Training-Induced Deactivation of the AT₁ Receptor Pathway Drives Autonomic Control and Heart Remodeling During the Transition From the Pre- to Hypertensive Phase in Spontaneously Hypertensive Rats

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Background: The effects of hypertension and exercise training (T) on the sequential interplay between renin-angiotensin system (RAS), autonomic control and heart remodeling during the development of hypertension in spontaneously hypertensive rats (SHR), was evaluated.

Methods and Results: Time course changes of these parameters were recorded in 4-week-old SHR submitted to a T or sedentary (S) protocol. Wistar Kyoto rats served as controls. Hemodynamic recordings were obtained in conscious rats at experimental weeks 0, 1, 2, 4, and 8. The left ventricle (LV) was collected to evaluate RAS gene and protein expression, cardiomyocytes’ hypertrophy and collagen accumulation. Pre-hypertensive SHR exhibited augmented AT₁-R gene expression; at 5 weeks, they presented with elevated pressure, increased LV angiotensinogen and ACE mRNA expression, followed by sympathoexcitation (from the 8th week onwards). Marked AT₁-R protein content, myocytes’s hypertrophy, collagen deposition and increased pressure variability were observed in 12-week-old sedentary SHR. In addition to attenuating all these effects, T activated Mas receptor expression augmented parasympathetic modulation of the heart, and delayed the onset and reduced the magnitude, but did not block the development of genetic hypertension.

Conclusions: The close temporal relationship between changes in the LV ACE-Ang II-AT₁-R axis, autonomic control and cardiac remodeling at both the establishment of hypertension and during exercise training reveals the essential role played by the AT₁-R pathway in driving cardiac remodeling and autonomic modulation during the transition from the pre- to hypertensive phase.

Key Words: Collagen content; Exercise training; Juvenile spontaneously hypertensive rats; Myocytes’ hypertrophy; Renin-angiotensin system

Essential hypertension is a multifactorial chronic disease involving complex interactions between oxidative stress, pro-inflammatory profile, autonomic and endothelial dysfunction, immune system hyperactivity and arterial remodeling. These deleterious responses are important risk factors for cardiovascular morbidity and mortality. Although more prevalent in advanced ages, some pathophysiological abnormalities such as altered autonomic control, increased cardiac sympathetic neurotransmission, endothelial dysfunction, and vascular endoplasmic reticulum stress were already observed in young- to middle-aged hypertensive individuals. Experimental evidence suggests that angiotensin II (Ang II) and angiotensin-(1-7) [Ang-(1-7)], the main active hormones of the renin-angiotensin system (RAS), are essential players in hypertension-induced abnormalities.

Among hypertension-induced end-organ lesions, hypertrophic cardiac remodeling is a well-known characteristic. This pathological process consists in concentric left ventricle (LV) hypertrophy, increased myocyte cross-sectional area, interstitial inflammation, extracellular collagen deposition, cardiac fibrosis, capillary rarefaction and apoptosis. Experimental studies have identified a close relationship between RAS expression, oxidative stress, local inflammation and cardiac remodeling in distinct experimental models of hypertension. These studies confirmed clinical findings that AT₁ receptor (AT₁-R) blockade reduced LV hypertrophy, highlighting the importance...
Table. Specifications of Oligonucleotides Used in the Present Study

| Gene    | Sequence (5'→3') | Length (base pairs) | Access number (ncbi.nlm.nih.gov) |
|---------|------------------|---------------------|---------------------------------|
| GAPDH   | S: GGG CAG CCC AGA ACA TCA T A: CCG TTC AGC TCT GGG ATG AC | 76 | NM 017008.4 |
| Aogen   | S: CAC GGA CAG CAC CCT ATT TT A: TGT TGT CCA CCC AGA ACT CA | 100 | NM 134432.2 |
| ACE     | S: GGG CGC AGG ACA CCA ACA TCA A: TCC TGG TTG ATC AGG GCC GCT T | 69 | NM 012544.1 |
| ACE2    | S: ACC CTT CCT ACA TCA GCC CTA CTG A: TGT CCA AAA CCT ACC CCA CAT AT | 73 | NM 001012006.1 |
| AT1R    | S: CAG TTT CCT GGA TGT GCT GA A: CCC AGA AAG CCG TAG AAC AG | 140 | NM 030985.4 |
| MasR    | S: TCC ACC AAG ACC TGC TAG GA A: TCT TGT GCT GGA CCA CTT CA | 208 | NM 012757.2 |

S and A indicate the sense and antisense sequences, respectively. Length is the amplicon size in base pairs; NM represents the access number from the ncbi.nlm.nih.gov site.

