Research Article **AJCRAR (2022) 5:19**

American Journal of Cardiology Research and Reviews
(ISSN:2637-4935)

Absence of oxidative stress and sirtuins recruitment on cardiac tissue post stress

Ana Elisa T.S. de Carvalho*, Marco A. Cordeiro, Luana S. Rodrigues, Daniela Ortolani, Regina C. Spadari*

Department of Biosciences, Federal University of São Paulo (UNIFESP), Santos, SP, Brazil

ABSTRACT

Stress has emerged as a factor associated with cardiovascular disease. Catecholamines released during the stress reaction by the sympathetic nerves and the adrenal medulla couple to β_1-and β_2-adrenoceptors in the cardiomyocytes membrane enhancing heart function in order to attend the organism demand. This might produce excessive reactive oxygen species what may culminate with oxidative stress and progression of several cardiac diseases. Sirtuins have been described as cardioprotective factors and important regulators of the cellular stress response in the heart. The aim of this work is to investigate the putative participation of oxidative stress and sirtuins in the heart of rats submitted to foot shock stress, an experimental model where there is up regulation of β_2-adrenoceptors and downregulation of β_1-adrenoceptors. The data have shown that in the myocardium of rats submitted to foot shock stress the H_2O_2 concentration, catalase and superoxide dismutase activity, NAD^+/NADH ratio, as well as the protein expression of sirtuins 1 and 3 were not altered. Pharmacological blockade of the β_2-adrenoceptors by ICI118,551, did not modify this scenario. It is concluded that foot shock stress does not cause disruptions in oxidative stress or redox state processes in the myocardium, and consequently, sirtuins are not recruited to stress response.

Keywords: oxidative stress, sirtuins, foot shock stress, β_2-adrenoceptor

***Correspondence to Author:**
R.C. Spadari and A.E.T.S. de Carvalho
Laboratório de Biologia do Estresse e Departamento de Biociências, Campus Baixada Santista, Universidade Federal de São Paulo (UNIFESP), Rua Silva Jardim, 136, sala 310, CEP 11020-015, Santos, SP, Brazil (aetscarvalho @ unifesp.br and regina.spadari @ unifesp.br)

How to cite this article:
Ana Elisa T.S. de Carvalho, Marco A. Cordeiro, Luana S. Rodrigues, Daniela Ortolani, Regina C. Spadari. Absence of oxidative stress and sirtuins recruitment on cardiac tissue post stress.American Journal of Cardiology Research and Reviews, 2022, 5:19.

eSciPub LLC, Houston, TX USA.

Introduction

Stress is the body response to any challenge or threat aimed to guarantee survival in adversity. However, the persistence of a stressful situation can increase the susceptibility to diseases, including cardiovascular diseases. Catecholamines released by the sympathetic nervous system - adrenal medulla during the stress reaction overstimulate β-adrenoceptors (β-AR) in the cardiomyocytes. As a result, β_1/β_2-AR ratio is altered in various experimental models of stress [1-5]. In the foot shock stress model such alteration is due to downregulation of β_1-AR and upregulation of β_2-AR [4,6]. Once activated, those receptors trigger intracellular signaling cascades that modulate cardiomyocytes life cycle as well as cardiac function [1]. As a result of the increased heart beating and developed force, there is an increase of cardiomyocytes metabolism and production of reactive oxygen species (ROS) [7]. Excessive ROS production in parallel with deficient antioxidant defense culminates in oxidative stress that has been described to contribute to the progression of several cardiac diseases, such as cardiac hypertrophy and heart failure [7-9].

Sirtuins have emerged as important regulators of intracellular redox status and, consequently, as tissue protector factors [10]. Sirtuins are a family of NAD^+-dependent

histone deacetylases that participate in the regulation of energy metabolism, reactive oxygen species production, and survival signaling pathways [11]. Till now, seven sirtuin isoforms are recognized [5]. In cardiomyocytes, the two main expressed sirtuin isoforms are sirtuin 1 (SIRT1) and sirtuin 3 (SIRT3) [11]. The sirtuins effects in cellular protection during stress are due to their control of FoxOs deacetylation [12,13], thus regulating physiological processes such as cell death, autophagy and oxidative stress [5,11,14].

Studies over the last decades suggest that SIRT3 plays an important role in protecting the heart from oxidative stress [5,15,16]. In addition, reduced levels of SIRT3 were reported in failing hearts and in cardiac fibrosis and contractile dysfunction [15,17,18]. SIRT1 expression seems to play a protective role in cardiomyocytes due the apoptosis inhibition and the expression of FoxO-dependent antioxidants enzymes [19-22].

