Spironolactone Effects on Myocardium Changes Induced by Thyroid Hormone in Rats

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Summary

Background: To study the possible role of aldosterone on thyroid hormone-induced myocardium hypertrophy, using spironolactone.

Objective: To evaluate morphological changes in the myocardium induced by thyroid hormone and the possible effects of spironolactone use on these alterations.

Methods: Forty Wistar rats were studied. The animals were allocated to four groups and were given: the vehicle used for dilution of the thyroid hormone (C); sodium levothyroxin at 50 μg/rat/day (H); spironolactone, 0.3 mg/kg/day (S); or thyroid hormone plus spironolactone (HS), at the same doses mentioned above, for 28 consecutive days. All the animals were weighed, had blood drawn for hormonal measurements and underwent ECG at the start and the end of the experiment. At the end of experiment all animals were euthanized, the weight of the left ventricle (LV) was determined and LV slices were obtained for morphological analysis.

Results: There was an increase in T3 levels, decrease of body weight and higher heart rate in the animals from group H. The LV weight was significantly higher in the H e HS groups. The histometric analyses that measured the diameter of the myocytes showed higher values in group H and a progressive decrease in groups HS, S and C, with a significant difference among all the groups. The addition of spironolactone decreased the transversal myocyte hypertrophy by 14.6%.

Conclusion: Rats treated with thyroid hormone present cardiac hypertrophy with increased LV weight and greater myocyte diameter. Spironolactone, when associated with thyroid hormone, can partially prevent this hypertrophy through mechanisms that are yet to be determined. (Arq Bras Cardiol 2007;89(6):360-361)

Key words: Myocardium; thyroxine; spironolactone; ventricular remodeling; cardiomegaly.

Introduction

The correlation between thyroid disease and cardiopathy was first established in 1825, when Caleb Parry noticed the association between the increased thyroid volume and heart failure[1]. It was only 50 years later that Robert Graves described the disease, which later would be named after him. The importance of the cardiac manifestations in thyrotoxicosis has been acknowledged worldwide[2,3], particularly those related to myocardial hypertrophy[4-7] and the presence of arrhythmias[8,9].

The hypertrophic action of the thyroid hormone (TH) on the heart occurs through a direct action, by modulating the protein synthesis in myocytes[10-12], as well as through indirect systemic effects[13,14], by causing important changes in hemodynamics[15,16].

In the last 30 years, it has been acknowledged that aldosterone is associated not only with the pathogenesis, but also with the progression of heart failure[17]. Results of the *CONSENSUS I* showed that elevated plasma aldosterone values are correlated with poor survival in patients with congestive heart failure (CHF) functional class IV and that the association of enalapril to the therapy for CHF reduced mortality by 27% when compared to the placebo group[18,19].

The activation of the renin-angiotensin-aldosterone system in arterial hypertension can lead to myocardium remodeling through the progressive accumulation of collagen in the interstitium and heart hypertrophy. This reactive fibrosis seems to be an important determinant of the diastolic dysfunction and the pathological hypertrophy[6,17].

More recent studies such as *RALES*[20] and *EPHESUS*[21] demonstrated that increased plasma aldosterone levels have deleterious effects on the cardiovascular system. A study carried out in rats showed that spironolactone prevents collagen proliferation in the myocardium after the infarction, which complements the previous findings[22]. In these animals, the reactive fibrosis that follows necrosis decreased the systolic and diastolic functions, inducing heterogeneity and electrical dispersion, which predisposes to arrhythmias[23].

The participation of the renin-angiotensin-aldosterone

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system[20,24,25] in myocardial remodeling has yet to be investigated in hypertrophy induced by the thyrotoxic state.

The aim of the present study was to verify whether there was such correlation with aldosterone and if it could be blocked by using a specific competitor for this hormone, spironolactone.

Methods

Animals - Forty male adult Wistar rats, evaluated under the same environmental conditions, were used in the study. All animals received humane care and had free access to food and water throughout the study. After an adaptation period of 1 week, the forty rats were sequentially distributed in four groups of 10 animals each, identified as: control (C), thyroid hormone (H), spironolactone (S) and thyroid hormone + spironolactone (HS) groups.

The groups underwent the following procedures:

Group C - 0.5 ml/day, intraperitoneally, of the vehicle used for the dilution of the thyroid hormone (buffer solution).

Group H - thyroid hormone diluted in buffer solution, at a dose of 50 ug/rat/day, intraperitoneally.

Group S - spironolactone, at a single daily dose of 0.3 mg/kg/day, subcutaneously, diluted in olive oil, in a 20 mg/ml solution.

Group HS - thyroid hormone at a dose of 50 ug/rat/day, intraperitoneally and spironolactone, at a single daily dose of 0.3 mg/kg/day, subcutaneously, diluted in olive oil, in a 20 mg/ml solution.

The experiment lasted 28 days for all groups and all animals were euthanized at the end.

All animals were weighed before and after the 28 days of treatment. The rats were anesthetized with ketamine, at a dose of 0.1 ml/100g, i.m., associated to xylazine chloride at a dose of 0.1 ml/100g, i.m.