Figure 1. Hypertension- and training-induced changes on functional parameters during the development of hypertension in spontaneously hypertensive rats (SHR). Sequential changes on running distance (A), mean arterial pressure (MAP, B), systolic arterial pressure (SAP) variability (C) and its low frequency (LF-SAP, D) and very low frequency (VLF-SAP, E) components in SHR and normotensive controls (Wistar Kyoto (WKY)). MAP, SAP-variability and respective components were measured in 7–10 rats/treatment point/group. Training/Sedentary protocols started with 60 SHR and 25 WKY. Significance (P<0.05): # vs. respective week 0; * vs. age-matched WKY; † vs. age-matched SHR-sedentary (S).
analyzed the temporal changes of resting arterial pressure (AP) and heart rate (HR), their variabilities and spectral components indicative of sympathetic and parasympathetic activity to heart and vessels. Wistar Kyoto rats (WKY) served as controls.

Methods

Young male SHR and WKY (21–22 days old) from the Animal Breeding Facility of the University of São Paulo were maintained in the Animal Facility of the Department of Physiology and Biophysics at a controlled room temperature (∼23°C), in a 12-h dark–light cycle and had free access to standard chow and water. All surgical procedures and experimental protocols were in accordance with the National Institutes of Health, Guide for Care and Use of Laboratory Animals. The study was approved by the Institutional Committee of Animal Care and Use of Biomedical Sciences Institute, University of Sao Paulo (nº 10, page 124, book 02).

After a 1-week adaptation period, the aerobic capacity was evaluated. SHRs were allocated to moderate exercise training (T) or sedentary (S) protocols for 8 weeks. At weeks 0, 1, 2, 4 and 8 (SHR) and weeks 0 and 8 (WKY), rats were chronically cannulated for hemodynamic record-
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Aerobic training increased the velocity attained by SHR-T (+52% and +96% at the 4th and 8th experimental weeks, respectively, P<0.001). In contrast, SHR-S and WKY-S did not exhibit significant alterations in aerobic capacity (Figure 1A). Four-week-old SHR and WKY (63 ± 2 g and 67 ± 3 g) exhibited a similar weight gain (+173 ± 6 g and +167 ± 5 g, respectively) during the 8 experimental weeks, with no difference between SHR-S and SHR-T.

Sequential Effects of Hypertension and Training on Arterial Pressure and Pressure Variability

At the beginning of the protocols, 4-week-old SHR exhibited similar MAP levels as age-matched WKY (110 ± 8 and 104 ± 5 mmHg, respectively, P=0.47; Figure 1B). SHR-S exhibited a small decrease in the first week, delayed the onset (significant increase only at the fourth experimental week) and reduced its magnitude (SHR-T=158 ± 3 mmHg, SHR-S=172 ± 3 mmHg, Figure 1B). Spectral analysis confirmed the efficacy of T in delaying and reducing the hypertensive effects. While SHR-S exhibited a progressive LF-SAP increase (significant from the fourth experimental week), SHR-T exhibited a reduction of approximately 50% (significant from the fourth experimental week).

**Results**

**Effects of Training on Treadmill Performance and Body Weight Gain**

Since the beginning of experiments, SHR showed better treadmill performance than age-matched WKY, as evidenced by the higher speed reached during maximal exercise tests (1.67 ± 0.05 vs. 0.63 ± 0.05 km/h, respectively, P<0.001, Figure 1A). Aerobic training increased the velocity attained by SHR-T (+52% and +96% at the 4th and 8th experimental weeks, respectively, P<0.001). In contrast, SHR-S and WKY-S did not exhibit significant alterations in aerobic capacity (Figure 1A). Four-week-old SHR and WKY (63 ± 2 g and 67 ± 3 g) exhibited a similar weight gain (+173 ± 6 g and +167 ± 5 g, respectively) during the 8 experimental weeks, with no difference between SHR-S and SHR-T.

