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Involvement of the atrial natriuretic peptide in the reduction of arterial pressure induced by swimming but not by running training in hypertensive rats

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ABSTRACT

The aim of this study was to compare, under resting conditions, the influence of chronic training in swimming or running on mean arterial pressure (MAP) and the involvement of the natriuretic peptide system in this response. Two-month-old male spontaneously hypertensive rats (SHR) were divided into three groups—sedentary (SD), swimming (SW) and running (RN)—and were trained for eight weeks under regimens of similar intensities. Atria tissue and plasma atrial natriuretic peptide (ANP) concentrations were measured by radioimmunoassay. ANP mRNA levels in the right and left atria as well as the natriuretic peptide receptors (NPR), NPR-A and NPR-C, mRNA levels in the kidney were determined by real-time PCR. Autoradiography was used to quantify NPR-A and NPR-C in mesenteric adipose tissue. Both training modalities, swimming and running, reduced the mean arterial pressure (MAP) of SHR. Swimming, but not running, training increased plasma levels of ANP compared to the sedentary group ($P < 0.05$). Expression of ANP mRNA in the left atrium was reduced in the RN compared to the SD group ($P < 0.05$). Expression of NPR-A and NPR-C in the kidneys of the SW group decreased significantly ($P < 0.05$) compared to the SD group. Although swimming increased ^{125}I-ANP binding to mesenteric adipose tissue, displacement by c-ANF was reduced, indicating a reduction of NPR-C. These results suggest that the MAP reduction induced by exercise in SHR differs in its mechanisms between the training modalities, as evidenced by the finding that increased levels of ANP were only observed after the swimming regimen.

1. Introduction

The importance of physical exercise for the control of hypertension is well documented and is the subject of guidelines from the American College of Sports Medicine [32]. A reduction in blood pressure in spontaneously hypertensive rats (SHR) has been found after chronic physical training by swimming [25,40] or running [19,45,46]. The mechanisms involved in the reduction of blood pressure (BP) could be dependent on the type of exercise training. There is evidence that the acute and chronic hemodynamic responses to swimming are different from the responses to running [1,9,43]. Studies have shown that water immersion causes an immediate translocation of blood from the dependent limbs and an increase in the intrathoracic blood volume that augments the cardiac output via increased end-diastolic and stroke volume due to the effect of increased cardiac muscle length on the contractile force of the cardiac muscle. The stretching of the atrium also results in a compensatory ANP secretion [30]. Thus, the reduction of blood pressure that is induced by exercise training could be involved in different neural or hormonal adaptations.

Atrial natriuretic peptide (ANP) is a hormone that promotes acute vasodilatation, natriuresis and diuresis with a consequent reduction in blood pressure [34]. Normotensive rats that received a prolonged infusion of ANP, resulting in increased plasma levels of this hormone, showed sustained hypotension [14]. Additionally, ANP knockout mice or natriuretic peptide receptor A (NPR-A) knockout mice have increased peripheral vascular resistance, hypertension and ventricular hypertrophy [22,28]. Moreover, elevated levels of ANP in hypertensive individuals could partially compensate for the high levels of vasoconstrictor hormones originating primarily from the renin–angiotensin–aldosterone system [41]. It is known that under physiological conditions, the primary stimulus for the secretion of ANP is the distension of the atrial chamber [7]. Among the factors that stimulate ANP secretion are increased concentrations of endothelin and vasopressin, tilting of the head downward [34] and immersion in water [26,39].

It has been shown that training by swimming increased the expression of ANP in the ventricles [8]. However, the lack of studies that compare both modalities of physical exercise on the natriuretic peptide system, particularly under conditions of arterial hyperten-

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sion, has made it difficult to understand the mechanisms that are involved in the reduction of blood pressure that is induced by exercise.

In the present study, we tested the hypothesis that different types of exercise training could lead to different changes in the natriuretic peptides system. We thought that even the swimming training, if chronically realized, could alter the ANP synthesis, secretion and bioavailability in the circulation. To compare the effect of both training modalities, we maintained both exercises at similar intensities by using the intensity of the maximal lactate steady state [11,33] to induce adaptations from predominantly aerobic activities.

2. Materials and methods

2.1. Ethical approval

The procedures were carried out in compliance with the guidelines for the ethical use of animals in scientific research as stated by the Federation of the Brazilian Society of Experimental Biology and were approved by the Ethics Committee for Animal Use of the Federal University of Espírito Santo.

2.2. Animals

The experiments were conducted on 21 spontaneously hypertensive male rats obtained from the Institute of Biomedical Sciences, University of São Paulo (270–300 g; 14 weeks old). The rats were housed with controlled temperature (22 °C), humidity (40%) and light cycles (12-h light/dark), had free access to tap water and were fed standard rat chow (Purina Labina, SP-Brazil) *ad libitum*. The animals were randomly divided into three groups: sedentary (SD, $n = 8$), run trained (RT, $n = 7$) and swim trained (ST, $n = 6$). The sedentary rats were handled five days/week to become accustomed to the experimental protocols.

