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AT₁ receptor participates in the cardiac hypertrophy induced by resistance training in rats

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CARDIAC HYPERTROPHY IS THE adaptation of the heart to altered mechanical or hormonal stimuli. Exercise training, such as resistance training, is a known hypertrophic stimulus for cardiac hypertrophy. In this regard, we studied the rat cardiac hypertrophy in response to a resistance training program in the presence and absence of an AT₁ blocker. The exercise protocol consisted of: 4 × 12 bouts, 5×/wk during 8 wk, with 65–75% of one repetition maximum. Left ventricle weight-to-body weight ratio increased only in trained and trained + high-salt diet groups (8.5% and 10.6%, P < 0.05) compared with control. Also, none of the pathological cardiac hypertrophy markers, atrial natriuretic peptide, and αMHC (α-myosin heavy chain)-to-βMHC ratio, were changed. ACE activity was analyzed by fluorometric assay (systemic and cardiac) and plasma renin activity (PRA) by RIA and remained unchanged upon resistance training, whereas PRA decreased significantly with the high-salt diet. Interestingly, using Western blot analysis and RT-PCR, no changes were observed in cardiac AT₁ receptor levels, whereas the AT₁ receptor gene (56%, P < 0.05) and protein (31%, P < 0.05) expressions were upregulated in the trained group. Also, cardiac Ang II concentration evaluated by RIA and by ELISA and RT-PCR was unchanged (23.27 ± 2.4 vs. 22.01 ± 0.8 pg/mg, P > 0.05). Administration of a subhypotensive dose of losartan prevented left ventricle hypertrophy in response to the resistance training. Altogether, we provide evidence that resistance-training-induced cardiac hypertrophy is accompanied by induction of AT₁ receptor expression with no changes in cardiac Ang II, which suggests a local activation of the RAS consistent with the hypertrophic response.

AT₁ receptor; renin-angiotensin system; cardiac hypertrophy; resistance training

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**METHODS**

**Animals.** Thirty-six Wistar rats were assigned into six groups \((n = 6 \text{ each})\): control (CO), control treated with losartan (CO+LOS), control plus high-salt diet (CO+SALT), trained (TR), trained treated with losartan (TR+LOS), and trained plus high-salt diet (TR+SALT). Losartan \((10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1})\) was administered in the drinking water at a known dose that blocks the AT\(_1\) receptor but does not decrease the blood pressure of normotensive rats \((20)\). Salt \((1\%\) was also administered in the drinking water at a known dose to inhibit the renin release but not to alter the blood pressure \((24)\). Salt loading was used only to uncouple local vs. systemic activation of the RAS. Animals were housed in standard cages and food and water were provided ad libitum. The environmental temperature was kept at \(23 \pm 1°C\), and a 12:12-h dark-light cycle was maintained throughout the experiment. Animals were weighed every week. All protocols and surgical procedures used were in accordance with the guidelines of the Brazilian College for Animal Experimentation and were approved by the Ethics Committee of the Institute of Biomedical Science of the University of São Paulo.