Although partially related to the regulation of mitochondrial function, energy homeostasis, and oxidative stress post cellular or mechanical cardiac stress [15,21,23], a comprehensive understanding of the participation of SIRT 1 and 3 in the heart response to physical and psychological stress is still missing. Here we aimed to elucidate whether oxidative stress and sirtuins 1 and 3 play a role in the heart of rats submitted to foot shock stress. Moreover, taking into account that in this stress model, there is an increase in the expression of β_2-AR, we also intended to investigate if the presence of β_2-AR will alter those parameters.

Methods

Animals and experimental groups

Male Wistar rats (*Rattus norvergicus*; 250-300 g, 12-week-old) purchased from the Center for the Development of Experimental Models (CEDEME), of the Federal University of São Paulo (São Paulo, SP, Brazil) were housed in cages in a temperature-controlled room (22ºC), with 12/12 h light/dark cycle, lights on at 7:00 a.m. Standard laboratory chow for rodents and tap water were available *ad libitum*. The rats were randomly distributed in four groups, 6 rats/group, as follows: untreated and unstressed (CO), untreated and stressed (ST), treated with ICI118,551 and unstressed (ICI) and treated with ICI118,551 and stressed (ICIST). All procedures were in accordance with the ethical profile of the institution and experimental protocols were approved by the Ethics Committee for Animal Use of the Federal University of São Paulo (CEUA/UNIFESP), protocol number 86613101116, in accordance with the Brazilian National Council for Control of Animal Experimentation (CONCEA, Brazil).

Stress protocol

The foot shock stress protocol was administered as previously described [4,24].The rats in the stressed groups (ST and ICIST) were submitted to foot shock sessions; rats in the non-stressed groups (CO and ICI-treated) were also placed in the foot shock cage, but did not receive foot shocks. A Plexiglas chamber (26 cm long x 21 cm wide x 26 cm high) provided with a grid floor consisting of stainless-steel rods (0.3 cm in diameter and spaced 1.0 cm apart) was used to apply the foot shocks. During the 30 minutes sessions, which occurred between 8:00 am and 11:00 am on three consecutive days, foot shocks were delivered by a constant current source controlled by a microprocessor-based instrument. The intensity of the current was 1.0 mA, with duration of 1.0 s, with pulses delivered at random intervals of between 5 and 25 s. The rats were returned to their standard cages after the first and second period in the Plexigas chamber. After the third session, the rats were immediately euthanized by decapitation. The hearts were harvested and the left ventricles were isolated and stored at -80^{o} C.

Rats treatment with ICI118,551

The rats in the ICI-treated groups, unstressed and stressed (ICI and ICIST,respectively) received during 5 days, 500 µg/kg/day, i.p., of ICI118,551((±)-1- [2,3-(Dihidro-7-metil-1H-inden-4-il) oxi]-3-[1-metilletil) amino]–2-butanol; Tocris Bioscience, Bristol, UK), a highly selective β_2-AR antagonist, i.p. The rats in the untreated groups (CO and ST) received injections of saline solution, i.p. The foot shock stress sessions began on the third day of the treatment with ICI or saline solution.

Western blot analysis

The left ventricles were homogenized in 1.0 mL of assay buffer (4°C) of the following composition: 1% Triton X-100 (BioRad, Hercules, CA, USA), 10 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 10 µg/ml Aprotinin (Amresco, Solon, Ohio), 1 mmol/L PMSF, 0.25 mmol/L sodium orthovanadate and 0.1% cocktail inhibitors protease (Sigma-Aldrich, St. Louis, MO, USA). The samples were centrifuged and the supernatant was collected and assayed for total protein concentration using the Bradford method (Bio Rad Laboratories, Hercules, CA, USA). One hundred micrograms of total protein were separated by Bolt Bis-Tris Plus Gel (Thermo Fisher Scientific, Waltham, MA, USA) and transferred to nitrocellulose membranes using iBlot 2 Dry Bloting System (Thermo Fisher Scientific, Waltham, MA, USA). Membranes were blocked with 4% BSA in TBS buffer containing 0.1% Tween 20, for 1 hour. The following primary antibodies (from Abcam, Cambridge, MA, USA (ab) and Santa Cruz Biotechnology, Dallas, TX, EUA (sc) were incubated at 4°C overnight: SIRT1 mouse monoclonal (ab110304); SIRT3 rabbit polyclonal (ab189860); GAPDH rabbit polyclonal (sc25778). The membranes were subsequently rinsed three times (5 minutes each) in buffer solution and then incubated with the respective HRP-conjugated secondary antibody 1:2000 dilution (Sigma-Aldrich, St. Louis, MO, USA) for 1 h, then rinsed in buffer. Using an enhanced chemiluminescence kit (Super Signal, West Pico Chemiluminescent Substrate (ThermoFisher Scientific, Rockford, IL, USA), the blots were developed on ChemiDoc XRS+ System (Bio Rad Laboratories, Hercules, CA, USA). Densitometric analyses were done using Image J Launcher software.

