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Myocardial hypertrophy induced by high salt consumption is prevented by angiotensin II AT2 receptor agonist

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Abstract *Background and aims:* Although many studies have reported the effects of AT1 receptor on dietary salt overload, the role of AT2 receptor in this model is far from completely elucidated. The present study aimed to better understand the role of AT2 receptor in cardiac structure alterations in response to chronic high salt intake in rats.
Methods and results: Male Wistar rats were fed a normal or high salt diet from weaning until 18 weeks of age. Both groups were subdivided into two groups. Starting at 7 weeks of age, rats were treated with or without compound 21 (0.3 mg/kg/day, n = 16), an AT2 receptor agonist. Metabolics and structural parameters were measured. BP, transverse cardiomyocyte and interstitial fibrose was higher in animals fed with high salt diet compared with normal salt fed animals.
Conclusion: Compound 21 prevented the development of cardiac hypertrophy and fibrosis, reduced the increase in blood pressure and prevented the lower weight gain in animals fed a high salt diet.

Introduction

High dietary salt intake is one of the main causes of hypertension and cardiovascular diseases [1,2]. Previous studies performed in our laboratory observed an association between high salt intake and myocardial hypertrophy and fibrosis in Wistar rats [3,4]. In addition, a higher cardiac angiotensin II (AngII) expression was observed in all groups fed high salt (HS) diet [3,4]. The role of the renin-angiotensin system (RAS) in the development of cardiac hypertrophy and fibrosis induced by a high salt diet is well established [3,4].

The expression of the AT1 receptor is increased in the left and right ventricles of male Wistar rats fed a high salt diet that develop cardiac hypertrophy, fibrosis and increased oxidative stress [4,5]. Although, several efforts were attempted to understand the role of the cardiac AT2 receptor in response to high salt intake, this subject needs additional studies for its full comprehension. Compound 21 is the first selective nonpeptide AT2 receptor-agonist to be reported and has a bioavailability of 20–30% after oral administration in rats [6]. Stimulation of the AT2 receptor with compound 21 in infarcted hearts of Wistar rats improves systolic and diastolic ventricular function [7]. Moreover, C21 protects the heart from early left ventricular thinning and rupture after a myocardial infarction [7].

In an effort to better understand the role of AT2 receptor in the heart of rats fed a high salt diet, the purpose of this study was to evaluate the effects of C21 in this experimental model.

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Methods

The experiments reported in this study were previously approved by the Committee of Research Projects Evaluation of the University of São Paulo School of Medicine, Brazil (certificate number 258/13).

Animals

Male Wistar rats obtained from the Institutional Animal Facility of the University of São Paulo School of Medicine were housed in a temperature-controlled environment at 25 °C on a 12-h (hr) light/dark cycle, with free access to food and water.

Rats were fed a normal (NS: 1.3% NaCl) or high salt (HS: 8% NaCl) diet (HarlanTekad, Madison, WI, USA) from weaning (3 weeks of age) to adulthood (18 weeks of age). All diets were pelleted. The only difference between the diets was their sodium chloride contents.

At 7 weeks of age, NS or HS subgroups started to received compound 21, an AT2 receptor agonist, orally active, non-peptide (Vicore Pharma AB, Göteborg, Sweden) treatment via daily gavaging until 18 weeks of age. It was verified before that compound 21 is active at 0.3 mg kg^{-1} day^{-1} dose in rats with myocardial infarction [7].

Experimental protocol

The body weight was measured weekly from 3 to 18 weeks of age. Blood pressure (BP) was measured at 6 weeks of age and every 2 weeks from 7 to 18 weeks of age. At 17 weeks of age, rats were individually housed in metabolic cages (model 650-0100, Nalgene Brand Products, New York, NY, USA) for 3 days. At 18 weeks of age, rats were decapitated for organ collection or perfused for histological analyses.

BP measurement

Blood pressure (BP) was measured in conscious animals using an oscillometric method (Kent Scientific, model RTBP 2045 with the RTBP 001 acquisition system, CT, USA).

Histological analysis

Cardiac fibrosis and cardiomyocyte diameter were measured in tissue sections stained respectively with Masson's trichrome and periodic acid Schiff. For details, see Supplementary data.