**Exercise protocol.** Animals were exercised following a model adapted from Tamaki et al. \((29)\). Rats fitted with canvas jackets were able to regulate the twisting and flexion of their torsos and were suspended in a standard position on their hind limbs. An electrical stimulation \((20 \text{ V}, 0.3\)-s duration, at 3-s intervals\) was applied to the legs repeatedly, which lifted the weight arm of the training apparatus. The TR groups were exercised by four sets of 12 repetitions with a 90-s rest period between each set, five times per week for 4 wk. This exercise regimen was started after 2 wk of adaptation. All training sessions were performed in a dark room. After measurement of the maximum weight lifted [one repetition maximum (1RM)] with the squat-training apparatus, the training load was set at 65–75% of 1RM. The 1RM was defined as the minimum load that the rats were unable to jump following electrical stimulation. On the day following, blood pressure and heart rate were recorded as described below, rats were killed by quick decapitation without prior anesthesia, and blood and tissue samples were harvested, frozen, and stored at \(-80°C\). To determine plasma renin activity the first 3 ml of trunk blood were rapidly collected in chilled glass tubes containing a mixture of protease inhibitors \([\text{potassium EDTA (25 mmol), } \cdot \text{phenylthiouline (0.44 mmol), pepstatin A (0.12 mmol), and } 4\text{-chloromercuribenzenic acid (1 mmol)}\) to prevent the in vitro production and degradation of angiotensin peptides \((1)\). The blood was centrifuged, and the plasma was separated and stored at \(-20°C\). In the heart, the blood was harvested and weighed. The weight of the left ventricles and the tibia length were determined, and the heart weight index of each animal (weight of left ventricles in milligrams per body weight in grams and weight of left ventricles in milligrams per tibia length in millimeters) was calculated. The ventricles, lung, kidney, and serum were frozen at \(-80°C\) and used within 1 mo for enzyme assay and mRNA and protein preparation.

**Arterial blood pressure and heart rate.** Twenty-four hours after the last training session and under pentobarbital anesthesia \((40 \text{ mg/kg ip})\), a polyethylene cannula \((PE-50)\) was inserted into the carotid artery and emerged through the back of the rat’s neck. During the experiment, this cannula was connected to a strain-gauge transducer \((P23 \text{ Df}; \text{ Gould-Statham})\). Twenty-four hours after the surgery, arterial blood pressure was recorded on a beat-to-beat basis \((AT/ CODAS)\) at a frequency of 100 Hz for 30 min in quiet, conscious, unrestrained rats. The data reported indicates the average of all values of systolic, diastolic, and mean arterial pressure over the entire recording period of 30 min; heart rate was taken from blood pressure pulse records.

**Plasma renin activity assay.** The plasma renin activity assay was measured by angiotensin radioimmunoassay, using a commercial kit \((REN-CT2; \text{ CIS Bio International, Gif-sur-Yvette, France})\). This assay permits direct measurement of plasma renin activity. Results were quantified in a gamma counter, and the enzyme activity was expressed as nanograms ANG I per milliliter per hour.

**ACE activity assay.** ACE activity in rat serum and tissue extracts were determined using Abz-FRK(DnpP)-OH derivatives as substrates by continuously measuring the fluorescence according to Alves et al. \((1)\). Tissue samples were quickly harvested, homogenized in Tris·HCl buffer, \(pH 7.0\), containing 50 mM NaCl and centrifuged at \(1,000 \text{ g}\) for 10 min. The assays were performed at \(37°C\) in 0.1 M Tris·HCl buffer, \(pH 7.0\), containing 50 mM NaCl and 10 \(μ\text{M}\) ZnCl\(_2\). Hydrolysis rate of the intramolecular quenched fluorogenic substrate Abz-YRK-(Dnp)p (10 mM) incubated with aliquots of tissues homogenate and serum for 30 min at \(37°C\) was assessed to obtain ACE enzymatic activity. Fluorescence increments along the time were read at 420 nm emission:320 nm excitation. Tissues and serum ACE activity were expressed as arbitrary fluorescence units \((\text{AFU})\)·min\(^{-1}\)·mg\(^{-1}\) of protein \(\times 1,000\). The protein content was determined by the Bradford method \((5)\) by using bovine serum albumin as the standard \((\text{Bio-Rad protein assay})\).
in the trained group to the level in the control group, the following formula was applied \( e = 2^{-\Delta \Delta Ct} \).

