Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2011;27:291-298

Accepted: February 02, 2011

Influence of Taurine on Cardiac Remodeling Induced by Tobacco Smoke Exposure

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Key Words
Ventricular function • Smoking • Heart failure

Abstract
Background/Aims: To investigate the effect of taurine on cardiac remodeling induced by smoking. Methods: In the first step, rats were allocated into two groups: Group C (n=14): control; Group T (n=14): treated with taurine (3% in drinking water), for three months. In the second step, rats were allocated into two groups: Group ETS (n=9): rats exposed to tobacco smoke; Group ETS-T (n=9): rats exposed to tobacco smoke and treated with taurine for two months. Results: After three months, taurine presented no effects on morphological or functional variables of normal rats assessed by echocardiogram. On the other hand, after two months, ETS-T group presented higher LV wall thickness (ETS=1.30 (1.20-1.42); ETS-T=1.50 (1.40-1.50); p=0.029), E/A ratio (ETS=1.13±0.13; ETS-T=1.37±0.26; p=0.028), and isovolumetric relaxation time normalized for heart rate (ETS=53.9±4.33; ETS-T=72.5±12.0; p<0.001). The cardiac activity of the lactate dehydrogenase was higher in the ETS-T group (ETS=204±14 nmol/mg protein; ETS-T=232±12 nmol/mg protein; p<0.001). ETS-T group presented lower levels of phospholamban (ETS=1.00±0.13; ETS-T=0.82±0.06; p=0.026), phosphorylated phospholamban at Ser16 (ETS=1.00±0.14;ETS-T=0.63±0.10;p=0.003), and phosphorylated phosfolamban/phospholamban ratio (ETS=1.01±0.17; ETS-T=0.77±0.11; p=0.050). Conclusion: In normal rats, taurine produces no effects on cardiac morphological or functional variables. On the other hand, in rats exposed to cigarette smoke, taurine supplementation increases wall thickness and worsens diastolic function, associated with alterations in calcium handling protein and cardiac energy metabolism.

Introduction

Cardiac remodeling may be defined as changes in the size, geometry, shape, composition and function of the heart. This process occurs in response to several stimuli including volume overload, hypertension, myocardial infarction and genetic mutations. Importantly, ventricular

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remodeling is recognized as a significant pathological process that results in progressive ventricular dysfunction and cardiovascular death. In addition, strategies to reverse or prevent further remodeling are the key to improving prognosis following several injuries [1-3].

Taurine is the most abundant free amino acid in the heart [4]. Several experimental and clinical studies showed that reduced taurine levels are associated with important cardiovascular alterations [5-8]. Likewise, taurine supplementation attenuated the cardiac remodeling induced by different injuries. Some theories have been proposed to account for the cardioprotective activity of taurine: it plays a role similar to diuretics since it promotes sodium and water excretion; it modulates several ions relevant to normal cardiac cell function; it protects the liposomal membranes against damage caused by free radicals and antagonic action due to angiotensin II [7-13]. Therefore, the cumulative evidence suggests that taurine might play a critical role in the cardiac remodeling process.

Recently, several studies have shown that exposure to tobacco smoke, or to its compounds, results in cardiac remodeling and compromised cardiac function [14-20]. However, the exact mechanisms involved in this phenomenon are not known. Given that taurine may modulate cardiac alterations following different injuries, we have hypothesized that cardiac remodeling induced by tobacco-smoke exposure would be modulated by taurine. Therefore, the objective of this study was to investigate the effect of taurine on cardiac remodeling induced by tobacco smoke exposure in rats.

Materials and Methods

Groups and Treatment

All experiments and procedures were performed in concordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee of our Institution.

In the first step, male Wistar rats weighing 200-250 g were allocated into two groups: Group C (n=14): control animals; Group T (n=14): animals treated with taurine (3% in drinking water). The planned observation period was three months.

In the second step, male Wistar rats weighing 200-250 g were allocated into two groups: Group ETS (n=9): rats exposed to tobacco smoke (40 cigarettes/day); Group ETS-T (n=9): rats exposed to tobacco smoke (40 cigarettes/day) and treated with taurine (3% in drinking water). The planned observation period was two months, given that after this follow-up period, the animals already presented cardiac abnormalities induced by smoking [14-18].