Determination of angiotensin II expression

Immunohistochemistry was performed on 5-μm-thick sections mounted on glass slides that had been precoated with 2% silane to determine the AngII expression in both ventricles. Sections were deparaffinized and rehydrated using conventional techniques. Thereafter, sections were immediately circled with a PAP pen (Vector Laboratories, −CA, USA) to form a waterproof barrier and were pre-incubated with an Avidin/Biotin blocking kit (Vector Laboratories, −CA, USA). Sections were then washed and incubated with a serum-free protein blocker (DAKO) for 30 min. Afterward, they were incubated with an anti-angiotensin II primary antibody (Peninsula, −CA, USA) overnight at 4 °C. Sections were washed and incubated with a LSAB-HRP kit (K0609) (DAKO North America, Inc., CA, USA) for 30 min.

The angiotensin II-positive areas were quantified using a point counting method. The total area of 2 slices per animal was examined at a magnification of 200 X (Nikon, Tokyo - Japan).

RNA isolation and expression of genes involved in the RAS in both ventricles

Gene expression was assessed using RT-PCR as previously described [8]. Primers used for RT-PCR are listed in Table 1S of the supplementary data.

Expression of proteins involved in the RAS

Protein expression was measured by Western blotting using the protocol described in Supplementary data.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used to compare three or more means. Changes in TcBP over time were analyzed using two-way ANOVA with Bonferroni's post hoc test. Results are presented as means ± SEM. Calculations were performed using Prism® 5.0 (GraphPad® Software). $P < 0.05$ was considered significant.

Results

BP

At six weeks of age, differences in BP were not observed between the 4 experimental groups. However, the BP was higher in the HS group beginning at 7 weeks than in other groups, and this profile was maintained until the 18th week. Additionally, the BP was lower in the HS + C21 group than in the HS group, with the exception of the 11- and 13-week-old animals, indicating an antihypertensive effect of compound 21. BP did not differ between NS and NS + C21 groups until 17 weeks, and at 18 weeks, the BP was lower in the NS + C21 group than in NS group (Fig. 1).

Food and water intake and 24-h urinary volume

The water intake and 24-h urinary volumes were higher in the HS and HS + C21 groups than in the NS and NS + C21 groups. No differences were observed between the NS and NS + C21 groups. The food intake was not different between the groups (Table 1).

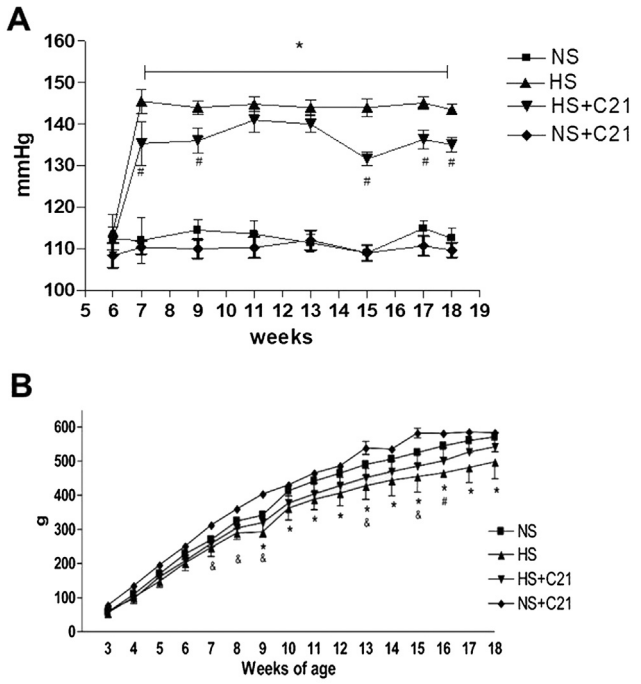

Figure 1 Tail-Cuff Blood Pressure (A) and Body Weight (B). Eighteen-week-old rats (n = 14–16) were fed diets with normal–or high-salt diet since weaning and treated with or without compound 21 (C21) since seven weeks of age. *P < 0.05 HS vs. NS; #P < 0.05 NS + C21 vs. NS; &P < 0.05 HS + C21 vs. NS.

Body weight

Body weight was lower in the HS group than in the NS group from 8 to 18 weeks of age. At 14 weeks, the body weight of the HS + C21 group was higher than the HS group. No differences were observed between NS and HS + C21 groups. At 7 weeks of age, a higher body weight was observed in the NS + C21 group than in the NS group (Fig. 1).

Heart rate, cardiac index, and LV and RV angiotensin II expressions

Heart rates did not differ between any experimental groups. The ratios between the cardiac mass (cardiac index), LV mass (LV index), and RV mass (RV index) corrected for tibia length measured in the 18-week-old Wistar rats were not different between groups. The angiotensin II expression in the LV was higher in the NS + C21 and HS groups than in the NS group. No difference was observed between the HS + C21 and NS groups. No differences in the angiotensin II expression in the RV were observed among all groups (Table 1).

