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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,

1 μl of mRNA from each sample was mixed in plastic tubes with a solution containing the following compounds: diethyl pyrocarbonate water (DEPC), the reverse primer of the target gene (ANP, NPR-A, or NPR-C) or the reverse primer of the normalizing gene or housekeeping gene (ribosomal subunit s26), oligo dT, triphosphate deoxyribonucleotide (dNTP), dithiothreitol (DTT), specific buffer (10×) and a solution containing the Moloney murine leukemia virus (MMLV) reverse transcriptase enzyme, according to the manufacturer's guidelines (Invitrogen, CA, USA). After this process, the plastic tubes were heated at a temperature of 40 °C for 60 min. After reaching room temperature, the tubes were stored at −20 °C.

For the atria, prior to reverse transcription, the samples were subjected to DNAse treatment. The treatment was performed by mixing 0.5 μg of total mRNA from the atria with 4 μl of water and 1 μl of mix buffer containing DNAse (1:1). This mixture was incubated for 15 min at room temperature; after this period, EDTA was added, which stopped the reaction. The samples were then heated to 65 °C for 10 min.

After building the cDNA, a PCR was performed to amplify the cDNA for ANP, NPR-A and NPR-C, using specific primers (ANP: GGA TTT CAA GAA CCT GCT AGA CTT and CAT CGG TCT GCT CGC TCA, NPR-A: ATC ACA GTG AAT CAC CAG CAG TTC AGA and AGA TGT TAA CTC TGC TTC CCT G, NPR-C: CCT ACA TTA TCG ACG AGA CCA AA and ACT CGC TCA TGG ATG CTG CCC TA). For this procedure, 2 μl of cDNA was added into wells of specific plates for real-time PCR, followed by 1.5 μl of sense primer (1 pmol/μl), 1.5 μl of antisense primer (1 pmol/μl), 10 μl of Power SYBR Green PCR Master Mix (Applied Biosystems, Warrington WA, UK) and 5 μl of DEPC water. Afterward, the plates were sealed and taken to the apparatus for the real-time measurement of gene expression in the thermocycler (ABI Prism 7000 SDS; Applied Biosystems, Warrington WA, UK) using the following thermal cycles: [stage 1], a cycle of 52 °C/2 min; [stage 2], a cycle of 95 °C/10 min; [stage 3], 40 cycles of 95 °C/0.15 min and 50 °C/1 min.

2.9. Autoradiography

The ANP receptor autoradiography has been described in detail [2,6]. Briefly, the rats were killed by decapitation, and the mesenteric adipose tissue was rapidly isolated, snap-frozen in isopentane at −18 °C, mounted on cryostat chucks and cut into 15-μm-thick sections at −30 °C. The sections were thaw mounted on pre-cleaned gelatin-coated slides and then stored at −80 °C until they were used. The frozen slide-mounted adipose tissue sections were acid washed by pre-incubation for 10 min at room temperature in a sodium acetate buffer in NaCl 0.15 M, pH 5.0, for the removal of peptides from the receptors and then incubated in 50 mM Tris–HCl buffer (pH 7.4) containing 0.1% polyethylenimine to reduce the binding of ^{125}I-ANP to the gelatin-coated slides. The sections were then incubated with 50 mM Tris–HCl buffer (pH 7.4) containing 150 mM NaCl, 5 mM $MgCl_2$, 40 pg/ml bacitracin, 0.5% BSA, and approximately 50 pM ^{125}I-ANP. The ability of 10^{-12}–10^{-6} M ANP to displace specific ^{125}I-ANP binding from NPR-A and NPR-C was determined. Considering that des[Gln18, Ser19, Gly20, Leu21, Gly22]ANP-(4–23)-NH_2 (cANF; Bachem, Torrance, CA), a truncated ANP, binds only to NPR-C in mammals [20], the displacement by 10^{-12}–10^{-6} M cANF (Bachem, Torrance, CA) was used to determine NPR-C. The difference between the displacement by ANP and c-ANF indicates ^{125}I-ANP binding to NPR-A. After 1 h incubation, the slides were placed in racks and transferred sequentially through four rinses, lasting for 1 min each, of cold 50 mM Tris–HCl buffer (pH 7.4) and finally dipped in distilled water to wash off the salts. The slides were rapidly dried and exposed to a PhosphorImager (Fujifilm, BAS-1800II, Tokyo, Japan), and the images were analyzed using the Image Gauge 3.12 software.