The ETS rats were exposed to cigarette smoke in a chamber (dimensions 95x80x65 cm) connected to a smoking device [14-18]. The smoke was drawn out of filtered commercial cigarettes (composition per unit: 0.6mg of nicotine; 8 mg of tar; and 8 mg of carbon monoxide) with a vacuum pump and exhausted into the smoking chamber. During the first week, the number of cigarettes was gradually increased from 5 to 10 cigarettes over a 30-min period, twice in the afternoon. After that, 10 cigarettes were used in each smoking trial, repeated four times/day, twice in the morning and twice in the afternoon.

This protocol has consistently shown that tobacco smoke exposure induces enlargement of the left ventricular chamber, and myocardial hypertrophy [14-18]. Given the corresponding carboxyhemoglobin levels, this protocol is similar to 3-4 pack/day in a human. On the other hand, this protocol did not result in hypoxia [15, 17].

Systolic Blood pressure

After the observation period, the tail systolic pressure was measured in each animal by use of a tail plethysmograph with a polygraph (Byo-Sistem PE 300, NARCO), a sensor placed in the proximal region of the tail, and an electro-sphygmomanometer to record tail pressure. The animals were warmed in a wooden box at 37°C with the heat generated by 2 incandescent lamps for 4 minutes and then transferred to an iron support, where the tail was exposed. In the proximal region of the tail, a sensor (KSM-microphone) was placed and coupled to the plethysmograph. Blood pressure was recorded on paper with the polygraph at a 2.5-mm/s velocity.

Echocardiographic Study

After the observation period, all animals were weighed and evaluated by a transthoracic echocardiographic exam. The exams were performed using a commercially available echocardiographic machine (Philips model TDI 5500) equipped with a 12 MHz phased array transducer. Imaging was performed utilizing a 60º sector angle and a 3 cm imaging depth. Rats were lightly anesthetized by intramuscular injection of a mixture of ketamine (50mg/kg) and xylazine (1mg/kg). All measurements were obtained according to the leading-edge method recommended by the American Society of Echocardiography/ European Association of Echocardiography [21]. Measurements represented the mean of at least five consecutive cardiac cycles. Left ventricle (LV) end-diastolic dimension (LVEDD) and posterior wall thickness (LVWT) were measured at maximal diastolic dimension, and the end-systolic dimension (LVESD) was taken at the maximal anterior motion of the posterior wall. The left atrium (LA) was measured at its maximal diameter and the aorta at the end of diastole. The LV systolic function was assessed by calculation of the ejection fraction [$(LVDD^3 - LVSD^3)/ LVDD^3$], fractional shortening index [(LVDD – LVSD)/ LVDD] x 100, cardiac output (CO) ($LVDD^3$- $LVSD^3$) x heart rate), flow velocity through the aorta (VAO), and cardiac index (CI) (CO divided by body weight). The velocities of transmitral diastolic flow (E and A velocities) were obtained from the apical four-chamber view. The E/A ratio, the isovolumetric relaxation time (IRT), and the isovolumetric relaxation time normalized to the heart rate ($IRT/RR^{0.5}$) were used as indices

of LV diastolic function.

Morphometric analysis

At the completion of the functional study, the right (RV) and left ventricles (including the interventricular septum) were dissected, separated, and weighed.

The morphometric analysis of the myocardium was performed as described previously [22-23]. LV myocyte cross-sectional area (CSA) was determined for at least 100 myocytes per slide stained with hematoxylin-eosin. The measurements were performed using a Leica microscope (magnification lens X400) attached to a video camera and connected to a personal computer equipped with image analyzer software (Image-Pro Plus 3.0, Media Cybernetics, Silver Spring, MD). LV interstitial collagen volume fraction (CVF) was determined for the entire picrosirius-red-stained cardiac section through an automated image analyzer (Image-Pro Plus 3.0, Media Cybernetics). On average, 35 microscopic fields were analyzed with an X40 lens. Perivascular collagen was excluded from this analysis.

Evaluation of cytokine production

Briefly 60 mg of cardiac tissue samples was homogenized and solubilized in 50 mM potassium phosphate buffer, pH 7.4; 0.3 M sucrose; 0.5 mM DTT; 1mM EDTA, pH 8.0; 0.3 mM PMSF; 10 mM NaF, and 1:100 protease inhibitor. Cytokine levels in cardiac homogenate were evaluated by ELISA according to manufacturer instructions (R & D Systems, Minneapolis, MN, USA). Sensitivities of ELISA for IFN-γ and TNF-α were 19 and 31 pg/mL, respectively.