Transverse cardiomyocyte diameter and interstitial fibrosis in both ventricles

In the LV, a larger transverse cardiomyocyte diameter was observed in the HS and HS + C21 groups than in the NS and NS + C21 groups, respectively, and a smaller diameter was observed in the HS + C21 group than in the HS group. In the RV, a larger transverse cardiomyocyte diameter was observed in the HS group than in NS, NS + C21 and HS + C21 groups. Myocardial Masson's trichrome staining revealed a greater extent of interstitial fibrosis in the LV of the HS group than in the other experimental groups. In the RV, the level of interstitial fibrosis was lower in the NS + C21 group than in the NS group, and the level in the HS group was higher than the HS + C21 group (Fig. 2).

Expression of gene and proteins involved in the RAS

Gene and protein expression of all components of RAS is on Supplementary data. There was no significative difference in gene and protein expression in left ventricle. C21 not differ gene and protein expression in right ventricle when compare with controls groups.

Discussion

One interesting finding of the present study is the ability of C21 to partially prevent the increase in BP induced by high salt intake. Similarly, it was already observed that C21 decreases blood pressure in obese rats fed with a high salt diet associated to higher natriuresis [9]. It was also observed that C21 increases vasorelaxation of resistance arteries in response to AT2 receptor stimulation [10,11]

Table 1 Body weight, cardiac mass and index, LV and RV mass and index, hematocrit, urinary and serum sodium levels, LV and RV angiotensin II expressions in 18-week-Old wistar rats in the NS, NS + C21, HS and HS + C21 groups.

	NS	N	NS + C21	N	HS	n	HS + C21	N
Body weight, g	571 ± 11.3	12	583 ± 11.8	12	497 ± 12.8[a]	12	542 ± 12.1	11
Cardiac index, g/cm	0.53 ± 0.0	15	0.51 ± 0.0	15	0.51 ± 0.0	16	0.52 ± 0.0	13
LV index, g/cm	0.33 ± 0.0	16	0.34 ± 0.0	16	0.32 ± 0.0	15	0.35 ± 0.0	15
RV index, g/cm	0.08 ± 0.0	16	0.07 ± 0.0	16	0.08 ± 0.0	15	0.08 ± 0.0	15
Cardiac mass, g	2.42 ± 0.2	16	2.35 ± 0.1	16	2.40 ± 0.1	15	2.41 ± 0.2	15
LV mass, g	1.52 ± 0.0	16	1.48 ± 0.1	16	1.54 ± 0.0	15	1.59 ± 0.1	15
RV mass, g	0.40 ± 0.0	16	0.35 ± 0.0	16	0.37 ± 0.0	15	0.37 ± 0.0	15
Food intake	13.5 ± 2.3	8	12.2 ± 0.8	8	15.9 ± 0.7	8	17.3 ± 2.1	8
Water intake	31.8 ± 1.6	8	30.0 ± 6.5	8	77.1 ± 9.1[a]	8	69.2 ± 9.4[a]	8
24-h urinary volume	14.0 ± 0.8	8	9.7 ± 1.0	8	41.9 ± 1.3[a]	8	38.4 ± 2.0[a]	8
Angiotensin II LV (% area)	0.35 ± 0.1	8	1.00 ± 0.4	8	1.56 ± 0.2[a]	6	1.53 ± 0.2[a]	7
Angiotensin II RV (% area)	0.44 ± 0.2	8	0.33 ± 0.1	8	0.70 ± 0.3	6	0.66 ± 0.3	7

[a] P < 0.05 compared with the NS and NS + C21 group; HS, high salt (8% NaCl); HS + C21, HS + compound 21 (0.3 mg/kg/day); LV, left ventricle; NS, normal salt (1.27% NaCl); NS + C21 (0.3 mg/kg/day); RV, right ventricle.