Table 1
Body weight before and after in sedentary, running and swimming exercise-trained spontaneously hypertensive rats.

Groups	Body weight (g)	
	At the beginning of the study	At the end of the study
Sedentary	294 ± 6.3	333 ± 8.1[a]
Swimming	280 ± 3.9	300 ± 4.9[a,b]
Running	282 ± 11.7	318 ± 8.1[a]

Effect of running or swimming training on the body weight of spontaneously hypertensive rats.
[a] $P < 0.01$ vs. at the beginning of the study in the same group.
[b] $P < 0.05$ vs. SD.

2.10. Statistical analysis

The experimental data were expressed as the means ± standard errors of the mean (SEM), and the statistical analysis was performed using GraphPad Prism 5. The variables that showed a normal distribution were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison *post-hoc* tests. Any changes in the inter- and intra-group body weight at the beginning and at the end of the study were compared by two-way ANOVA followed by Dunnet's multiple comparison *post-hoc* tests. The variables without a normal distribution were analyzed by Kruskal–Wallis tests followed by Dunn's multiple comparison tests. The values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Body weight

As shown in Table 1, all animals began the training period with similar body weights and this was different after the exercise training period in all groups ($P < 0.01$). However, at the end of the training period, animals from the SW group, but not from the RN group, showed lower body weight compared to those from the SD group ($P < 0.05$).

3.2. Blood pressure and heart rate

Table 2 shows that the basal MAP of the SW and RN groups were significantly reduced compared to the SD group. Similar results were observed for diastolic pressure (DP) and systolic pressure (SP). No significant differences in basal HR were observed between the experimental groups.

3.3. Plasma and atria ANP

Fig. 1 shows that swimming but not running training significantly increased plasma ANP levels (A) compared to the SD group. Fig. 2 also shows that neither training modality altered the concentration of ANP in the right (B) or left atria (C).

Table 2
Systolic, diastolic, and mean arterial pressure and heart rate in sedentary, running and swimming exercise-trained spontaneously hypertensive rats.

Groups	SP (mmHg)	DP (mmHg)	MAP (mmHg)	HR (bpm)
Sedentary ($N = 7$)	210 ± 15	120 ± 6.7	162 ± 10	356 ± 14
Swimming ($N = 6$)	163 ± 5.8[a]	87 ± 5.5[a]	125 ± 5.9[a]	311 ± 13
Running ($N = 6$)	148 ± 15[a]	79 ± 9.2[b]	109 ± 11[b]	348 ± 26

Data are means ± SE. SP, systolic pressure; DP, diastolic pressure; MAP, mean arterial pressure; HR, heart rate. Statistically significant differences in one-way ANOVA followed by Dunnet's *post-hoc* test are indicated.
[a] $P < 0.05$ vs. sedentary group.
[b] $P < 0.01$ vs. sedentary group.