Cardiac energy metabolism

LV samples of 200 mg were weighed and homogenized in 5 ml of cold phosphate buffer (0.1M, pH 7.4) containing 1mM ethylenediaminetetraacetic acid (EDTA). Tissue homogenates were prepared in a motor-driven Teflon-glass Potter-Elvehjem tissue homogenizer (1min, 1000 rpm) immersed in ice water. The homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant was analyzed for total protein, lactate dehydrogenase (LDH; E.C.1.1.1.27.), β- hydroxyacyl-coenzyme A dehydrogenase (HADH; E.C.1.1.1.35.) and citrate synthase (CS; E.C.4.1.3.7.). The assay medium for LDH contained 50mM Tris-HCl buffer (pH 7.5), 0.15 mM nicotinamide adenine dinucleotide in reduced form and 1mM pyruvate (omitted in the C group). For CS activity the assay medium consisted of 50mM of Tris-HCl buffer (pH 8.1), 0.3mM acetyl-coenzyme A, 0.1mM 5,5-dithio-bis-2-nitrobenzoic (DTNB) and 0.5 mM oxaloacetate (omitted in the group C). HADH was assayed in a medium containing 50mM Tris-HCl buffer (pH 7.0), 5mM EDTA, 0.45mM reduced nicotinamide adenine dinucleotide and 0.1 mM acetyl-coenzyme A [24].

Myosin isoform distribution

Electrophoretic separation of myosin heavy chain (MHC) isoforms α and β was determined as previously reported by Vescovo et al [25]. In brief, 60mg of cardiac tissue samples was homogenized and solubilized in 50 mM potassium phosphate buffer, pH 7.4; 0.3 M sucrose; 0.5 mM DTT; 1mM EDTA, pH 8.0; 0.3 mM PMSF; 10 mM NaF, and 1:100 protease inhibitor. Sample buffer consisting of 0.5 M Tris, pH 6.8 and 100% glycerol was added to 8 μg of the samples. They were loaded into wells with 0.05% bromophenol blue. Analytical SDS-polyacrylamide gel electrophoresis of MHC was performed on 8% polyacrylamide slabs with 37.5% glycerol. Running buffer was composed of 50 mM Tris, 384 mM glycine pH 8.3, and 0.2% SDS. Separation of MHC was performed in a Bio-Rad apparatus at 70 V for 36 h, with 20ºC throughout the electrophoretic procedure. The gel was removed and fixed in 50% methanol and 7% acetic acid for 10 min. Gel was then stained with 0.1% comassie brilliant blue in 5% acetic acid-40% methanol and destained with 40% methanol-7% acetic acid. Staining and destaining were performed at room temperature on a rotatory shaker. The gel was photographed and MHC isoforms α and β were analyzed by LabWorks™ Analysis Software.

Western blot analysis

Antibodies to Serca2 ATPase, mouse IgG1 1: 2500 (ABR, Affinity BioReagents, Golden, CO, USA), Phospholamban, mouse IgG2a 2 μg/ml (ABR, Affinity BioReagents, Golden, CO, USA) and Phophoslamban phospho-Ser16, rabbit *I*gG 1:5000 (Badrilla, Leeds-UK) were utilized.

