA (30 %), after 20 min. The amount of inorganic phosphate released was determined by the method of Lanzetta et al. [15] and Na^+, K^+ -ATPase activity was calculated as the difference between "total" and ouabain-sensitive Na^+, K^+ -ATPase activity. All reagents were purchased from Sigma (St. Louis, MO).

Statistical Analyses

All data are expressed as means \pm SEM. The statistical analyses of hemodynamical (blood pressure, heart rate) and biochemical (Na^+, K^+ -ATPase and blood lactate) data were carried out by two-tailed unpaired Student's T test. The Pearson's correlation coefficient was determined for systolic, diastolic, mean blood pressure and Na^+, K^+ -ATPase activity. A $P < 0.05$ was considered significant.

RESULTS

Table 1 shows the effect of a 12-week swimming training program on the resting heart rate, systolic, diastolic and mean blood pressure of spontaneously hypertensive rats. Statistical analysis revealed that trained animals presented lower systolic [$t(14)=3.82$; $p<0.05$], diastolic [$t(14)=3.13$; $p<0.05$] and mean [$t(14)=4.27$; $p<0.05$] blood pressure and resting heart rate [$t(14)=5.00$; $p<0.05$] measures than their age-matched sedentary counterparts. The effect of swimming training on erythrocyte Na^+, K^+ -ATPase activity and on blood lactate content of SHR subjected to a 90 min swimming challenge is also shown in Table 1. Statistical analysis revealed that trained animals presented higher erythrocyte Na^+, K^+ -ATPase activity than age-matched sedentary counterparts [$t(14)=2.37$; $p<0.05$]. Moreover, trained animals presented lower lactate levels immediately after the 90-min swimming challenge than sedentary counterparts [$t(14)=3.49$; $p<0.05$], indicating that the training program increased animals' aerobic resistance. In addition, systolic blood pressure ($r=-0.7$, $P<0.05$; Fig. 1), diastolic blood pressure ($r=-0.67$, $P<0.05$; Fig. 2) and mean blood pressure ($r=-0.74$, $P<0.01$; Fig 3) negatively correlated with Na^+, K^+ -ATPase activity.

DISCUSSION

In the present study, we show that SHR rats subjected to a 12-week swimming program present lower blood pressure and higher erythrocyte Na⁺,K⁺-ATPase activity than age-matched controls. A significant body of evidence suggests that exercise decreases blood pressure by multiple mechanisms, including the decrease in peripheral resistance and sympathetic tone [10]. It is interesting that previous studies have proposed that increased viscosity and rigidity of RBC membranes may contribute to increased peripheral resistance in hypertension [6]. Erythrocyte membrane fluidity depends on Na⁺,K⁺-ATPase activity, which is inhibited in hypertensive patients [16]

and in spontaneously hypertensive rats [3]. The mechanisms underlying such an inhibition are still obscure, but it has been reported that plasma membrane Na⁺,K⁺-ATPase may be inhibited by ouabain-like endogenous inhibitors [2,17], which are decreased by physical exercise [18] or antihypertensive agents, which can enhance Na⁺,K⁺-ATPase activity [17].

However, specifically in what concerns the findings of the current study, the possibility that physical exercise may have decreased ouabain-like inhibitors and increased Na⁺,K⁺-ATPase activity seems unlikely, because ghost preparation involves exhaustive washing that remove these substances [12]. Moreover, alterations of the antioxidant status and increased lipoperoxidation have been also proposed as a cause of Na⁺,K⁺-ATPase inhibition in erythrocyte membranes [16]. Regarding this point, it is worth noting that aerobic exercise improves erythrocyte free radical defense status, and decreases plasma TBARS levels [19] in humans and rodents [20], suggesting that increasing free radicals defenses may play a role in the currently reported increase of erythrocyte Na⁺,K⁺-ATPase activity. It is

TABLE 1. Hemodynamical parameters and on erythrocyte Na⁺,K⁺-ATPase activity.

<i>Variable</i>	<i>Control (n=8)</i>	<i>Trained (n=8)</i>
Systolic blood pressure (mm Hg)	218.9 ± 3.2	198.8 ± 4.3*
Diastolic blood pressure (mm Hg)	163.8 ± 5.1	141.9 ± 4.6*
Mean blood pressure (mm Hg)	190.0 ± 3.4	168.4 ± 3.4*
Resting heart rate (bpm)	357.7 ± 5.3	305.9 ± 9.4
Na ⁺ ,K ⁺ -ATPase (nmol Pi/mg protein/min)	4.9 ± 0.6	6.9 ± 0.5*
Plasma lactate (mM)	5.7 ± 0.6	3.1 ± 0.2*

Values are means ± SE. *Significant difference compared to control group at P<0.05.

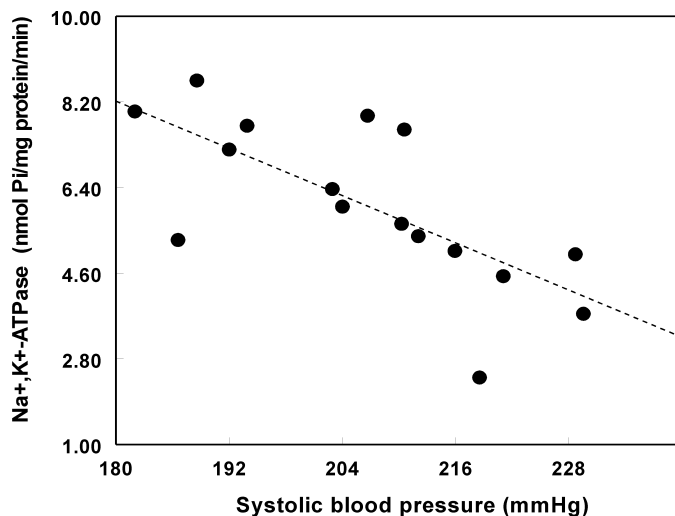


Figure 1. Na⁺,K⁺-ATPase activity (nmol Pi/mg protein/min) negatively correlates with systolic blood pressure. *P*<0.05 by the Pearson's correlation coefficient (*r*=-0.65; *n*=16).