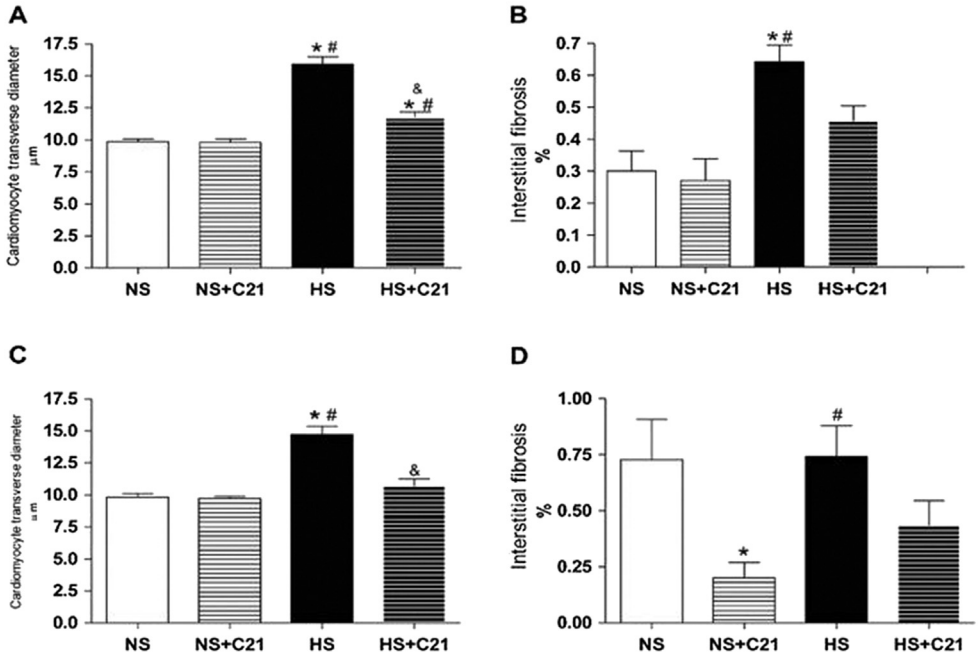

Figure 2 Transverse Cardiomyocyte Diameter and Interstitial Fibrosis. Cardiomyocyte diameters and interstitial fibrosis in the left ventricle (LV) (A and B) and right ventricle (RV) (C and D) of 18-week-old (n = 6–8) fed with normal salt (NS) or high salt (HS) diet that were treated with or without compound 21 (C21). *P < 0.05 vs. NS group; #P < 0.05 vs. NS + C21 group; &P < 0.05 vs. HS group.

possibly explaining the result of C21 on blood pressure in the present study. Further studies should be performed to evaluate a possible therapeutic effect of C21 in hypertensive humans.

It is possible that the dose of C21 used may not be sufficient to achieve a maximum stimulation of AT2 receptor. Therefore, a study examining a gavage dose response curve for compound 21 is needed to better understand the partial hypotensive response of the dose of C21 used in this study.

The higher food intake observed in both the HS and HS + C21 groups is a confirmation of previous findings from our group. High salt intake increases food intake, despite the lack of increase in body weight. This phenomenon is due to higher energy expenditure observed in rats fed with high sal diet [12].

In the present study, the body weight of the animals fed a high salt diet was lower compared to normal salt fed rats beginning at eight weeks of age and persisted until the end of the study. This observation confirms previous findings from our laboratory were a high energy expenditure was observed in rats fed with high salt diet [12]. Interestingly, compared to the NS group, the lower weight gain observed in the HS group was prevented by C21. Since we have previously observed a relationship between high salt intake and higher energy expenditure [12], it can be speculated that C21 decreases brown adipose tissue activity.

The cardiac index and mass were not different among groups. The left ventricle mass was reported to be normal in 20% of patients with hypertrophic cardiomyopathy, as detected by magnetic resonance imaging [13]. Based on this previous study and the present results, cardiac mass may not be the best indicator of cardiac hypertrophy, since the HS group displayed a larger transverse cardiomyocyte diameter. In other hand, results from transverse cardiac diameter determination showed that C21 protected left and right ventricles from cardiac hypertrophy in Wistar rats fed a high salt diet. Many studies have reported an improvement in cardiac structure elicited by AT2 receptor stimulation [14–16]. The present study is the first to show the protective role of compound 21 in cardiac hypertrophy induced by high salt intake.

A higher interstitial fibrosis was observed in the LV of the HS group than in all other groups. C21 protected the hearts of animals fed a high salt diet from fibrosis in LV. A similar effect of AT2 receptor stimulation has been observed in other studies, which showed an improvement in cardiac fibrosis by reducing the expression of fibronectin and collagens I and III in the heart. In RV no difference in interstitial fibrosis was observed between HS and NS fed animals. However a lower interstitial fibrosis was observed in C21 treated rats fed NS and HS. This result was unexpected. Perhaps, C21 might initiate a positive feedback in healthy animals impairing the normal fibrosis production in right ventricle. Nevertheless, additional studies are required to clarify this finding.

Confirming previous reports [3,4], in the present study, cardiac angiotensin II expression was increased in both groups fed high salt diet.

In conclusion, salt-induced cardiac hypertrophy was prevented by C21. In addition, C21 ameliorated the effect of salt overload on BP. Thus, C21 may be an accessory therapeutic treatment for salt-induced hypertension.

Conflicts of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2018.11.001.

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