Left ventricle tissue was analyzed by Western blot to quantify the protein expression of Serca2, phospholamban and phosphorylated phospholamban at Ser[16]. Briefly, liquid nitrogen frozen ventricles isolated from ETS (n=5) and ETS-T (n=5) rats were homogenized in a buffer containing 50 mmol/L potassium phosphate buffer (pH=7.0), 0.3 mol/L sucrose, 0.5 mmol/L DTT, 1 mmol/L EDTA (pH=8), 0.3 mmol/L PMSF, 10 mmol/L NaF, and phosphatase inhibitor cocktail (1:100; Sigma-Aldrich). The homogenate was centrifuged at 4°C for 20 min at 12,000g. The supernatant was collected and total protein content was determined by the Bradford Method. Samples were subjected to SDS-PAGE in polyacrylamide gels. After electrophoresis, proteins were electro-transferred to nitrocellulose membrane. Equal loading of samples (50 μg) and even transfer efficiency were monitored through the use of 0.5% Ponceau *S* staining of the blotted membrane. The blotted membrane was then blocked (5% nonfat dry milk, 10 mM Tris·HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) for 2 h at room temperature and incubated with specific antibodies overnight at 4°C. Binding of the primary antibody was detected by peroxidase-conjugated secondary antibodies (rabbit or mouse depending on the protein, 1:10,000, for 1:30 h at room temperature) and developed by enhanced chemiluminescence (Amersham Biosciences) detected by autoradiography. Quantification analysis of blots was performed with the aid of Scion Image software (Scion based on NIH Image). Targeted bands were normalized for cardiac β-actin.

Statistical analysis

Comparisons between groups were made by Student's *t* test for parameters with normal distribution. Otherwise, groups were compared by the Mann-Whitney U test. Data were expressed as mean ± SD or medians (including the lower quartile and upper quartile). Data analysis was carried out with SigmaStat for Windows v2.03 (SPSS Inc, Chicago, IL). The significance level was considered 5%.

Results

Step One

Considering the final body weight (C = 439 ± 48 g, T = 449 ± 23 g; p>0.05) there were no differences between the groups.

The echocardiographic data revealed no intergroup differences in heart rate (C = 309 ± 86 beats/min, T = 298 ± 30 beats/min; p>0.05), LA (C = 5.38 ± 0.76 mm, T = 5.50 ± 0.79 mm; p>0.05), DDVE (C = 8.01 ± 0.53 mm, T = 7.94 ± 0.45 mm; p>0.05), DSVE (C = 3.53 ± 0.71 mm, T = 3.42 ± 0.70 mm; p>0.05), LV mass index (C = 1.79 ± 0.25 g/kg, T = 1.77 ± 0.26 g/kg; p>0.05), LVWT (C = 2.93 ± 0.35 mm, T = 3.17 ± 0.36 mm; p>0.05), fractional shortening (C = 56.1 ± 6.76 %, T = 57.0 ± 7.71 %; p>0.05), LVWT shortening velocity (C = 37.8 ± 4.15 cm/s, T = 37.2 ± 3.90 cm/s; p>0.05), E wave (C = 79.6 ± 7.23 cm/s, T = 83.8 ± 13.9 cm/s; p>0.05), A wave (C = 52.2 ± 13.9 cm/s, T = 45.3 ± 10.8 cm/s; p>0.05), E/A ratio (C = 1.59 ± 0.30, T = 1.97 ± 0.71; p>0.05), TRIV (C = 23.4 ± 4.33 ms, T = 23.0 ± 3.82 ms; p>0.05) or $TRIV/RR^{0,5}$ (C = 52.9 ± 9.43, T = 50.9 ± 6.66; p>0.05). These results suggest that, in normal rats, taurine administration does not result in cardiac morphological or functional alterations assessed by echocardiogram.

Step Two

The groups did not differ as to final body weight (ETS = 301 (293-303) g, ETS-T = 303 (296-308) g; p>0.05) or the tail systolic pressure (ETS = 116 ± 12 mmHg, ETS-T = 106 ± 15 mmHg; p>0.05).

The morphological data assessed by echocardiogram are shown in Table 1 and Fig. 1. Taurine supplementation increased LV wall thickness. As to the other variables, there were no differences between the groups.

The functional data assessed by echocardiogram are shown in Table 2. The heart rate was lower in the ETS-T group. The groups did not differ in relation to the systolic function variables. On the other hand, the ETS-T group presented lower E and A waves but higher E/A ratio, isovolumetric relaxation time, and isovolumetric relaxation time normalized for heart rate, suggesting worsened diastolic function in comparison with the ETS group. Importantly, there are some factors influencing ventricular relaxation. For that reason, we analyzed some variables related to diastolic dysfunction including collagen amount, calcium handling protein, and cardiac energy metabolism.