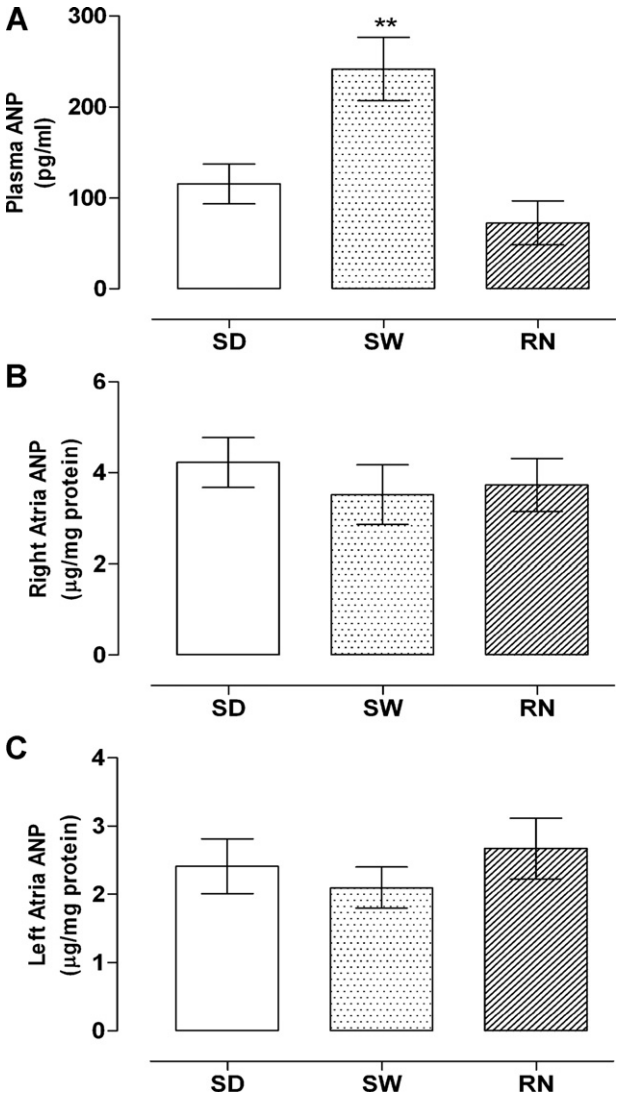

Fig. 1. Plasma ANP and atria ANP after chronic training in running or swimming of SHR. Plasma ANP levels (A), concentration of ANP in the RA (B) and LA (C) after chronic training in running or swimming of SHR. Values are means ± SEM. Sedentary group ($n = 7$) and trained groups ($n = 6$). Statistically significant differences in one-way ANOVA followed by Dunnet's *post-hoc* test are indicated. $^{*}P < 0.05$ vs. sedentary group.

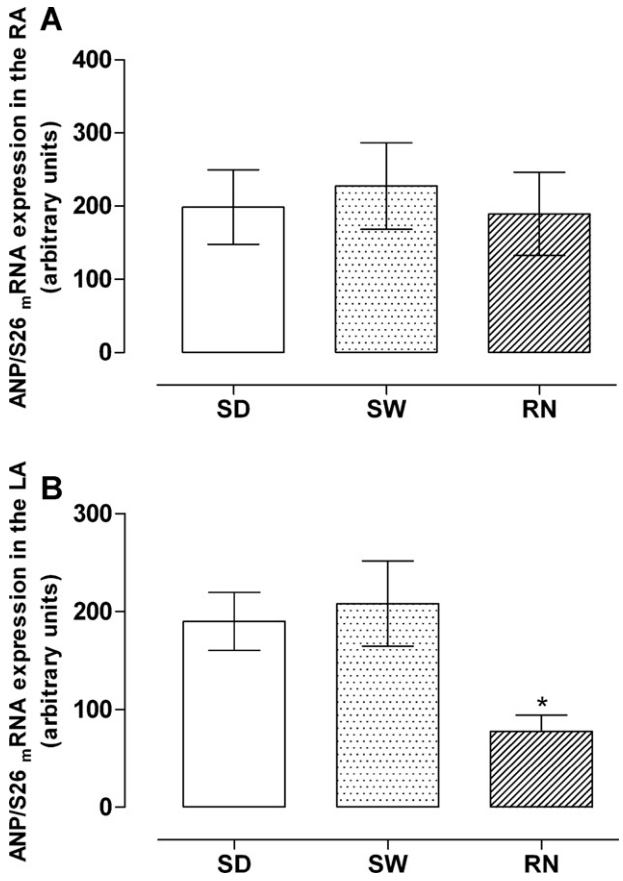

Fig. 2. mRNA expression of ANP in the atria after chronic training in running or swimming of SHR. The mRNA expression of ANP normalized by s26 mRNA levels in the RA (A) and LA (B) after of chronic training in running or swimming of SHR. Values are means ± SEM. Sedentary group ($n = 6$) and trained groups ($n = 6$). Statistically significant differences in non-parametric Kruskal–Wallis followed by Dunn's *post-hoc* test are indicated. $^{*}P < 0.05$ vs. sedentary group.

3.4. ANP mRNA expression

Fig. 2 shows no difference in the mRNA expression of ANP in RA between the SD and the trained groups. However, mRNA levels of ANP in the LA were significantly lower in the RN group, but not in the SW group, compared to the SD group.

3.5. NPR-A and NPR-C mRNA expression in the kidney

As shown in Fig. 3A, the gene expression of NPR-A in the kidney was significantly lower in the SW compared to the SD group. However, the expression of NPR-A in the RN group compared to the SD group did not reach significance. Similarly, only the gene expression of NPR-C was significantly decreased in the SW group, but not in the RN group, when compared to the SD group (Fig. 3B).