Gene expression measurements by RT-PCR

Total RNA was isolated from a fragment of no more than 100 mg of the left ventricle using TRIzol Reagent (Invitrogen, Carlsbad, CA, EUA), according to the manufacturer's instructions. The final volume of the samples was 30 µL of water treated with 0.1 % dietilpyrocarbonate (DEPC UltraPure; Invitrogen, Carlsbad, CA, USA); these were stored at -80^{o}C overnight. The RNA concentration and degree of purity were determined in Nanodrop 2000 c (Thermo Scientific, Waltham, MA, USA) under 260/280 nm. Samples were treated with DNAse (Deoxyribonuclease Amp Grade I, Invitrogen, Carlsbad, CA, EUA), according to the manufacturer´s instructions. After,

complementary DNA (cDNA) was obtained, using the High-Capacity cDNA Reverser Transcription kit (Applied Biosystems, Carlsbad, CA, USA), according to the manufacture indication, in a final volume of 20 µL. Primer sequences (Thermo Fisher Scientific, Waltham, MA, USA) were as follow: SIRT1 (forward: 5´-TGACTTCAGATCAAGAGATGGTAT-3´; reverse: 5´-TGGCTTGAGGATCTGGGA GAT-3´); SIRT3 (forward: 5´-ATGGAAAGCTGGATGGACAG-3´; reverse: 5´-AGGTCTCCCTGGGAATAGGA-3´); GAPDH (forward: 5´- ACTCACGGCAAATTC AACGG-3´; reverse: 5´- AGTTGGGATAGGGCCTCTCTTG-3´). Glyceraldeide triphosphate desidrogenase (GAPDH) was used to normalize the results. Real time-PCR was done using SYBRGreen PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in a Step One Plus Real Time PCR System (Applied Biosystems, Foster City, CA, USA). The results were normalized in relation to the values obtained for the endogenous gene and were expressed as 2-ΔΔCT.

Antioxidants enzyme activities

Enzymatic activities were determined in the left ventricle tissues using commercial assays. Catalase activity was detected by colorimetric assay using Catalase Activity Assay Kit (ab83464 - Abcam, Cambridge, MA, USA), according to the manufacturer's instructions. The catalase activity was calculated based on standard curve data, the amount of H_2O_2 in sample wells, the reaction time and the sample volume used. The results were expressed as nmol/min/mL. Superoxide dismutase activity (SOD) was detected by colorimetric assay using Superoxide Dismutase Activity Assay Kit (ab65354 - Abcam, Cambridge, MA, USA), according to the manufacturer's instructions. The SOD activity was calculated based on the three control blanks as the inhibition activity of xanthine oxidase by SOD. The results were expressed as inhibition rate %.

NAD^+/NADH assay

The intracellular nicotinamide adenine dinucleotide oxidized (NAD^+) and reduced (NADH) were determined in the left ventricle tissues by colorimetric assay using NAD^+/NADH Assay Kit (ab65348 - Abcam, Cambridge, MA, USA), according to the manufacturer's instructions. The concentration of total NAD (NADt) and NADH in the samples was calculated based on standard curve data and sample volume used. NAD^+ concentration was calculated by subtraction of NADH data from NADt data, and NAD^+/NADH ratio could be calculated. The results were expressed as ng/mg protein.

Hydrogen peroxide concentration assay

The hydrogen peroxide (H_2O_2) concentration was determined in the left ventricle tissues by colorimetric assay using Hydrogen Peroxide Assay Kit (ab102500 - Abcam, Cambridge, MA, USA), according to the manufacturer's instructions. The concentration of H_2O_2 in the samples was calculated based on standard curve data and sample volume used. The results were expressed as pmol/ µl.

Statistical analysis

The results were expressed as means ± s.e.m. Student's t-test was used to compare two groups; one-way ANOVA was used to compare three groups. Differences were considered significant at $p \leq 0.05$. Statistical analyses were done using Prism v.8 (GraphPad Software Inc., San Diego, CA).

Results

The foot shock stress protocol in rats promotes significant increment in corticosterone plasma level and consistent increase in β_2-AR protein expression [4,25].

In order to explore the balance of oxidative stress in the cardiac tissue post foot shock stress, H_2O_2 production and antioxidant enzymes activity were evaluated. There were no significant differences in H_2O_2 production (Figure 1A) or in the activity of catalase and SOD (Figures 1B and 1C) in the left ventricle tissue of stressed rats (ST) in comparison to untreated

unstressed rats (CO). To characterize the β_2-AR participation on oxidative balance, non-stressed rats were treated with the selective β_2-AR antagonist, ICI188,551. β_2-AR blockade did not change the H_2O_2 production or antioxidant enzymes activity (ICI; Figure 1).