	Group ETS (n=9)	Group ETS-T (n=9)	p
LA (mm)	3.88 ± 0.39	3.89 ± 0.24	0.943
LA/BW (mm/kg)	13.2 ± 1.12	12.9 ± 0.84	0.570
Ao (mm)	3.56 ± 0.30	3.49 ± 0.27	0.628
LVEDD (mm)	6.62 ± 0.48	6.53 ± 0.74	0.767
LVEDD/BW(mm/kg)	22.5 ± 1.23	21.7 ± 2.71	0.429
LVWT (mm)	1.30 (1.20-1.42)	1.50 (1.40-1.50)	0.029
LVWT / LVEDD	0.20 ± 0.03	0.23 ± 0.03	0.05
LVESD (mm)	2.98 ± 0.42	2.90 ± 0.58	0.748
LVESD/BW(mm/kg)	9.81 ± 1.00	9.90 ± 2.11	0.915

Table 1. Morphological data assessed by echocardiogram. ETS: animals exposed to tobacco smoke; ETS-T: animals exposed to tobacco smoke and treated with taurine; LA: left atrium; BW: body weight; LV: left ventricle; Ao: aorta; LVEDD: LV end-diastolic dimension; LVESD: LV end-systolic dimension; LVWT: LV posterior wall thickness. Data are expressed as mean ± SD or medians (including the lower and upper quartiles).

	Group ETS (n=9)	Group ETS-T (n=9)	p
HR (beats/min)	341 ± 37	285 ± 49	0.015
E (cm/s)	79.3 ± 7.72	66.1 ± 11.8	0.013
A (cm/s)	71.2 ± 14.5	49.7 ± 12.3	0.004
E/A	1.13 ± 0.13	1.37 ± 0.26	0.028
IRT (ms)	22.6 ± 1.43	33.2 ± 4.28	<0.001
$IRT/RR^{0.5}$	53.9 ± 4.33	72.5 ± 12.0	<0.001
EF	0.91 ± 0.02	0.90 ± 0.03	0.405
FS	56.3 ± 4.00	54.6 ± 5.21	0.443
CO (l/min)	0.25 ± 0.05	0.22 ± 0.05	0.228
CI (l/min/kg)	0.84 ± 0.17	0.72 ± 0.17	0.144
VAO (cm/s)	76.0 ± 9.16	76.5 ± 13.5	0.920

Table 2. Functional data assessed by echocardiogram. ETS: animals exposed to tobacco smoke; ETS-T: animals exposed to tobacco smoke and treated with taurine; HR: heart rate; E: peak velocity of early ventricular filling; A: peak velocity of transmitral flow during atrial contraction; IRT: isovolumetric relaxation time. $IRT/RR^{0.5}$: isovolumetric relaxation time normalised for heart rate; EF: ejection fraction; FS: fractional shortening; CO: cardiac output; CI: cardiac index; VAO: flow velocity through the aorta. Data are expressed as mean ± SD.

	Group ETS (n=9)	Group ETS-T (n=9)	p
LVW (g)	0.59 ± 0.02	0.61 ± 0.04	0.178
RVW (g)	0.17 ± 0.02	0.17 ± 0.02	0.837
LVW/BW(mg/g)	2.02 ± 0.11	2.04 ± 0.14	0.787
RVW/BW(mg/g)	0.59 ± 0.08	0.58 ± 0.06	0.843
CSA (μm^2)	239 ± 15.4	232 ± 19.5	0.424
CVF (%)	1.50 ± 0.66	1.88 ± 0.86	0.302

Table 3. Morphometric data. ETS: animals exposed to tobacco smoke; ETS-T: animals exposed to tobacco smoke and treated with taurine; LVW: left ventricular weight; RVW: right ventricular weight; BW: body weight; CSA: cross-sectional area; CVF: interstitial collagen volume fraction. Data are expressed as mean ± SD.