Blood samples were collected from all anesthetized animals at times 0 and 4 weeks after the use of the drugs described in the experimental protocol. The samples were used for hematocrit determination as well as T3 and T4 measurement (by chemiluminescence). At basal time, blood samples were obtained by cutting each rat's tail tip and collecting the blood drop by drop, up to a volume of 0.8 ml. After the four-week intervention, the blood was collected through cardiac puncture, before the animals were humanely euthanized.

Heart rate was measured at the basal moment and after the four-week interval by conventional ECG. At the end of the predicted time, the rats were euthanized under anesthesia, the thorax was opened and the heart was removed, profusely washed with Ringer Lactato® and fixed in formaldehyde solution at 10%. After 24 hours, the heart was weighed on a precision scale; the left ventricle was separated, together with the interventricular septum and weighed.

Thereafter the sample was kept in alcohol 70% until its inclusion in paraffin.

The analysis was carried out considering certain aspects of optical microscopy and histometry from the digitalization of the slide pictures to measure the transversal diameter of the myocytes.

Drug dilution techniques - To dilute the thyroid hormone, a PBS buffer solution (Na2HPO4, 50mM at pH 7.4)[26] was used. Spironolactone was dissolved in acetone and then diluted in olive oil. Acetone evaporation was carried in an ultrasound bath.

Left ventricle (LV) weight - The weight of the LV was determined together with the interventricular septum. To avoid interference caused by the modifications of body weight on the LV, the expected weight of this chamber was determined for any final weight of the animal. This was calculated based on the LV weight in the control group and the final weight of the animals. A linear regression analysis was carried out and a straight line equation was obtained, which allows the calculation of the expected LV weight for any rat with any weight. Once the expected weight of LV was defined in each group, the comparison with the respective weight observed after the treatment was performed.

Histomorphometry - The images were obtained from a binocular optical microscope and all the images were captured by a video camera at 40X magnification. Image selection for capture and digitalization was carried out manually.

The morphometry of the acquired and digitalized pictures was performed using adequate software. Five captures of different fields were acquired from each of the five slices obtained from the LV of each animal, chosen according to the place where more cells could be visualized at the cross-section. Of these, the smallest diameters were measured in 4,759 cells in the 40 rats studied.

Statistical analysis - Analysis of variance (ANOVA) was used to compare the studied groups, complemented by Tukey's test, when necessary. This test was applied to HR, T3 measurement and myocyte diameter. Student's *t* test for non-independent samples was used when the weight of the animals was compared at the beginning and at the end of the experiment in each group and when the expected and observed LV weights were compared for each studied group. Statistical significance was set at $p \leq 5\%$.

Ethics - All animals received humane care throughout the experiment according to the guidelines established by "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D. C., 1996) and the Ethical Principles in Animal Experimentation of the Brazilian College of Animal Experimentation (COBEA).

Results

Animals' weight - There was no significant difference regarding the weight of the animals in groups C and HS before and after treatment. In group H, the final weight was significantly lower than the initial one. Conversely, the final weight was higher than the initial one in group S. The

percentage differences regarding the weight of the animals from the beginning to the end of the study were 12.53% of weight loss for group H and 8.63% of weight gain for group S.

Total T3 - Final total T3 was 104 ± 9 ng/dl in group C, 173 ± 40 ng/dl, in group H, 102 ± 9 ng/dl in group S and 180 ± 44 ng/dl in group HS. These values were significantly higher in groups H and HS.

Final Heart Rate (HR) in beats per minute (BPM) - The final HR in groups C, H, S and HS were, respectively, 215 ± 19 bpm, 309 ± 23 bpm, 211 ± 2 bpm and 301 ± 48 bpm.

Final HR was significantly higher in groups **H** and **HS**.

LV weight - As shown in Table 1, the observed LV weight was significantly higher than the expected LV weight only in groups H and HS.

Histomorphometry - The measurement of the diameter of the muscular fibers in groups C, H, S and HS are significantly different among them, as shown in Table 2.

Group C presented the smallest fiber diameters (13.90 ± 3.00μ) and group H (20.95 ± 4.29μ) presented the largest diameters. Group S also presented increased fiber diameter when compared to group C.

Table 2 – Mean and SD of the diameters of myocytes in micra (μ) in the control (C), thyroid hormone (H), spironolactone (S) and thyroid hormone + spironolactone (HS) groups

Value	Myocyte diameter (μ)			
	C	H	E	HE
Mean	13.90	20.95 *	16.00 *	16.95 *
SD (a)	3.00	4.29	3.05	3.80
N	1,165	1,109	1,215	1,240

*SD - standard deviation; N - number of myocytes measured * p ≤ 5%.*

Discussion

Several experimental models have been used to study the multiple aspects involved in ventricular remodeling. In this context, the direct or indirect participation of substances that integrate the neurohormonal axis, activated in situations of low blood output, is involved. Vasoconstriction (noradrenaline, angiotensin II, endothelin I and arginine-vasopressin) and vasodilation substances (natriuretic peptides, bradykinin) integrate such axis. Little is known, however, on the participation of the thyroid hormone[27] in ventricular remodeling[24,26].