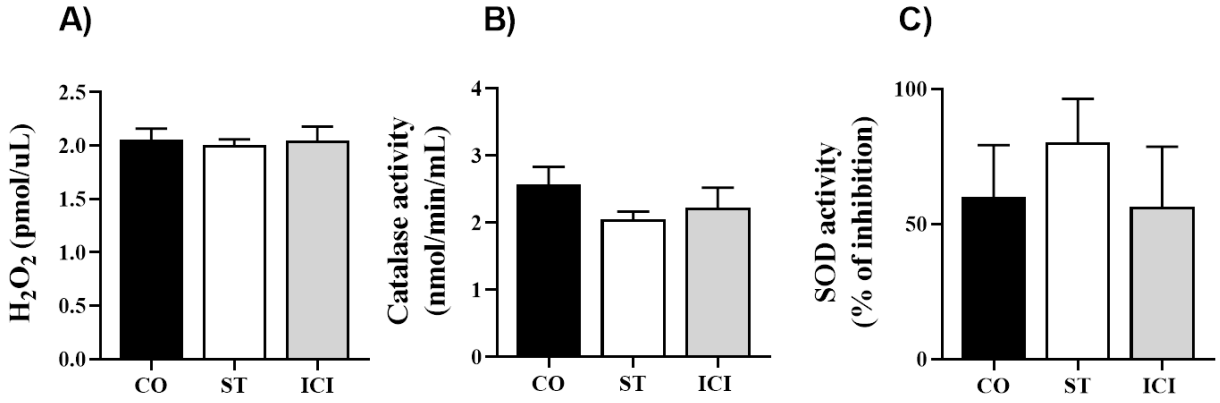

Figure 1. Production of H_2O_2 (A) and activity of catalase (B) and superoxide dismutase (C) in the left ventricle of untreated and unstressed rats (CO), untreated and stressed rats (ST) and ICI-treated unstressed rats (ICI). The bars indicate mean ± s.e.m. of 6 rats/group. There were no significant differences between groups, $p>0.05$, one-way ANOVA.

The NAD^+/NADH ratio is an important indicator of the cellular redox state [26]. Intracellular reduced and oxidized NAD concentrations were measured to characterize the redox state on the myocardium post stress. The quantitative analysis showed no significant differences in NAD^+ (Figure 2A) or NAD^+/NADH ratio (Figure 2B) in the myocardium of untreated rats submitted to stress (ST) or unstressed ICI-treated (ICI) in comparison with untreated unstressed rats (CO) (Figure 2).

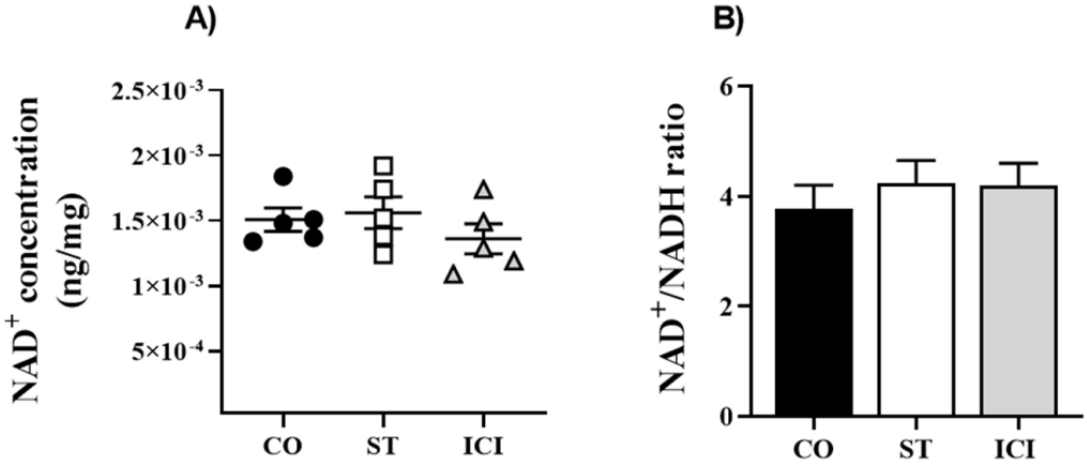

Figure 2. NAD^+ concentration (A) and NAD^+/NADH ratio (B) in the left ventricle of untreated and unstressed rats (CO), untreated and stressed rats (ST) and ICI-treated unstressed rats (ICI). The bars indicate mean ± s.e.m. of 6 rats/group. There were no significant differences between groups, $p>0.05$, one-way ANOVA.