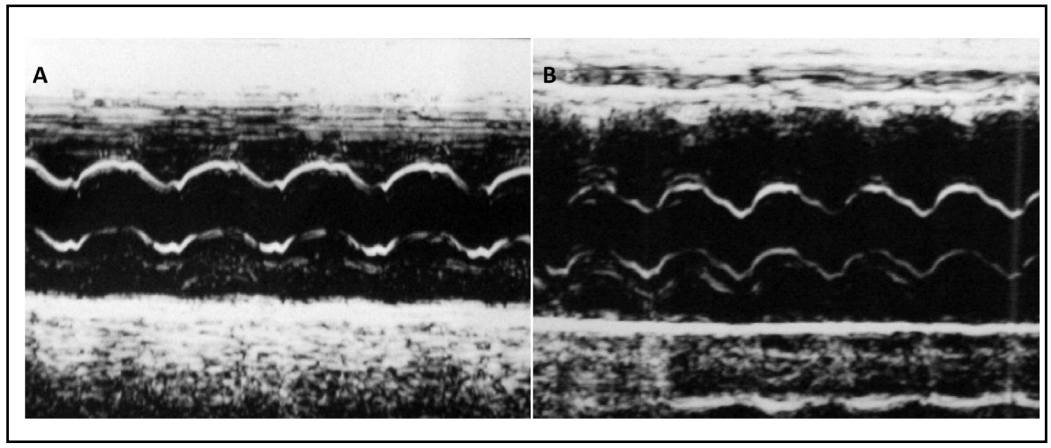

Fig. 1. Echocardiographic image. A: animals exposed to tobacco smoke; B: animals exposed to tobacco smoke and treated with taurine.

The morphometric data reveal no differences between the groups, as displayed in Table 3.

The biochemical data are shown in Table 4. The enzymatic activity of the lactate dehydrogenase was higher in ETS-T group. In relation to cytokine production, there were no intergroup differences in IFN-γ or TNF-α cardiac levels. Likewise, considering the heavy myosin isoform distribution, the groups did not differ.

The calcium homeostasis proteins are shown in Table 5 and Fig. 2. The ETS-T group presented lower levels of phospholamban, phosphorylated phospholamban, and phosphorylated phospholamban/phospholamban ratio. On the other hand, the groups did not differ in SERCA 2a levels.

	Group ETS (n=5)	Group ETS-T (n=5)	p
CS (nmol/mg protein)	157 ± 16	147 ± 22	0.291
HADH (nmol/mg protein)	169 ± 42	153 ± 49	0.482
LDH (nmol/mg protein)	204 ± 14	232 ± 12	<0.001
TNF-α(pg/mg)	162 (80-246)	174 (138-183)	1.00
IFN-γ (pg/mg)	146 (84-197)	159 (137-160)	1.00
β (%)	55.3 ± 9.53	61.6 ± 7.39	0.276
β/α ratio	1.32 ± 0.50	1.70 ± 0.63	0.326

Table 4. Biochemical data. ETS: animals exposed to tobacco smoke; ETS-T: animals exposed to tobacco smoke and treated with taurine; CS: citrate synthase; LDH: lactate dehydrogenase; HADH: β- hydroxyacyl-coenzyme A dehydrogenase; TNF: tumor necrosis factor; IFN: interferon; β: heavy myosin isoform β. Data are expressed as mean ± SD or medians (including the lower and upper quartiles).

	Group ETS (n=5)	Group ETS-T (n=5)	p
SERCA-2a/β actin	1.00 ± 0.13	1.02 ± 0.21	0.822
PLB/β actin	1.00 ± 0.13	0.82 ± 0.06	0.026
PPLB Ser16/β actin	1.00 ± 0.14	0.63 ± 0.10	0.003
PPLB Ser16/PLB	1.01 ± 0.17	0.77 ± 0.11	0.050

Table 5. Calcium homeostasis proteins. ETS: animals exposed to tobacco smoke; ETS-T: animals exposed to tobacco smoke and treated with taurine; PLB: phospholamban; PPLB Ser16: phosphorylated phospholamban at serine 16. Data (arbitrary units) are expressed as mean ± SD.

Discussion

The objective of this study was to analyze the effect of taurine supplementation on smoking-induced ventricular remodeling. Our results showed that in rats exposed to cigarette smoke, taurine supplementation did not change systolic function. On the other hand, taurine increased wall thickness and worsened diastolic function in smoking rats. In addition, this effect was associated with alterations in calcium handling protein, and cardiac energy metabolism.

Previous experiments have shown that exposure to tobacco smoke induces cardiac morphological and functional irregularities [14-20]. However, the mechanisms involved in this process induced by exposure to cigarette smoke are still incompletely understood [26].