3.6. Autoradiography

The ability of natriuretic peptide receptors to bind ^{125}I-ANP was investigated in mesenteric adipose tissue by *in vitro* autoradiography. Unlabeled ANP displaces ^{125}I-ANP bound to both receptors, NPR-A and NPR-C, and c-ANF displaces ^{125}I-ANP bound specifically to NPR-C. The displacement of ^{125}I-ANP from NPR-A can be inferred by the difference between ANP and cANF displacements. ^{125}I-ANP bound reversibly and with high affinity to the mesenteric adipose tissue of all groups, but as Fig. 4A–C shows, the SW group presented higher total ^{125}I-ANP binding compared to the other groups. Unlabeled ANP almost completely inhibited ^{125}I-ANP binding to the mesenteric adipose tissue of the SD group. A high displacement rate was also observed using c-ANF, which indicates a high level of NPR-C in the mesenteric adipose tissue of SHR. The percentage of displacement by ANP in the SW group was similar to the SD group, but the displacement by c-ANF was reduced, indicating a reduction of NPR-C (Fig. 4A, B, D and E). Although no difference in total binding was observed in the RN group compared to the SD group, displacement by ANP or c-ANF was reduced, indicating a reduction in the specific receptors, NPR-A and NPR-C, respectively (Fig. 4C and F).

4. Discussion

This study demonstrated for the first time that chronic swimming and running training promote significant changes in endogenous ANP of SHR at rest through alterations in the synthesis and bioavailability of ANP as well as within its gene expression receptors. The data showed increased plasma ANP levels in the SW group and decreased ANP expression in the LA only in the RN group.

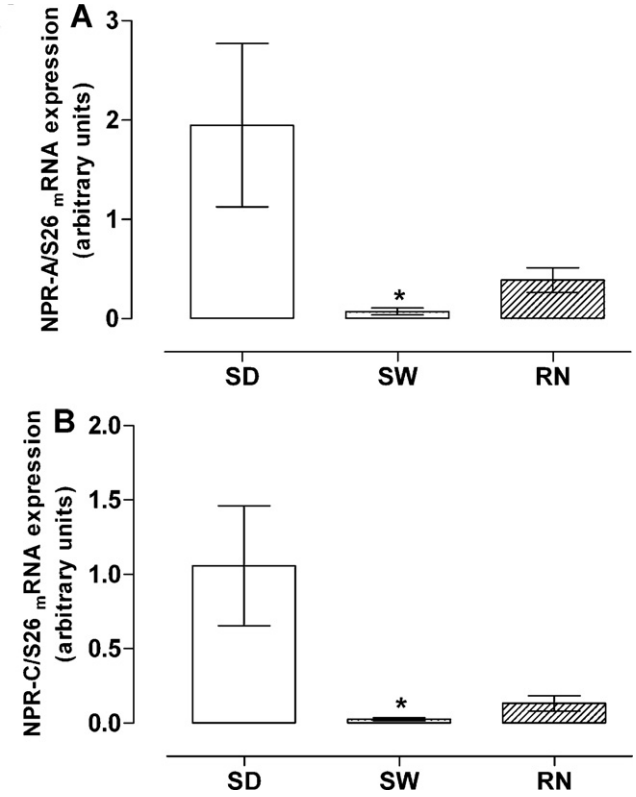

Fig. 3. mRNA expression of natriuretic peptide in receptor in the kidney after chronic training in running or swimming of SHR. The mRNA expression of NPR-A (A) and NPR-C (B) normalized by s26 mRNA levels (arbitrary units) in the kidney after of chronic training in running or swimming of SHR. Values are means ± SEM. Sedentary group ($n = 6$) and trained groups ($n = 6$). Statistically significant differences in non-parametric Kruskal–Wallis followed by Dunn's *post-hoc* test are indicated. *$P < 0.05$ vs. sedentary group.

In the kidney, a decrease in NPR-A such as in NPR-C gene expression was only noticed in the SW group; however, swimming increased ^{125}I-ANP binding to mesenteric adipose tissue and displacement by c-ANF was reduced, indicating a reduction of NPR-C.