**Measurement of ANG II in heart tissue.** Hearts were homogenized in lysis buffer (0.1 M sodium phosphate, 0.34 M sucrose, 0.3 M NaCl) containing a mixture of protease inhibitors and centrifuged at 10,000 g, 4°C, 10 min. The supernatant was collected, and it was passed through phenylsilica cartridges (Sep-Pak C18 columns; Waters), and the absorbed angiotensin was eluted with methanol. Eluate was dried in a vacuum centrifuge and the pellet was resuspended in EIA buffer, mixed, and centrifuged at 3,000 g for 10 min at 4°C. ANG II levels were determined by ELISA, according to the manufacturer’s instructions (SPI-BIO). The protein content was determined by the Bradford method (5) by using bovine serum albumin as the standard (Bio-Rad Protein Assay).

**RESULTS**

**Body weight.** Table 1 shows the body weight of the animals at the beginning and at the end of the study. There were no differences in the body weight among all groups neither before nor after the training protocol. Furthermore, all groups had the body weight increased over the period except the TR+SALT group, whose increase was not significant (\( P > 0.05 \)).

**Maximal strength.** Table 1 also shows the values of the absolute 1RM before (week 1) and after training (week 4). It can be observed that all groups began the training protocol with similar 1RM. However, this value increased similarly in all groups (TR, 113%; TR+LOS, 212%; TR+SAL, 131%). Analyzing the 1RM load normalized by the body weight, it can be observed that the animals began lifting approximately threefold of their body weight and finished the training period lifting ~5.5-fold of their body weight (\( P < 0.05 \)).

**Hemodynamics parameters.** To confirm that the doses of losartan and salt used in this study would not influence the results, some hemodynamic parameters, such as blood pressure and heart rate, were measured directly using an intra-arterial cannula. Table 1 shows the resting values of mean blood pressure and heart rate. Both blood pressure and heart rate remained the same in all groups, regardless of the treatment. Although resting blood pressure was unchanged, we have also measured the blood pressure achieved during one session of exercise training. As already shown by many human studies, one resistance training session induces increase in blood pressure. We have also found in this animal model that systolic blood pressure peaks around 180 mmHg, and these peaks were also similar apart from the administration of losartan or salt (data not shown).

**Cardiac hypertrophy.** Left ventricular weight-to-body weight ratio was ~8.5% and 10.6% greater in TR (2.15 ± 0.05 mg/g) and TR+SALT (2.19 ± 0.06 mg/g) groups, respectively, compared with the CO group (1.98 ± 0.04 mg/g) (Fig. 1A, \( P < 0.01 \)). Among trained groups, left ventricular hypertrophy was not observed in the animals that received the AT1 blocker losartan (TR+LOS, 1.99 ± 0.03 mg/g, Fig. 1A), which suggests a role for local RAS in exercise-induced cardiac hypertrophy. Also, there were no differences among the control groups, regardless of the treatment (CO vs. CO+SALT, 1.97 ± 0.05 mg/g vs. CO+SALT, 2.00 ± 0.04 mg/g). To confirm these findings, heart weight was also normalized to tibia length (Fig. 1B). An increase of 9.6% in cardiac hypertrophy in the TR (22.8 ± 0.9 mg/mm) compared with CO (20.8 ± 1.8 mg/mm) was found.

To better understand whether hypertrophy is physiological or pathological, two markers of pathological hypertrophy, ANP expression and αMHC-to-βMHC ratio, were analyzed and both were not altered after the resistance training (Fig. 3A).

**RAS.** Renin and ACE are the two main enzymes that regulate the formation of the peptide ANG II. Table 2 (ACE) and Fig. 2 (renin) show the activities of these enzymes. ACE activity was decreased by losartan treatment, while no effect could be observed due to the resistance training. ACE activity was assessed in serum and locally in lung, kidney, and heart but has been lowered only by losartan treatment in the serum. Furthermore, no influence could be observed in the renin activity in response to resistance training. However, as expected, losartan treatment increased renin activity possibly due to the blockade of the negative feedback on renal juxtaglomerular cell production and increased release of renin (\( P < 0.01 \)); whereas salt diet almost abolished its activity, as has already been demonstrated by our group (24) (\( P < 0.01 \)). The lower ACE activity by losartan treatment in the serum suggests a compensatory mechanism to increased renin activity in this group.