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Sequential Effects of Hypertension and Training on LV RAS Gene and Protein Expression

Knowing that cardiac RAS is a key regulator of LV remodeling in hypertensive individuals, we investigated the sequential effects of hypertension and exercise on gene and protein expression of vasoconstrictor and vasodilator RAS axes during the establishment of hypertension. Compared to age-matched WKY-S, pre-hypertensive SHR exhibited smaller angiotensinogen (Aogen), ACE and MasR (−65%, −82% and −66%, respectively, *P*<0.05, Figure 3A, 3B, 3E), but a higher LV AT1R mRNA level (+78%, *P*>0.05, Figure 3C). While mRNA expression of precursor and other RAS components did not change in WKY-S during the experimental weeks, Aogen and ACE mRNA of the SHR-S showed significant increases at the first and second experimental weeks (+2.3- and +2.7-fold vs. basal levels, corresponding to 5 and 6 weeks of age, Figure 3A and 3B, respectively) when MAP started raising. These changes were not maintained throughout the fourth to eighth experimental weeks. In the SHR-S, AT1R, ACE2 and MasR mRNA levels were not significantly changed during the 8 experimental weeks (Figure 3C–3E). Importantly, aerobic training mitigated and shortened the transient increase in Aogen mRNA expression exhibited by SHR-S (Figure 3A) and altered the time course changes of both RAS axes during the transition up to the eighth experimental week (Figure 2B). Spontaneous baroreflex sensitivity showed an increasing trend in the SHR-T when compared to SHR-S group (Figure 2F).

Sequential Effects of Hypertension and Training on HR, Pulse Interval (PI) Variability and Spontaneous Baroreflex Function

Juvenile rats exhibited, at the beginning of protocols, high resting HR that was reduced with advancing age in all groups. Four-week-old SHR showed higher values than age-matched WKY (437±31 and 371±8 beats/min respectively, *P*<0.05, Figure 2A). HR falls were observed in both SHR groups since the fourth experimental week, but HR reduction was greater in SHR-T from the fourth week on, when values attained the same range exhibited by WKY-S (SHR-T=331±8, WKY-S=322±7 beats/min). The higher resting HR in juvenile SHR was accompanied by smaller PI variability (−42%, Figure 2B) mainly due to reduced HF-PI (−59% vs. age-matched WKY, Figure 2D). Spontaneous baroreflex sensitivity was already depressed in juvenile SHR-S (−50% vs. WKY-S, Figure 2F). During the experimental protocol SHR-S showed significant increases in LF and LF/HF ratio that were abrogated by training (Figure 2C, 2E). Exercise training also augmented and almost normalized HF-PI from the fourth to eighth experimental week (Figure 2D), thus progressively increasing PI variability to a similar level exhibited by WKY at the eighth experimental week (Figure 2B). Spontaneous baroreflex sensitivity showed an increasing trend in the SHR-T when compared to SHR-S group (Figure 2F).
from the pre-hypertensive to hypertensive phase. There was a transient ACE increase in SHR-T during the first experimental week (corresponding to 5 weeks of age) that was abrogated by training from the second week on (Figure 3B). The marked T-induced decrease in AT1R mRNA expression, significant since the fourth experimental week (~80% from basal levels, Figure 3C), also contributed to the downregulation of the vasoconstrictor-trophic-oxidative-inflammatory RAS axis. At the end of the protocols, SHR-T AT1R mRNA expression was lower than that for age-matched SHR-S. In addition, the contraregulatory axis was simultaneously augmented in the LV of the SHR-T; there was a marked increase in ACE2 mRNA expression from 4 up to 8 weeks training (~2-fold increase, Figure 3D) accompanied by increased MasR mRNA expression that peaked at the fourth experimental week (4-fold increase, Figure 3E) and was still 2.7-fold higher than basal levels at the end of protocols.

Gene expression changes were confirmed by sequential analyses of LV AT1R and MasR protein expression. In arbitrary normalized units, AT1R of pre-hypertensive SHR was 5-fold higher than MasR values (3.99 ± 1.45 vs. 0.80 ± 0.08, Figure 4A, 4B). At the beginning of protocols, there was a trend for higher AT1R and smaller MasR in SHR-S vs. WKY-S (Figure 4A, 4B, P > 0.05). AT1R expression was not changed in WKY-S; in contrast, it was largely increased in SHR-S at the eighth experimental week (+3.4-fold, Figure 4A). This increase was completely blocked in SHR-T, whose value was like that of WKY-S. During the experimental protocols, MasR protein expression increased in all groups (~1.7-fold at the eighth week), with a large transient compensatory increment in the SHR-S at the fourth week (~2.4-fold, Figure 4B). Training-induced changes in SHR receptors’ expression during the transition from the pre-hypertensive to hypertensive phase were more pronounced in the vasoconstrictor (marked AT1R reduction at the eighth week) than in the vasodilator axis (mild MasR increase at the fourth experimental week). As depicted in Figure 4C, the high arbitrary normalized values of AT1R relative to respective MasR yielded positive AT1R/MasR ratios during the 8 experimental weeks. SHR-S AT1R/MasR ratio was slightly but not significantly higher than WKY-S at the pre-hypertensive phase (week 0), but largely increased at the eighth experimental week (rats aged 3 months). Interestingly, 8 weeks of aerobic training did not block the establishment of hypertension (although it did reduce it, Figure 1B), but it was largely effective to reduce the AT1R/MasR ratio back to normotensive levels.
Sequential Changes on Cardiac Remodeling