An initial relevant finding of our study was that treatment with taurine did not affect the systolic function variables associated with cigarette smoking. In fact, smoking rats supplemented with taurine presented similar cardiac output, cardiac index, ejection fraction and fractional shortening in comparison with smoking animals that did not receive taurine. In addition, the mechanical properties of the heart are correlated with MHC isoform distribution. In different animal models of cardiac injuries, cardiac remodeling was associated with a significant increase in β-MHC expression. Importantly, this MHC redistribution was associated with decreased cardiac function [27]. In our study, the absence of abnormalities in the systolic function of ETS-T animals was associated

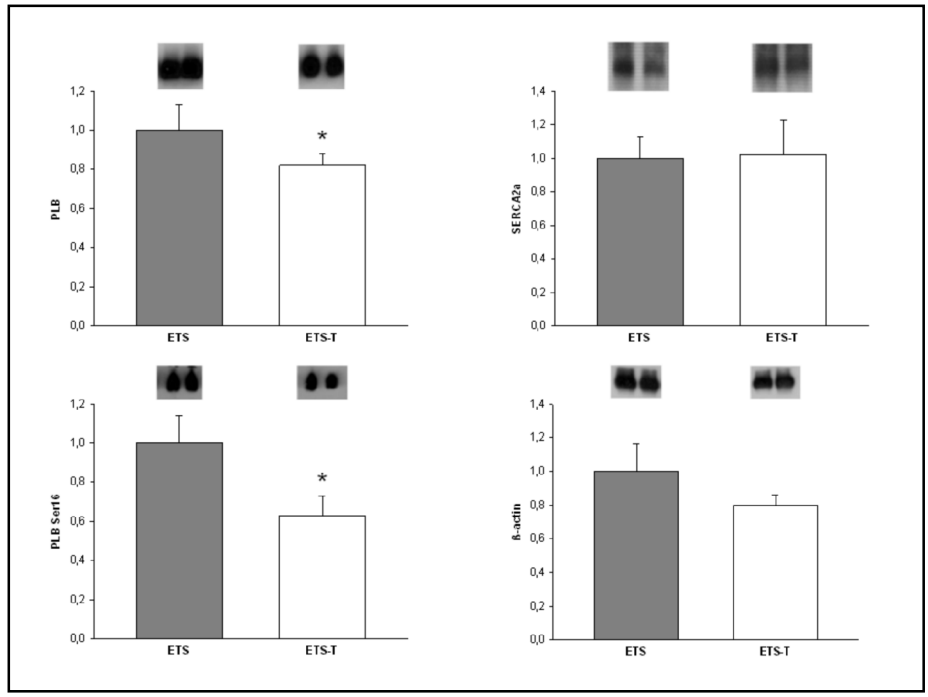

Fig. 2. Calcium homeostasis proteins. ETS: animals exposed to tobacco smoke; ETS-T: animals exposed to tobacco smoke and treated with taurine. * P<0.05.

with a lack of alterations in MHC isoform distribution. Therefore, our data strongly support a non-participation of taurine in systolic function abnormalities induced by exposed to cigarette smoke.

Another important issue is the fact that taurine supplementation augmented the LV wall thickness. Alterations in ventricular mass, volume, and geometry after cardiac injury can be interpreted as an expression of the remodeling process. In fact, regardless of the complexity of the remodeling process, measurements to assess cardiac remodeling include heart size, shape and mass [1-3]. Therefore, our data would indicate that taurine supplementation increases the hypertrophic process during exposure to tobacco smoke.

In some heart failure models, cytokine production can modulate left ventricular remodeling. Indeed, increased IFN-γ and, especially, TNF-α levels are associated with left ventricular dysfunction, cachexia, activation of fetal gene programs, apoptosis, hypertrophy, and fibrosis [28, 29]. In this study, taurine was not associated with altered cytokine levels. Therefore, at this point, our study suggests a non-participation of these cytokines in the deleterious taurine effects.

One noteworthy finding in the present study was that taurine worsened diastolic function in smoking rats. In fact tobacco-smoke expose was associated with alterations in several variables of diastolic function. At this point, we must consider that the heart rate differed between the groups. This variable interferes with diastolic function variables such as the E and A waves. Importantly, after normalization for heart rate, there was also evidence of abnormalities in the isovolumetric relaxation time index, signifying worsened diastolic function after taurine supplementation.