Figure 3A shows the RT-PCR results of the gene expression of AGT, ACE, and AT1a receptor in the heart between CO and TR groups. mRNA level of ACE was not altered as already suggested by ACE activity, but AGT decreased 30% (\( P = 0.066 \)) and AT1a receptor increased 56% (\( P < 0.05 \)).

In regard to the protein expression, it can be observed that resistance training has no effect on the protein expression of both the AT2 receptor and ANG II (Fig. 3, B and C). Furthermore, the ANG II concentration evaluated by ELISA confirms this result (CO, 23.27 ± 2.4 pg/mg; TR, 22.01 ± 0.8 pg/mg).

### Table 1. Body weight, absolute 1RM, mean blood pressure, and heart rate

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<th></th>
<th>CO</th>
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Data are reported as means ± SE of 6 animal in each group. 1RM, 1 repetition maximum; CO, control; CO+LOS, control treated with losartan; CO+SALT, control + 1% salt diet; TR, trained; TR+LOS, trained treated with losartan; TR+SALT, trained + 1% salt diet. * \( P < 0.05 \) 8th wk vs. 1st wk.
However, there was an overexpression of the AT$_1$ receptor protein (31%, Fig. 3B), which is in agreement with the increase in the AT$_{1a}$ mRNA.

**DISCUSSION**

In this study, we have focused on the role of RAS in the cardiac hypertrophy induced by resistance training in rats. It was observed that AT$_1$ receptor expression increases in response to training without any alterations in other components of the RAS. Furthermore, the treatment with an AT$_1$ receptor blocker losartan prevented this adaptive hypertrophy. The implications of the results are twofold: first, AT$_1$ stimulation is necessary for the development of cardiac hypertrophy in response to mechanical stretch during exercise; second, the mechanical stretch due to resistance training increases AT$_1$ expression. Other cardiovascular adaptations in this animal model have already been described by our group in previous publications (2, 3). Using this animal model of resistance training, we have recently shown that resistance training induces the development of concentric cardiac hypertrophy without ventricular dysfunction or cavity reduction, analyzed by echocardiography, showing that this stimulus leads to physiological cardiac hypertrophy. In this study, we have also analyzed markers of pathological hypertrophy. ANP expression and the αMHC-to-βMHC ratio are both increased during cardiac hypertrophy (16). Hypertension and other forms of cardiac stress are important regulators of higher expression of these markers. However, although the resistance training is also a cardiac stressor, these markers were not altered, which agree with our previous results of ventricular function (3).

The resistance training due to increased cardiac workload during the exercise sessions is a good in vivo model for studying pressure overload on cardiac cells. It has become clear that external load is sensed by cardiac cells, and this stimulus is converted into intracellular signals, which ultimately leads to cardiac hypertrophy; however, the mechanisms involved are less understood (35). It has been observed in vitro that stretch of cultured neonatal rat myocyte induces hypertrophy that is inhibited by ACE inhibitors or AT$_1$ receptor blockers (23). In addition, it has been suggested that mechanical stretch causes a direct release of humoral factors, such as...
ANG II and endothelin-1, from cardiac myocytes and that these factors act as initial mediators of the stretch-induced hypertrophic response (28). However, other authors have failed to find considerable amounts of ANG II in the medium after mechanical stretch (31), and others, even finding ANG II in the medium, observe that its amount is far below the amount needed to induce near-maximal hypertrophic effects (25). Recently, in a elegant study, Zou et al. (37) directly demonstrated that the medium from cardiomyocytes conditioned to mechanical stretch did not activate intracellular AT1 receptor-dependent pathway, which also demonstrated that even if the cells had released ANG II, it would not be capable of activating AT1 receptors. In the present study, we did not observe any alteration in local cardiac ANG II concentration after resistance training, which is in agreement with the lack of alteration in ACE and renin activity and decreased AGT gene expression (P = 0.066). Interestingly, in a very recent study, Xiao et al. (33) observed that mice expressing ACE only in the heart showed local heart ANG II concentration twice that of wild-type mice but that cardiac hypertrophy induced by aortic banding did not differ between the genotypes. Altogether, these findings and the ones of the present study suggest that it is very unlikely that ANG II plays a role in the cardiac hypertrophy after resistance training.