Sirtuins activation is dependent of perturbations in NAD^+ concentration [10,26]. In the myocardium

of rats submitted to stress compared to untreated and unstressed rats there was upregulation of SIRT1, whereas SIRT3 gene expression remains unaltered (Figures 3A and 3C). Despite the upregulation of SIRT1 gene expression, there were no significant differences in protein expression of SIRT1 or SIRT3 (Figures 3B and 3D). There were also no differences with β_2-AR blockade.

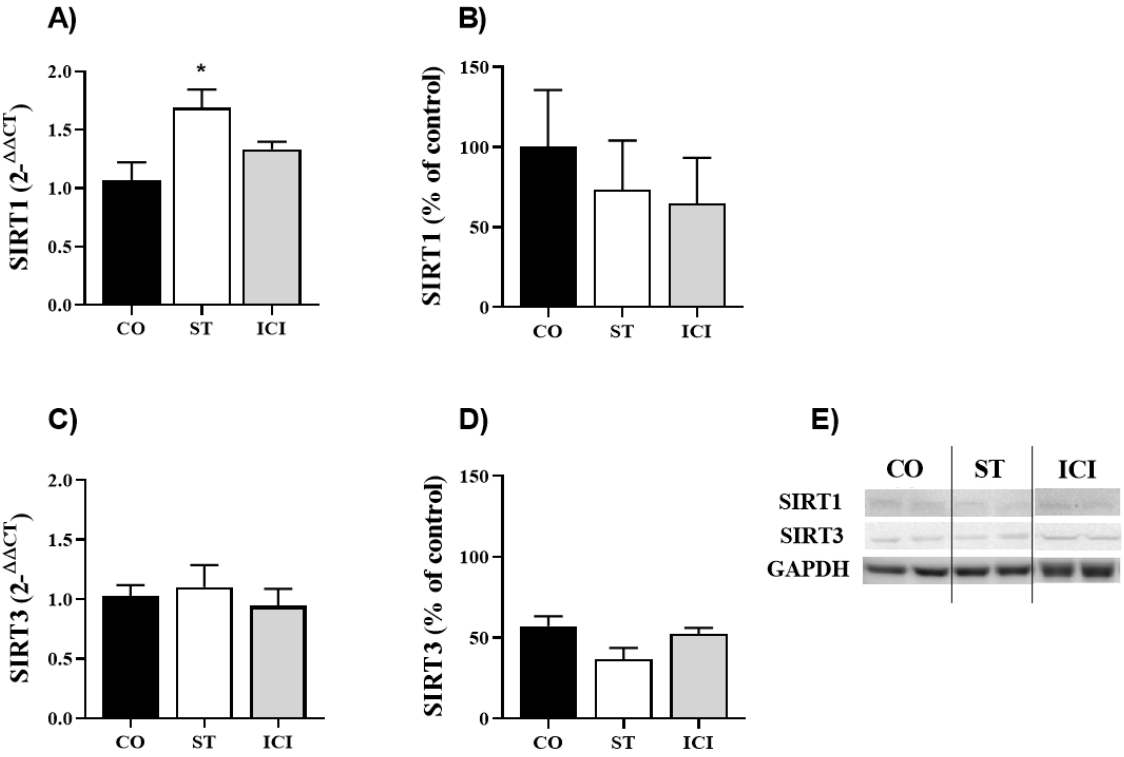

Figure 3. Gene and protein expressions of SIRT1 (A and B) and SIRT3 (C and D) in the left ventricle of untreated and unstressed rats (CO), untreated and stressed rats (ST) and ICI-treated unstressed rats (ICI). The bars indicate mean ± s.e.m. of 6 rats/group. *p≤0.05 compared to control, Student's t test. The representative Western blots images are in panel E.

NAD^+/NADH ratio and sirtuins expressions were also measured in the myocardium of rats pre-treated with ICI188,551 and submitted to foot shock stress. The stress under β_2-AR blockade did not change NAD^+/NADH ratio or the expression of SIRT1 and SIRT3 (Figure 4).

Discussion

The data presented here have shown that foot shock stress did not change the balance of oxidative stress or redox state in the myocardium of rats. Indeed, the results indicate absence of abnormal H_2O_2 production or changes in NAD^+/NADH ratio. Furthermore, β_2-AR expression seems to not interfere on this.

Evidences from our group demonstrate that stress induces changes in the proportion of β-AR subtypes expressed in the rat heart [4]. The upregulation of β_2-AR is accompanied by super-sensitivity of the chronotropic and inotropic responses to non-selective and selective β_2-AR agonists [4]. Moreover, the expression of the PI3K catalytic unit and Akt phosphorylation were persistently reduced in the myocardium of rats submitted to stress [25]. Recently, we have also demonstrated that stress induces changes in the expression of several genes codifying proteins related to the β-AR signaling cascade in the heart and that β_2-AR modulates those changes [27]. Despite that, the present data have shown that in this stress model, the ROS production

and the intracellular electron flux were not altered. This is positive for the heart because the exacerbated ROS production can impair the cardiac contractile function by modifying proteins involved in this process [9,28]. In heart failure, beyond the high mitochondrial ROS production, there is an overall deficiency in the endogenous antioxidant mechanisms, e.g., with reduction in SOD/catalase activity or NAD+ levels [9].