RAS hyperactivity and LV hypertrophy characterized the chronic hypertension.\textsuperscript{11,13,22,25} To uncover the time-course changes in LV remodeling during the establishment of hypertension and the effects of exercise, we measured the LVW/BW ratio and the sequential changes in LV myocytes’ diameter. Pre-hypertensive SHR already exhibited larger LVW/BW than age-matched WKY (4.07±0.36 vs. 3.22±0.07 mg/g, Figure 5B). LVW/BW ratio was reduced in all groups during the 8 experimental weeks, without significant differences between them. At the beginning of protocols, LV myocytes’ diameter was also higher in SHR vs. WKY (8.6±0.2 μm vs. 4.5±0.1 μm, respectively, Figure 5A and 5C). Similar to LVW/BW, myocytes’ diameter of both SHR-S and SHR-T decreased from the first to fourth experimental week, but the establishment of hypertension reversed this effect: SHR-S diameters’ size started to increase markedly from the fourth week on, while SHR-T only showed a small increase. At the end of protocols, SHR-T exhibited smaller myocytes’ diameter than SHR-S (8.5±1.0 vs. 12.2±0.1 μm, respectively, Figure 5C).

Knowing that collagen accumulation contributes to cardiac remodeling in hypertension,\textsuperscript{10,13,14,26} we also analyzed the temporal changes of collagen content during the transition from the pre- to hypertensive phase. There was no difference in LV collagen content between juvenile SHR and age-matched controls (Figure 6A and 6B). While collagen content was not changed in WKY-S, the establishment of hypertension augmented LV collagen deposition that was more precocious and intense in SHR-S and milder in the SHR-T, specifically at the end of protocols when training was effective in reducing AT1R/MasR ratio (compare Figures 6B and 4C).

Discussion

The present set of data confirmed the involvement of the vasoconstrictor-trophic-pro-oxidative-pro-inflammatory RAS axis in SHR LV hypertrophy and that pre-hypertensive exercise training attenuates both hypertension and cardiac remodeling. In addition, it identified the sequential mechanisms that drive the hemodynamic and autonomic control and the LV remodeling during the transition from the pre-hypertensive to hypertensive phase. Our findings are as follow: (1) at the pre-hypertensive phase, young SHR exhibits lower Aogen, ACE and MasR expression, but an already increased AT1R mRNA expression; (2) the development of hypertension starts earlier in SHR-S (at

![Figure 6. Hypertension- and training-induced changes on left ventricle (LV) collagen deposition during the development of hypertension in spontaneously hypertensive rats (SHR). (A) Photomicrographs taken from sedentary (S) and trained (T) SHR and normotensive controls (Wistar Kyoto (WKY)) depicting myocytes' collagen content at different time points. (200x magnification). Sequential changes on LV collagen deposition (B) in SHR and WKY. Measurements were made in 3–4 rats/time point/group. Significance (P<0.05): †vs. respective week 0; *vs. age-matched WKY; †vs. age-matched SHR-S.](image-url)
approximately the fifth week of age) and occurs simultaneously with increased LV Aogen and ACE expression, which is suggestive of high Ang II availability, and augmented LV collagen deposition; (3) the pressure increase in SHR-S is accompanied by reduced spontaneous baroreflex sensitivity, progressive augmentation in the sympathetic modulation of heart/vessels since the fourth experimental week (8 weeks old), and by increased AT1R expression, augmented pressure variability and deleterious LV remodeling at the end of protocols (12 weeks old); and (4) exercise training abrogates the initial hypertension-induced increase in Aogen and ACE expression and the late augmentation in AT1R, improves the expression of the contraregulatory RAS axis, decreases the sympathetic and increases the parasympathetic modulation of the heart, blocks LV myocytes’ hypertrophy and collagen deposition, while delaying and reducing vasomotor sympathetic activity, pressure levels and pressure variability. As expected, exercise training also increases PI variability and reduces resting HR, two important markers of trainability.