Our findings might be explained by recent studies suggesting that mechanical load can directly activate the AT1 receptor even in the absence of ANG II. A number of studies have addressed the role of the AT1 receptor in cardiac hypertrophy, using genetically modified mice models overexpressing AT1 receptor in the heart (14, 26, 32). Van Kats et al. (30) used transgenic mice that had cardiac levels of ANG II 20- to 50-fold greater than those seen in control groups with no detectable increase in circulating ANG II. These transgenic mice showed no evidence of cardiac hypertrophy at 3 mo of age. In the same study, using another type of transgenic mice that overexpressed a degradation-resistant form of ANG II, the hormone levels reached 100 times the normal levels and began to spill into the circulation. In spite of this, the animals again did not develop cardiac hypertrophy, which was only observed when the blood pressure began to increase in response to the increased circulating ANG II. Recent results by Crowley et al. (7) have demonstrated that cardiac remodeling correlates with increases in blood pressure and that in the absence of an increase in blood pressure, hearts did not undergo remodeling even under ANG II administration for 4 wk. These studies suggest different mechanisms for pressure-mediated and ANG II-mediated effects through AT1 in vivo.

Frank et al., in 2008 (13), demonstrated that a stretch-specific gene program exists and is mediated, at least in part, by the AT1 receptor, but that is different from that induced by pharmacological intervention, such as phenylephrine, endothelin, or even ANG II induction. Earlier, Malhotra et al. (22) had already described that mechanical stretch and ANG II differentially regulate the RAS in cardiac myocytes in vitro. These differences in the intracellular program induced by stretch or by ANG II might be due to the fact that the AT1 receptor does not only couple to the G protein family, but also to unconventional intracellular signaling, such as JAK2-STAT1/2 and ATIR-associated proteins. These different effects mediated by pressure and ANG II may have important clinical implications since they imply that the RAS may be important in conditions that AT1 receptor levels are increased.

In concert with the findings described above, our results demonstrated that although resistance training did not significantly modify ANG II concentration, protein and mRNA expression of AT1 receptor increased. Furthermore, when the AT1 receptor was blocked, but not with systemic RAS inhibition, cardiac hypertrophy was prevented, suggesting a key role to the local RAS in this adaptation. The cardiac hypertrophy observed in the present study might be explained by the
activation of the AT1 receptor caused by repeated chronic mechanical stretch due to the resistance training. This finding regarding physiological cardiac remodeling independent of ANG II concentration is in agreement with another study of our group that did not observe differences in cardiac hypertrophy between mice harboring one to four copies of the ACE gene when submitted to physical training (11).

During the revision of this manuscript, Yasuda et al. (36) described the molecular mechanisms of AT1 receptor activation by mechanical stress. Using substituted cysteine accessibility mapping technique, they have observed that the AT1 receptor might be disrupted by mechanical stretch independently of ANG II and that the counterclockwise rotation of the transmembrane 7 domain might cause activation of intracellular signaling pathways.

**Perspectives and Significance**

This study shows for the first time in the literature that local RAS contributes to the development of physiological cardiac hypertrophy in response to resistance training and that the AT1 receptor plays a key role on this adaptation. It allows future studies to investigate the mechanisms and the intracellular signaling by which different exercise training leads to different cardiac hypertrophy patterns, such as concentric and eccentric hypertrophy. Furthermore, this study suggests that the activation of RAS can also be observed even in physiological adaptations induced by resistance training in addition to the well-known action of the RAS in the development of many cardiovascular diseases.

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