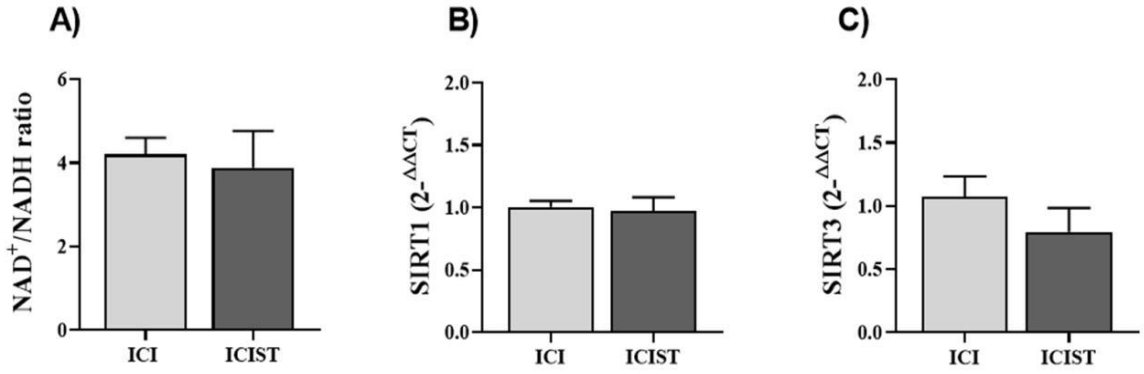

Figure 4. NAD^+/NADH ratio (A), SIRT1 (B) and SIRT3 (C) gene expressions in the left ventricle of ICI-treated rats unstressed (ICI) and stressed (ICIST). The bars indicate mean ± s.e.m. of 6 rats/group. There were no significant differences between groups, $p>0.05$, Student's t test. The representative Western blots images are in panel C.

SIRT1 and SIRT3 exert cardioprotective roles under pathological circumstances [15,29,30] and are important regulators of the cellular stress response in the heart [5,21,30]. These sirtuins have similar effects on cellular stress response through the FoxOs activity [12,13]. The FoxOs transcriptional activity is controlled by their transit between the nucleus and the cytoplasm [31]. Phosphorylation of FoxOs by Akt promotes the translocation of these factors from the nucleus to the cytoplasm. In contrast, deacetylation of FoxOs factors by sirtuins inhibits the phosphorylation-dependent nuclear export and FoxOs are maintained inside the cellular nucleus, thus promoting the transcription of FoxO-dependent genes [12,15]. Although Akt phosphorylation is reduced in the myocardium of rats submitted to foot shock stress [25], the absence of altered sirtuins activities suggest that FoxOs did not participate in the stress response.

Sirtuins are strictly dependent on NAD^+/NADH ratio [10]. NAD^+ is an essential cofactor for oxidative metabolic processes to cellular energy production through being reduced to NADH. In the mitochondria, NADH donates H^+ to electron transport chain and promotes ATP generation. Thus, NAD^+/NADH ratio affects the main energy metabolic pathways and free radicals production playing as a redox state sensor and an endogenous antioxidant [26]. It has already been reported that reduction in NAD^+ levels can dysregulate oxidative phosphorylation balance and ROS production [26,32]. Moreover, the increment in NAD^+ levels follows the increase in sirtuins activity [33]. The higher gene expression of SIRT1 in the myocardium of stressed rats did not result in any alteration in NAD^+ concentration, independently of β_2-AR, suggesting none consequence in sirtuins activation. This was corroborated by absence of changes in SIRT1 and SIRT3 protein expressions. Besides that, no changes were observed in the activity of antioxidant enzymes, catalase and SOD. Both SIRT1 and SIRT3 overexpression stimulate antioxidant mechanisms, as MnSOD and catalase enzymes [15,21].

The above-mentioned findings suggest that the

stress resistance is a major characteristic associated to sirtuins activation into the heart, due to their stimulation of antioxidants mechanisms able to preserve heart function. The data here reported have shown that the foot shock stress model did not cause disruptions in oxidative stress or redox state in the myocardium, and consequently, sirtuins were not recruited to stress response.

Disclosures of potential conflicts of interest

No conflicts of interest, financial, or otherwise are declared by the authors.