The present experiment was designed with the objective of studying the effects of TH on cardiac hypertrophy. The induction of the thyrotoxic state in rats is simple[27] and the confirmation of this condition can be obtained by the elevated T3 levels, increased HR and body weight loss.

The total duration of exposition to the TH was 28 days. One could ask whether the structural heart changes would be present after such a short period of time. An interesting study by KLEIN[24] showed an increase in the heart contractile proteins after 4, 7, 10 and 14 days of T4 therapy, with the stabilization of the hypertrophy model from this point on.

The cardiac hypertrophy in our model of thyrotoxicosis was clearly demonstrated by the increase in the LV weight, which was, on average, 17.3% higher in the group treated with thyroid hormone. Similar results were also detected by other studies. This increase in the LV weight seems to be the result of direct and indirect actions of the thyroid hormone on the myocardium, which are expressed by the increase in protein synthesis[25], with consequent myocyte hypertrophy. The thyroid hormone alters not only the myocyte, but also the collagen matrix[28]. At optical microscopy (OM), the mean diameter of the myocytes was 50.68% higher than that of the control group.

As described by other authors[26-29] in similar studies, no areas of necrosis were identified in the analyzed material.

The addition of spironolactone to the TH did not interfere with the T3 levels and did not modify the HR behavior, of which elevation in thyrotoxicosis is triggered by direct and indirect mechanisms of the thyroid hormone[21]. Spironolactone did not interfere with the LV weight in relation to the control group, but when it was associated with TH, there was a decrease in the hypertrophy. This effect of spironolactone on the TH-induced hypertrophy has not been previously described, although its fibrosis prevention effect that occurs in systemic arterial hypertension (SAH) models is well-known[6,17].

Table 1 – Expected and observed LV weight values in the control (C), thyroid hormone (H), spironolactone (S) and thyroid hormone + spironolactone (HS) groups

Rat	C		H		S		HS	
	Observed weight	Expected weight	Observed weight	Expected weight	Observed weight	Expected weight	Observed weight	Expected weight
Mean	0.92	0.92	1.08 *	0.94	1.00	1.02	1.15 *	1.03
SD (a)	0.07	0.05	0.07	0.03	0.05	0.04	0.08	0.05

*SD - standard deviation; * p ≤ 5%.*

At the OM analysis, spironolactone decreased the diameter of the myocytes by 14%, on average, when compared to the group that received only TH. These results confirm the hypothesis that this drug is able to reduce the hypertrophy induced by the thyroid hormone and reinforces its role in ventricular remodeling, not only by reducing the fibrosis, but also acting on the muscular component, as previously demonstrated in SAH models[30,31]. Recently, it has been recognized that aldosterone is associated not only with the pathogenesis, but also with the progression of heart failure[20,31], by facilitating disproportional patterns of myocardial hypertrophy and fibrosis. If the blocking of aldosterone by spironolactone was able to partially prevent the hypertrophy induced the thyroid hormone, it can be presumed that the hormonal system, to which aldosterone is integrated, can be involved in the myocardial alterations that occur in thyrotoxicosis. The connection between these actions might be related to the G-protein family, an extensive group of substances that exist in the plasmatic membrane and mediate catecholamine action. It is known that the cell growth process involves the activation of the receptor family coupled to these GTP-bound proteins, which constitute the largest group of known proteins that integrate the cell membrane and are involved with the signal transduction of some hormones[32]. If the thyroid hormone acts by activating the G-protein and that can trigger angiogenesis and myocyte hypertrophy[14], it is possible that spironolactone blocks these actions, at least partially, modulating the hypertrophy in SAH[17] as well as in that induced by thyroid hormone.

The outcome of a large clinical trial – the Randomized Aldactone Evaluation Study (RALES)[20] – in which spironolactone reduced the mortality by 30% and the hospitalization of patients with advanced heart failure, when associated to the standard therapy for this disease, by 35% corroborates the importance of blocking aldosterone in the clinical evolution of this disease[20,33].

Additionally, a more recent study – the Eplerenone Post Acute Myocardial Infarction Efficacy and Survival Study (EPHESUS)[21], which associated eplerenone, a selective blocker of aldosterone, to the conventional treatment in patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure, also showed a significant reduction in the morbimortality. The effect of the eplerenone, reducing hypertrophy and heart failure in rats with salt-sensitive hypertension, can be attributed, at least in part, to the attenuation of the oxidative stress in the myocardium and in the coronary vascular inflammation induced by the glucocorticoid-mediated mineralocorticoid receptor activation[34-37].

If the undesirable actions of hyperthyroidism on the myocardium are modulated, the improvement in the hemodynamic conditions produced by the thyroid hormone might be important in the control of CHF.

A noteworthy aspect was the increase in the myocyte diameter when they were exposed to spironolactone. This fact must be investigated in further studies with this drug.

In conclusion, rats treated with thyroid hormone demonstrate cardiac hypertrophy with increased LV weight and myocyte diameter. The association of spironolactone to the thyroid hormone partially prevents this hypertrophy. The mechanisms responsible for the modifications in the LV weight and myocyte diameter are yet to be elucidated.

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