2.3. Swimming training

Swimming training was performed in an apparatus adapted for rats that contained warm water (30–32 °C) and was kept at a depth of 50 cm. The training consisted of swimming sessions five days/week for 60 min for 8 weeks. The swimming time on the 1st day was 20 min, which was increased daily by 10 min until it reached 60 min on the 5th day. From the second week onwards, the exercise duration was kept constant and the rats were worn caudal dumbbells that weighed 2% of their body weight. The caudal weight was gradually increased until it was 5% on the 6th week and was thereafter kept constant [11,12,17,21]. All of the rats were weighed weekly to adjust the weight of the dumbbells.

2.4. Running training

The running training was performed on a motorized treadmill (Insight, São Paulo, Brazil) 5 days/week for 8 weeks, with the speed and duration progressively increased. The rats began training at 15 m/min for 20 min/day. The speed was gradually increased such that by the end of the 1st week, the animals ran at 15 m/min for 60 min/day. Thereafter, the duration was maintained but the speed was gradually increased. By the 6th week, the rats ran at 24 m/min for 60 min/day [33], and this exercise program was maintained until the end of the study.

2.5. Measurement of arterial blood pressure and heart rate at rest

Forty-eight hours after the end of the exercise training sessions, body weight, BP and heart rate (HR) at rest were measured. For this procedure, on the day before the measurement, a catheter that was filled with saline (PE-50) was inserted into the left femoral artery while the subject was under anesthesia (ketamine 70 mg/kg, xylazine 10 mg/kg). The free end of the catheter was exteriorized at the cervical dorsal area. For the BP measurement, the arterial catheter was attached to a 40-cm polyethylene catheter during the 40-min recording period in quiet, conscious rats, allowing the rats' complete freedom of movement in the cage. The BP was recorded by a pressure transducer coupled to a MP-100 System Guide (model MP100-CE; Biopac Systems, Santa Barbara, CA, USA). The HR was calculated instantaneously from the intervals of pressure pulses.

2.6. Collection of tissues and plasma

After the measurement of BP and HR, the rats were decapitated and 5 ml of blood was collected in pre-chilled tubes containing heparin sulfate and protease inhibitors: 10^{-5} mol/l ethylenediaminetetraacetic acid (EDTA), 10^{-5} mol/l phenylmethylsulphonyl fluoride (PMSF), and 0.5×10^{-5} mol/l pepstatin A. The blood was centrifuged at 4 °C and 2500 rpm (Eppendorf, Hamburg, Germany) for 15 min. The plasma was stored at −80 °C. The right and left atrial appendages, kidneys and mesenteric adipose tissue were removed, frozen in liquid nitrogen and stored at −80 °C.

2.7. Dosage of ANP

The dosages of ANP were performed by a double-antibody radioimmunoassay (RIA) as described by Gutkowska et al. [13]. The plasma was thawed, centrifuged for 5 min at 19,400 × *g* and 4 °C, and the ANP was extracted using Sep-Pak C18 columns (Waters Associates, Milford, MA, USA). The columns were activated with 8 ml of acetonitrile and washed with 8 ml of 0.2% ammonium acetate, pH 4.0. Afterward, 1 ml of plasma was infused into the column followed by 5 ml of 0.2% ammonium acetate. Finally, the absorbed ANP was eluted with 3 ml of 60% acetonitrile in 0.2% ammonium acetate, evaporated (Speed-Vac, Eppendorf, Hamburg, Germany) and stored at −20 °C for quantification by RIA.

To measure the ANP tissue concentrations, each half of the right (RA) and left atria (LA) was thawed and placed in a tube that was filled with 0.1 M acetic acid and protease inhibitors (10^{-5} M EDTA, 10^{-5} M PMSF and 0.5×10^{-5} M pepstatin A, all purchased from Sigma). The samples were then homogenized and centrifuged at 20,000 × *g* for 30 min at 4 °C, and the supernatant was diluted (final dilution: 1:2000) in phosphate buffer (0.01 mol/l sodium phosphate, 0.14 mmol/l bovine serum albumin, 0.1% Triton X-100, 0.1 mol/l NaCl and 0.01% sodium azide at pH 7.4) for ANP dosage. The ANP was measured by RIA as was previously described by Gutkowska et al. [13] using a specific antibody that was donated by Jolanta Gutkowska. All of the samples were measured in the same assay, and the intra-assay coefficient of variation was <10%. The protein content of the tissue was determined using the Bradford method [3].

2.8. Gene expression of ANP mRNA and the NPR receptors (NPR-A and NPR-C)

The total mRNA was extracted from the atria and kidneys by a guanidine isothiocyanate method as was previously described by Chomczynski and Sacchi [5].

The mRNA expression of ANP and its receptors (NPR-A and NPR-C) was determined by real time-PCR. The gene expression of ANP was evaluated in the RA and LA, and the NPR-A and NPR-C expression was determined in the right kidney. The cDNA was synthesized by the reverse transcription of mRNA. For this process,