Acknowledgement

This work was supported by grants to R.C.S. from São Paulo Research Foundation (FAPESP 2014/00314-8; FAPESP 2016/20777-8) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 424114/2016-0). A.E.T.S.C was recipient of fellowship from FAPESP (2016/20784-4). M.A.C. and L.S.R. were recipients of fellowships from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors are in debt with Dra Flavia Pidone for the technical assistance.

Abbreviations:

NAD^+: nicotinamide adenine dinucleotide oxidized; NADH: nicotinamide adenine dinucleotide reduced; FoxO: forkhead transcriptional factor; ICI: ICI118,551((±)-1- [2,3-(Dihidro-7-metil-1H-inden-4-il) oxi]-3-[1-metilletil) amino]–2-butanol

References

[1]. Santos IN, Spadari-Bratfisch RC. Stress and cardiac beta adrenoceptors. Stress, 2006, 9 (2): 69-84. DOI: 10.1080/10253890600771858.

[2]. Rehsia NS, Dhalla NS. Mechanisms of the beneficial effects of beta-adrenoceptor antagonists in congestive heart failure. Experimental and Clinical Cardiology, 2010, 15 (4): e86-e95. PMC3016066.

[3]. Lymperopoulos A, Rengo G, Koch WJ. Adrenergic nervous system in heart failure: pathophysiology and therapy. Circulation Research, 2013, 6: 739-753. DOI: 10.1161/CIRCRESAHA.113.300308.

[4]. Moura AL, Hyslop S, Grassi-Kassisse DM, Spadari RC. Functional β_2-adrenoceptors in rat left atria: effect of foot-shock stress. Canadian Journal of Physiology and Pharmacology, 2017, 9: 999-1038. DOI: dx.doi.org/10.1139/cjpp-2016-0622.

[5]. Spadari RC, Cavadas C, De Carvalho AETS, Ortolani D, de Moura AL, Vassalo PF. Role of beta-adrenergic receptors and sirtuin signaling in the heart during aging, heart failure, and adaptation to stress. Cellular and Molecular Neurobiology, 2018, 1: 109-120. DOI: 10.1007/s10571-017-0557-2.

[6]. Spadari RC, Bassani RA, De Moraes S. Supersensitivity to isoprenaline and epinephrine in right atria isolated from rats submitted to a single swimming session. General Pharmacology: The Vascular System, 1988, 1: 129-35. DOI: 10.1016/0306-3623(88)90018-3.

[7]. Santos CXC, Anilkumar N, Zhang M, Brewer AC, Shah AM. Redox signaling in cardiac myocytes. Free Radical Biology & Medicine, 2011, 50: 777–793. DOI:10.1016/j.freeradbiomed.2011.01.003.

[8]. Hill MF & Singal PK. Antioxidant and oxidative Stress changes during heart failure subsequent to myocardial infarction in rats. American Journal of Pathology, 1996, 148 (1): 291–300. PMC1861605.

[9]. van der Pol A, van Gilst HW, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. European Journal of Heart Failure, 2019, 21: 425– 435. DOI:10.1002/ejhf.1320.

[10]. Corbi G, Conti V, Russomanno G, Longobardi G, Furgi G, Filippelli A, Ferrara N. Adrenergic signaling and oxidative stress: a role for sirtuins? Frontiers in Physiology, 2013, 8 (4): 324. DOI: 10.3389/fphys.2013.00324.

[11]. Tanno M, Kuno A, Horio Y, Miura T. Emerging beneficial roles of sirtuins in heart failure. Basic Research in Cardiology, 2012, 107(4): 273. DOI: 10.1007/s00395-012-0273-5.

[12]. Frescas D, Valenti L, Accili D. Nuclear trapping of the forkhead transcription factor FoxO1 via Sirt-dependent deacetylation promotes expression of glucogenetic genes. The Journal of Biological Chemistry, 2005, 280 (21): 20589–20595. DOI: 10.1074/jbc.M412357200.

[13]. Sundaresan NR, Samant SA, Pillai VB, Rajamohan SB, Gupta MP. SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. Molecular and Cellular Biology, 2008, 28 (20): 6384–6401. DOI: 10.1128/MCB.00426-08.

[14]. Sciarretta S, Hariharan N, Monden Y, Zablocki

D, Sadoshima J. Is autophagy in response to ischemia and reperfusion protective or detrimental for the heart? Pediatric Cardiology, 2011, 32 (3): 275–81. DOI: 10.1007/s00246-010-9855-x.

[15]. Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A, Gupta MP. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. The Journal of Clinical Investigation, 2009, 119: 2758– 2771. DOI: 10.1172/JCI39162.

[16]. Pillai VB, Sundaresan NR, Jeevanandam V, Gupta MP. Mitochondrial SIRT3 and heart disease. Cardiovascular Research, 2010, 88 (2): 250–256. DOI: 10.1093/cvr/cvq250.