We did not observe any influence of physical training by running or swimming on HR at rest in SHR. Previously, Schaible and Scheuer had shown decreases in HR after eight weeks of training on running and swimming in normotensive animals [37]. Besides using hypertensive rats, the intensity of training used in our study was different. We used the intensity of the maximal lactate steady state (i.e., the highest intensity at which aerobic metabolism still predominates over anaerobic metabolism) [11,33]. This was done so that both training modalities had similar intensities and in order to promote adaptations from predominantly aerobic activities.

The reduction of the BP observed in the SW group was associated with increased plasma levels of ANP, a hormone that promotes vasodilatation and decreases peripheral vascular resistance [34]. These data indicate that the recognized role of resistance exercise in lowering the BP in hypertensive individuals [32] may work through a different mechanism and that ANP would be primarily involved in physical activities that were performed in the water.

In fact, these data show that the recognized role of predominantly aerobic exercise in lowering blood pressure in hypertensive individuals [32] may work through different mechanisms, in which the ANP would be primarily involved in physical activities that were performed in the water. In a study conducted by Melo et al., ANP-knockout animals developed severe hypertension. A blockage of the autonomic nervous system with hexamethonium caused a decrease in blood pressure to levels that were similar to those of the control animals [23]. Another study that used an animal model that was characterized by high basal sympathetic tones, such as SHR, showed that the infusion of ANP promotes a considerable hypotensive effect when compared to the control animals, with no change in cardiac output, intravascular volume, sodium, or water excretion [18]. These data show that ANP is an important mediator in the attenuation of cardiovascular sympathetic tone and, if tonically active, may be involved in the chronic vasodilation mechanism. Thus, it becomes the most likely factor to explain the decrease in blood pressure induced by ANP in chronic conditions. This is an important finding because, to date, there is no evidence of the efficiency of the hormone on other mechanisms that regulate blood pressure, such as electrolyte balance [24].

Another hypothesis that can be considered is the role of ANG II in the secretion ANP. Exercise training decreases the sympathetic drive [4,35] to the heart and consequently decreases the local ANG II synthesis [31]. An earlier study showed that ANG II produced in the heart decreases the secretion of ANP by the atria [27]. However, this hypothesis is unlikely because both modalities decrease the sympathetic drive and there was an increase in ANP levels in the SW group only. Finally, there is evidence that increased cardiac and plasma BNP levels result in elevated plasma ANP levels in mice with deletion of NPR-A in the heart [15]. However, these alterations by BNP due to transient myocardial ischemia, like that which occurs during acute exercise, are inconclusive [10,47] and might not explain our data because we analyzed chronic conditions.

Physiological behavior is different in an aquatic environment than in a terrestrial environment; thus, chronic swimming training decreased NPR-C expression in the kidney and mesenteric adipose tissue, resulting in increased plasma levels of its hormone, findings which were not found in chronic running training. In physical activities performed in an aquatic environment, such as swimming, body position in the water, hydrostatic pressure and body temperature regulation are all factors that trigger different behaviors in the body than those induced by activities performed on land, such as running [28]. In the water, the decreased gravitational force and increased central venous pressure facilitate venous return, which in turn stretches the atrial chambers, increasing the expression and secretion of ANP [42]. This mechanism has also been demonstrated using SHR as an animal model [36]. However, in our study, the higher level of ANP in the plasma of the swimming trained group could be accounted for by either higher secretion or lower degradation. Because no alterations were observed in the storage of ANP or mRNA in the right and left atria of swimming trained rats, the higher plasma levels of ANP may be due to a decrease in degradation by NPRC in the kidneys and adipose tissue.

Our study found no change in the plasma levels of ANP in the RN compared to the SD group. In contrast, previous data from running trained normotensive rats found changes in the plasma concentrations of ANP with no difference in the atria [16]. Differences in the intensity, the duration of training and the species of rat that was studied could be responsible for the differences that were found. Furthermore, another study of normotensive rats found that increases in the intensity of exercise were accompanied by increased plasma levels and concentrations of ANP in cardiomyocytes [29]. Besides showing the same methodological differences as in the previous study, the technique of cardiac ANP analysis and the time of collection (immediately vs. 48 h after the last session) may explain the differences found in our study.