There are some factors influencing ventricular relaxation. For example, relaxation is influenced by load. In addition, the cytosolic calcium level must decrease, a process requiring ATP and phosphorylation of phospholamban. Furthermore, given the importance of the viscoelastic properties of the myocardium, the collagen amount and the myocardial hypertrophy can influence relaxation.

With regard to the potential mechanisms involved in the adverse taurine effects on diastolic function, some studies provide evidence that exposure to tobacco smoke results in impaired vasodilatory function and increased blood pressure [15, 17, 30]. However, in our study, taurine did not change the blood pressure in smoking animals. Therefore, we could deduce that the blood pressure abnormalities did not contribute to the deleterious effects of taurine in this experimental model.

It is widely accepted that ventricular remodeling may be associated with alterations in the interstitial matrix structure. Different models of cardiac remodeling have revealed an abnormal collagen accumulation, resulting in myocardium fibrosis. Importantly, this abnormal collagen accumulation is associated with myocardial dysfunction, beginning with diastolic dysfunction followed by abnormalities in systolic function [31-33]. Interestingly, in our study, taurine did not change the collagen amount

in comparison to rats exposed to tobacco smoke. Thus, our data suggest that the collagen amount is not involved in the deleterious effect of taurine in this model.

Another potential mechanism involved in taurine's action is cardiac energy metabolism. Under normal conditions, the oxidation of fatty acid covers around 70% of the cardiac energy required. In several cardiac remodeling models, there is a shift in cardiac substrate utilization that includes a decline in fatty acid oxidation associated with an increase in the utilization of glucose [34]. This phenomenon can be associated with decreased phosphocreatine levels. As a consequence, there is decreased activity in ion pumps which are dependent on ATP, such as SERCA. It is noteworthy that in our study a worsened diastolic function was associated with an increase in glycolysis. Importantly, this shift in energy metabolism induced by taurine was also found in another cardiac injury model [35]. Therefore, our data suggest that metabolic remodeling can participate in the effects of taurine in this model.

Calcium (Ca^{2+}) is the most important ion related to diastolic function. In fact, Ca^{2+} must be removed from cytosol to allow relaxation. After contraction, there is a reuptake of Ca^{2+} into the sarcoplasmatic reticulum by SERCA2 and removal of Ca^{2+} from the cell by the Na^{+}/Ca^{2+} exchange. The activity of SERCA2 is regulated by phospholamban. In its unphosphorylated state, phospholamban inhibits SERCA2 activity. However, after phospholamban phosphorylation at Ser16 by protein kinase A, this inhibition is diminished, increasing Ca^{2+} uptake [36, 37]. Crucially, in our study, the worsened diastolic function induced by taurine was associated with decreased phosphorylated phospholamban levels and a lower phosphorylated phospholamban/phospholamban ratio. Therefore, we can conclude that Ca^{2+} handling abnormalities might be involved in the diastolic dysfunction induced by taurine in rats exposed to tobacco smoke.

Another potential mechanism involved in the remodeling process induced by smoking is cell death. In fact, the role played by apoptosis on ventricular remodeling has been studied in several models of cardiac injuries [2, 3]. Unfortunately, in our study, we did not analyze apoptosis. However, considering that myocyte progressive loss is an important modulator of cardiac alterations in different models, apoptosis might play a role in cardiac remodeling induced by exposure to tobacco smoke.

Finally, taurine has been shown to attenuate remodeling induced by different cardiac injuries, such hypertension. Actually, the deleterious effect of taurine, observed in our study, was a surprise. However, this model has several particularities. For example, more than 4,720 compounds have been identified in cigarette smoke. Thus, despite speculative, it is possible that taurine might present deleterious interaction with one or more compounds of cigarette. In addition, the mechanisms involved in these effects are unknown. Taurine is accepted as an antioxidant. In previous work of our group, beta-carotene (another antioxidant) supplementation resulted in adverse ventricular remodeling after acute myocardial infarction [38]. It is possible that under particular situations, antioxidants can present deleterious cardiac effects. However, this explanation is speculative and should be addressed in future studies.

In conclusion, taurine produces no cardiac effects in normal rats. In rats exposed to cigarette smoke, taurine supplementation does not change systolic function. On the other hand, taurine augments wall thickness and worsens diastolic function in smoking rats. In addition, this effect is associated with alterations in calcium handling protein, and cardiac energy metabolism.

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