[17]. Grillon JM, Johnson KR, Kotlo K, Danziger RS. Non-histone lysine acetylated proteins in heart failure. Biochimica et Biophysica Acta, 2012, 1822(4): 607-14. DOI: 10.1016/j.bbadis.2011.11.016.

[18]. Koentges C, Bode C, Bugger H. SIRT3 in Cardiac Physiology and Disease. Frontiers in Cardiovascular Medicine, 2016; 3: 38. DOI: 10.3389/fcvm.2016.00038.

[19]. Alcendor RR, Kirshenbaum LA, Imai S, Vatner SF, Sadoshima J. Silent information regulator 2alpha, a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes. Circulation Research, 2004, 95 (10): 971–980. DOI: 10.1161/01.RES.0000147557.75257.

[20]. Pillai JB, Isbatan A, Imai S, Gupta MP. Poly(ADP-ribose) polymerase-1-dependent cardiac myocyte cell death during heart failure is mediated by NAD+ depletion and reduced Sir2alpha deacetylase activity. The Journal of Biological Chemistry, 2005, 280 (52): 43121–30. DOI: 10.1074/jbc.M506162200.

[21]. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, Tian B, Wagner T, Vatner SF, Sadoshima J. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circulation Research, 2007,100:1512–1521. DOI: 10.1161/01.RES.0000267723.65696.4a.

[22]. Hsu CP, Zhai P, Yamamoto T, Maejima Y, Matsushima S, Hariharan N, Shao D, Takagi H, Oka S, Sadoshima J. Silent information regulator 1 protects the heart from ischemia/reperfusion. Circulation, 2010, 122:2170–2182. DOI: 10.1161/CIRCULATIONAHA.110.958033.

[23]. Matsushima S & Sadoshima J. The role of sirtuins in cardiac disease. American Journal of Physiology: Heart and Circulatory Physiology 309 (9): H1375–H1389, 2015. DOI:10.1152/ajpheart.00053.2015.

[24]. Ortolani D, Oyama LM, Ferrari EM, Melo LL, Spadari-Bratfisch RC. Effects of comfort food on food intake, anxiety-like behavior and the stress response in rats. Physiology and Behaviour, 2011, 103(5): 487-492. DOI: 10.1016/j.physbeh.2011.03.028.

[25]. Cordeiro MA, Rodrigues LS, Ortolani D, de Carvalho AET, Spadari RC. Persistent effects of subchronic stress on components of ubiquitin-proteasome system in the heart. Journal of Clinical and Experimental Cardiology, 2020, 11: 676. DOI: 35248/2155-9880.20.

[26]. Elkhwanky MS & Hakkola J. Extranuclear sirtuins and metabolic stress. Antioxidants & Redox Signaling, 2018, 28 (8): 662-676. DOI: 10.1089/ars.2017.7270.

[27]. de Carvalho AETS, Cordeiro MA, Rodrigues LS, Ortolani D, Spadari RC. Stress-induced differential gene expression in cardiac tissue. Scientific Reports, 2021, 11: 9129. DOI: 10.1038/s41598-021-88267-8.

[28]. Takimoto E & Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. Hypertension, 2007, 49 (2): 241–248. DOI: 10.1161/01.HYP.0000254415.31362.a7.

[29]. Wang L, Quan N, Sun W, Chen X, Cates C, Rousselle T, Zhou X, Zhao X and Li J. Cardiomyocyte-specific deletion of Sirt1 gene sensitizes myocardium to ischaemia and reperfusion injury. Cardiovascular Research, 2018, 114 (6): 805–821. DOI: 10.1093/cvr/cvy033.

[30]. Luo G, Jian Z, Zhu Y, Zhu Y, Chen B, Ma R, Tang F and Xiao Y. Sirt1 promotes autophagy and inhibits apoptosis to protect cardiomyocytes from hypoxic stress. International Journal of Molecular Medicine, 2019, 43 (5): 2033-2043. DOI: 10.3892/ijmm.2019.4125.

[31]. Huang H & Tindall DJ. Dynamic FoxO transcription factors. Journal of Cell Science, 2007, 120: 2479–2487. DOI: 10.1242/jcs.001222.

[32]. Gomes AP, Price NL, Ling AJY, Moslehi JJ, Montgomery MK, Rajman L, White JP, Teodoro JS, Wrann CD, Hubbard BP, Mercken EM, Palmeira CM, De Cabo R, Rolo AP, Turner N, Bell EL, and Sinclair DA. Declining NAD+ induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell, 2013, 155 (7): 1624–1638. DOI: 10.1016/j.cell.2013.11.037.

[33]. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nature Reviews Molecular Cellular Biology, 2012, 13: 225–38. DOI: 10.1038/nrm3293.