The alterations of plasma ANP levels cannot be attributed only to the atria's ability to express, synthesize and secrete ANP. The plasma ANP levels may also be affected by its clearance by the NPR-C receptor. Upon analyzing the expression of NPR-C in the kidneys, a significant decrease was found only in the SW group when compared to the SD group, which could explain the lower degradation of ANP and the increase in the plasma levels. However, there was

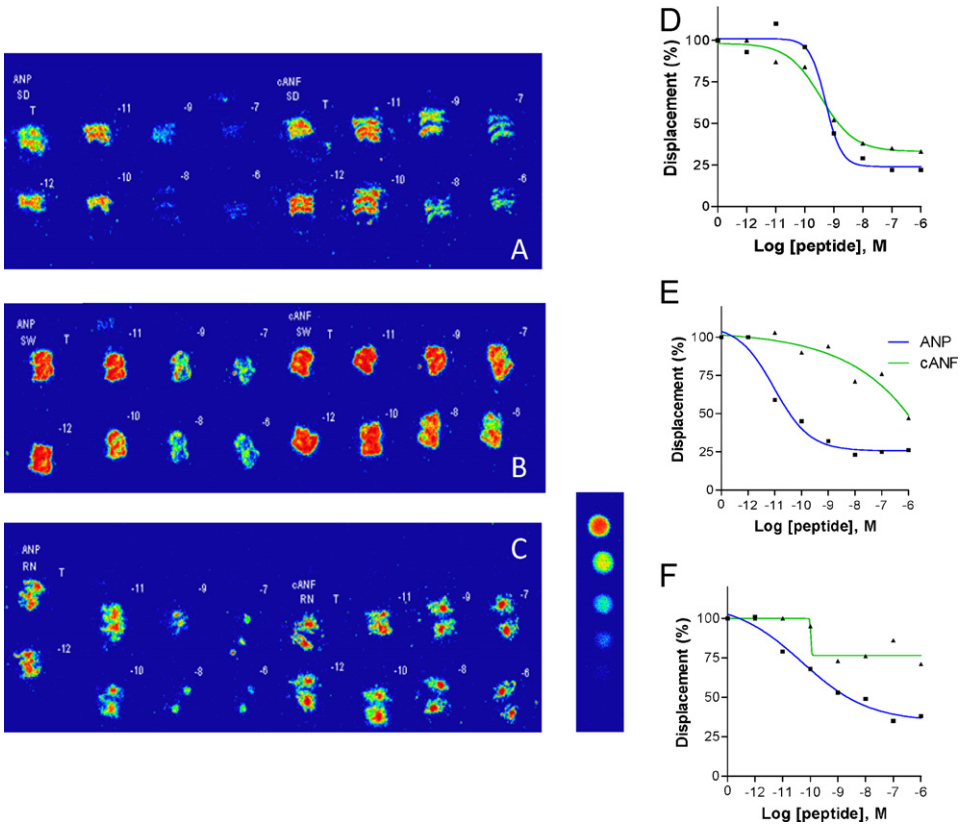

Fig. 4. Binding of NPR-C in the mesenteric adipose tissue by *in vitro* autoradiography using C-ANF as specific ligand of this receptor. Pseudocolor representation of ^{125}I-ANP binding sites in mesenteric adipose tissue of SD, SW, RN groups. Total ^{125}I-ANP binding and inhibition by 10^{-12}–10^{-6} M concentrations of unlabeled ANP and c-ANF in SD (A), SW (B), RN (C) groups. Displacement curves of ^{125}I-ANP binding calculated from color intensities (pixels) using Image Gauge program for SD (D), SW (E) and RN (F) groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a statistically significant decrease in NPR-A expression in the SW compared to the SD group. The downregulation of its receptor in the kidney could be the result of the increase in the ANP plasma levels.

Shanshan et al. found an increased expression of NPR-A in the kidney of normotensive racing rats that were trained over eight weeks, although they found a decrease in NPR-C concentrations [38]. Moreover, Suda et al. did not find changes in the density of receptors for ANP in the kidneys, adrenal glands and lungs in Wistar normotensive rats that were trained on running for 6–7 weeks [44]. However, unlike ours, the Suda et al. study did not specifically evaluate each subtype of receptor for ANP; it assessed only the total density of the receptors.

In conclusion, this study showed that exercise training – swimming or running – of the same intensity reduced the arterial pressure in SHR. However, the reduction of MAP that was induced by swimming, but not by running, was associated with an increase in ANP, a hormone with a well-known role as an anti-hypertensive agent, indicating that different mechanisms could be involved in the same response depending on the type of physical training.

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