The pivotal role of RAS in the maintenance of hypertension, autonomic dysfunction and cardiac hypertrophy in the SHR is well known.27 Increased plasma and tissue RAS expression, high Ang II availability, sympathetic hyperactivity, oxidative stress and pro-inflammatory profile, myocytes’ hypertrophy and increased LV collagen synthesis were observed in the SHR.3,13,18,26,28,30 Pharmacological studies showed that ACE inhibitors, AT1R receptor antagonists and RAS silencing attenuated hypertension and heart hypertrophy,31 and that AT1R antisense gene therapy reduced cardiac hypertrophy independently of pressure changes.25 Blockade of the AT1R also reduced Ang II intracellular signaling, oxidative stress, myocytes’ hypertrophy, LV fibrosis and collagen deposition in the SHR.32,33

As those studies were conducted in adult/aged and already hypertensive SHR, we focused our observations in the transition from the pre- to hypertensive phase. Our data indicated an already increased AT1R mRNA expression in young pre-hypertensive SHR-S. The augmented gene expression of both Aogen and the ACE-Ang II-AT1R RAS axis at the onset of hypertension are decisive to trigger the progressive increase in pressure (since the fifth week of age, Figure 7A) and sympathoexcitation (increased further in SHR-S aged 8 weeks). Moreover, a deleterious cardiac remodeling and a marked augmentation in LV AT1R protein content were observed when SHR were 12 weeks old (Figure 7A). The transient compensatory increase in the contraregulatory axis was not enough to block the increased activity of the vasoconstrictor-trophic-pro-oxidative-pro-inflammatory RAS axis, which worsened LV function and cardiac fibrosis. The essential role played by the vasoconstrictor axis was previously confirmed by the blockade of these effects following AT1R antagonism.34-36 It was also shown that collagen content changes occurred independently of pressure changes.38 The early increase in the ACE-Ang II-AT1R axis could also contribute to augment the sympathetic activity to the heart, which is an additional stimulus to potentiate the late myocyte hypertrophy and collagen deposition. Indeed, the elevated resting HR in pre-hypertensive SHR indicated an already high cardiac sympathetic activity, which was further increased from the eighth week of age on (Figure 7A).

Interestingly, myocytes’ diameter of pre-hypertensive SHR was almost 2-fold higher than that of age-matched normotensive controls. Keeping with the known developmental tendency of the biological aging progress,37 myocytes’ diameter and LVW/BW ratio, as well as resting HR, decreased with age (Figure 2A and Figure 5B,5C). However, the SHR-S myocytes’ diameter reduction is interrupted and reversed by the development of hypertension, attaining a high value in the chronic phase. At this time point, pressure variability, AT1R expression and LV collagen content were

Figure 7. Schematic representation of temporal changes of AT1- and Mas receptors expression and left ventricle remodeling in sedentary (A) and trained (B) spontaneously hypertensive rats (SHR) during the transition from the pre- to hypertensive phase. The black numbers on the X-axis indicate the experimental weeks and red numbers in parenthesis represent the age of rats at that time point. The longitudinal pink arrows represent the incidence of hypertension; the arrows pointing upwards indicate the time point in which sympathetic (pink) and parasympathetic activity (green) starts to increase. The relative width and size of arrows are indicative of the magnitude of changes.
also largely augmented (Figure 7A). As shown by Kudo et al., exaggerated pressure variability superimposed on hypertension aggravated the deleterious cardiac remodeling via Ang II-mediated chronic inflammation.

Figure 7B depicts an important finding of this study. Aerobic training did not interfere with the development of genetic hypertension, but delayed the onset by 3 weeks, reduced its magnitude and postponed the increase in vaso-motor sympathetic activity by 4 weeks. Importantly, it offset the hypertension-induced rise in cardiac AT1R expression and facilitated MasR expression from the fourth experimental week on. These training-adaptive changes increased parasympathetic modulation of the heart (fourth experimental week), decreased and postponed hypertension-related sympathoexcitation (Figure 7B), thus normalizing HR variability and reducing pressure variability, essential mechanisms to reduce end-organ damage. Indeed, increasing HR variability and reducing pressure variability, 

Conclusions

Our data that analyzed the temporal effects of RAS axes in LV remodeling during the transition from the pre- to hypertensive phase in sedentary and trained SHR uncover the pivotal role played by aerobic training in protecting the heart against deleterious remodeling. Repetitive exercise is accompanied by early downregulation of the ACE-Ang II-AT1R axis followed by upregulation of the contraregulatory RAS axis, both contributing to improve the autonomic control and to avoid myocytes' hypertrophy and LV collagen deposition. These adaptive responses contribute to a delay and a decrease in the magnitude of hypertension, reducing the risk for end-organ damage, even in the presence of hypertension.

Disclosures

The authors declare no potential conflicts of interest concerning the research, authorship and/or publication of this article.

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