particularly interesting that antioxidant defense increase has been observed only in aerobic exercise programs, and that trained animals presented lower plasma lactate levels than their control counterparts immediately after a 90-min swimming challenge (Table 1). Since the decreased plasma lactate levels suggest an improved aerobic capacity of trained animals compared to sedentary counterparts, it is tempting to speculate that exercise-induced redox alterations may have contributed for the presently reported increase in erythrocyte Na^+, K^+ -ATPase activity. However, we cannot rule out that trained rats may have improved their swimming efficiency over 12 weeks, which could also have contributed to lower LA levels in the challenge.

In present study we observed that the systolic (Figure 1), diastolic (Figure 2) and mean blood pressure (Figure 3) negatively correlated with Na^+, K^+ -ATPase activity. This finding is particularly remarkable, since the more was Na^+, K^+ -ATPase reduced, the more animals presented elevated blood pressure, evidenced by the significant negative correlation between these variables, which suggests a possible role for Na^+, K^+ -ATPase inhibition in blood pressure increase.

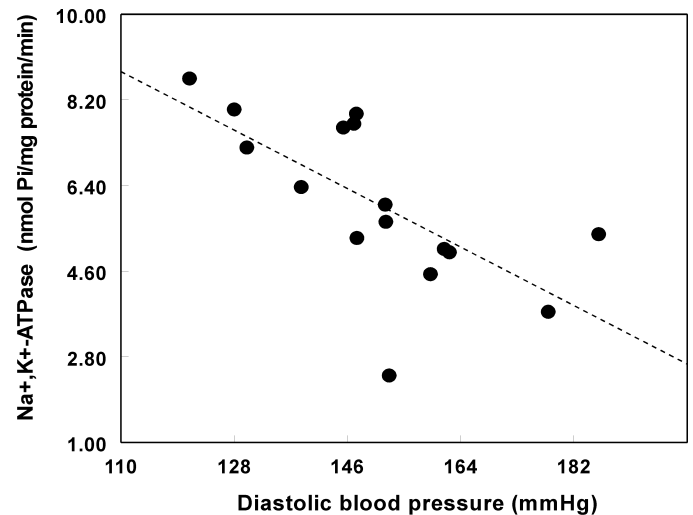


Figure 2. Na^+, K^+ -ATPase activity (nmol Pi/mg protein/min) negatively correlates with diastolic blood pressure. $P < 0.05$ by the Pearson's correlation coefficient ($r = -0.61$; $n = 16$).

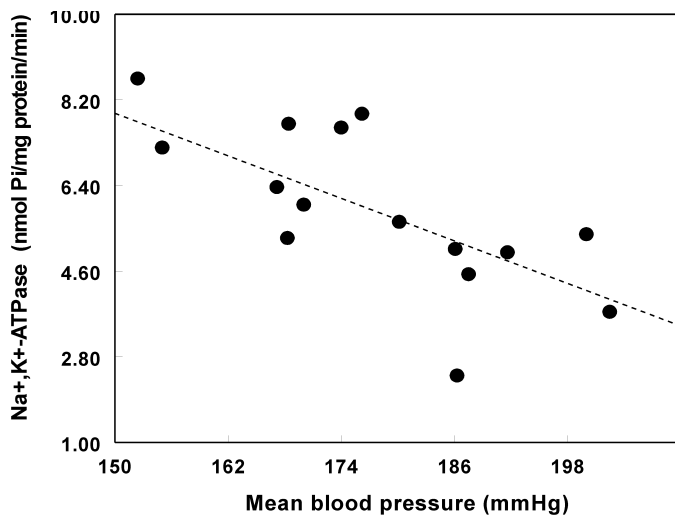


Figure 3. Na^+, K^+ -ATPase activity (nmol Pi/mg protein/min) negatively correlates with mean blood pressure. $P < 0.01$ by the Pearson's correlation coefficient ($r = -0.72$; $n = 16$).

Finally, one must be aware that the currently reported elevated erythrocyte Na^+, K^+ -ATPase activity in trained rats and decrease of arterial pressure induced by physical exercise does not imply a cause-effect relationship between them. Although it is tempting to establish such a relationship, caution regarding this point is recommended, since it has been reported that increases in arterial pressure values may alter the pattern of erythrocyte Na^+, K^+ -ATPase subunit expression [21]. Accordingly, it has been proposed that Ca^{+2} channel blockers-induced increase of erythrocyte Na^+, K^+ -ATPase is secondary to the reversal of an hypertensive state [2]. Therefore, we cannot rule out that the currently reported increase of erythrocyte Na^+, K^+ -ATPase is secondary to the reversal of an hypertensive state, particularly because physical exercise has been reported to decrease peripheral resistance by other mechanisms, such

as improvement in endothelium-dependent vessel relaxation and insulin sensitivity [22].

CONCLUSIONS

In the present study, we report the beneficial effects of chronic swimming exercise on the blood pressure of spontaneously hypertensive rats, which negatively correlated with erythrocyte Na⁺,K⁺-ATPase activity. We suggest that Na⁺,K⁺-ATPase activity increase may underlie the beneficial effect of chronic swimming on blood pressure, but more studies are necessary to clarify this point.

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