Cell Calcium 72 (2018) 26–38

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

Underlying mechanism of the contractile dysfunction in atrophied ventricular myocytes from a murine model of hypothyroidism

Dolores Montalvo[a,1], Perla Pérez-Treviño[a,1], Katheryne Madrazo-Aguirre[a], Fabio A. González-Mondellini[a], Hipólito O. Miranda-Roblero[a], Diego Ramonfaur-Gracia[a], Mariana Jacobo-Antonio[b], Maritza Mayorga-Luna[b], Norma. L. Gómez-Víquez[b], Noemí García[a], Julio Altamirano[a,*]

[a] Tecnologico de Monterrey, Escuela de Medicina, Av. Morones Prieto No. 3000 Pte., Monterrey, N.L., 64710, Mexico
[b] Departamento de Farmacobiologia, CINVESTAV-IPN sede Sur., D.F. 14330, Mexico

ARTICLE INFO

Keywords:
SERCA
Phospholamban
Ryanodine receptors type 2
Transverse tubules
Cardiac atrophy

ABSTRACT

Hypothyroidism (Hypo) is a risk factor for cardiovascular diseases, including heart failure. Hypo rapidly induces Ca^{2+} mishandling and contractile dysfunction (CD), as well as atrophy and ventricular myocytes (VM) remodeling. Hypo decreases SERCA-to-phospholamban ratio (SERCA/PLB), and thereby contributes to CD. Nevertheless, detailed spatial and temporal Ca^{2+} cycling characterization in VM is missing, and contribution of other structural and functional changes to the mechanism underlying Ca^{2+} mishandling and CD, as transverse tubules (T-T) remodeling, mitochondrial density (D_{mit}) and energy availability, is unclear. Therefore, in a rat model of Hypo, we aimed to characterize systolic and diastolic Ca^{2+} signaling, T-T remodeling, D_{mit}, citrate synthase (CS) activity and high-energy phosphate metabolites (ATP and phosphocreatine).

We confirmed a decrease in SERCA/PLB (59%), which slowed SERCA activity (48%), reduced SR Ca^{2+} (19%) and blunted Ca^{2+} transient amplitude (41%). Moreover, assessing the rate of SR Ca^{2+} release (dRel/dt), we found that early and maximum dRel/dt decreased, and this correlated with staggered Ca^{2+} transients. However, dRel/dt persisted during Ca^{2+} transient relaxation due to abundant late Ca^{2+} sparks. Isoproterenol significantly up-regulated systolic Ca^{2+} cycling. T-T were unchanged, hence, cannot explain staggered Ca^{2+} transients and altered dRel/dt. Therefore, we suggest that these might be caused by RyR2 clusters desynchronization, due to diminished Ca^{2+}-dependent sensitivity of RyR2, which also caused a decrease in diastolic SR Ca^{2+} leak. Furthermore, D_{mit} was unchanged and CS activity slightly decreased (14%), however, the ratio phosphocreatine/ATP did not change, therefore, energy deficiency cannot account for Ca^{2+} and contractility dysregulation. We conclude that decreased SR Ca^{2+}, due to slower SERCA, disrupts systolic RyR2 synchronization, and this underlies CD.

1. Introduction

It is well established that hypothyroidism (Hypo) profoundly impairs heart performance, by decreasing cardiac contractility and cardiac output (by reducing heart rate and stroke volume) [1,2]. These cardiac alterations develop rapidly, within a few weeks [3], and are the consequence of an abnormal pattern of gene expression in the myocardium, triggered by decreased thyroid hormones (TH) action, particularly triiodothyronine (T_3), directly on the heart [3–6], but also, indirectly, by inducing peripheral vasoconstriction and decreasing blood volume,

Abbreviations: α-MHC, α-Myosin heavy chain; β-MHC, β-Myosin heavy chain; β-AS, β-adrenergic stimulation; CICR, Ca^{2+}-induced Ca^{2+} release; CL, cell length; CS, citrate sintasa; CV, cell volume; CW, cell width; CD, contractile dysfunction; Ctrl, Control; CSA, cross-sectional area; $\Delta F/F_0$, peak amplitude of field-stimulated cytosolic Ca^{2+} transient; $\Delta F_{Caff}/F_0$, peak amplitude of caffeine-evoked cytosolic Ca^{2+} transient; D_{mit}, mitochondrial density; dRel/dt, rate of SR Ca^{2+} release; ECC, excitation-contraction coupling; FL, femur length; HF, Heart failure; HW, heart weight; Hypo, hypothyroidism; ISO, isoproterenol; κ_{Decay}, rate of decay of the cytosolic Ca^{2+} transient; λ_{Exc}, Emission wavelength; λ_{Emi}, Excitation wavelength; LCC, L-type Ca^{2+} channels; I_{Ca}, L-type Ca^{2+} channels current; LVDP, Left ventricular developed pressure; +dLVP/dt, maximum rate of rise of intraventricular pressure; −dLVP/dt, minimum rate of rise of intraventricular pressure; MPI, myocardium performance index; PTU, 6-propyl-2-thiouracil; SR, sarcoplasmic reticulum; RyR2, ryanodine receptors type 2; SL, saline solution; SR, sarcoplasmic reticulum; PLB, phospholamban; PCr, phosphocreatine; TG, thapsigargin; T-Tubules, Transverse Tubules; TT_{Period}, T-Tubule period; TT_{Power}, T-Tubule power; T_3, Triiodothyronine

* Corresponding author.
E-mail address: altamirano@itesm.mx (J. Altamirano).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ceca.2018.01.005
Received 2 November 2017; Received in revised form 18 January 2018; Accepted 31 January 2018
Available online 05 February 2018

causing a chronic decrease in cardiac hemodynamic preload [2,3,7–9]. Interestingly, the cardiac gene expression pattern in Hypo, considered of "fetal type" [10,11], resembles in many aspects that of heart failure (HF) [1,4,5,9,11,12], suggesting that untreated Hypo may lead to HF development [12,13].

In ventricular myocytes, among the affected genes in Hypo and HF are those of important Ca^{2+} handling proteins, particularly a decrease in the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) to phospholamban (PLB) ratio (SERCA/PLB) [11,12,14–16], and might largely explain the blunted Ca^{2+} transients and contractile dysfunction (CD) at the cellular level [14,17], however, the participation of other alterations cannot be ruled out. Furthermore, the spatial and temporal properties of systolic and diastolic Ca^{2+} cycling in Hypo have not been studied in detail, and those are important because they determine the magnitude and kinetics of cell contractility and relaxation, and SR Ca^{2+} content, and might even affect cell electrophysiology, among other important cellular functions. Moreover, detailed knowledge of local Ca^{2+} signaling would allow better understanding of the underlying molecular mechanisms of Hypo cardiac dysfunction. In this regard, Hypo induces important structural and functional changes in ventricular myocytes, however, their specific role in Ca^{2+} signaling dysregulation and CD, particularly at early stages of Hypo development, has not been thoroughly assessed. On the one hand, in humans [3] and rodents, Hypo rapidly decreases cardiac mass, and this is due to myocytes atrophy rather than a decrease in myocytes number [2,11,12,14,15], and this is accompanied by major changes in internal cellular structure and molecular composition [2,11,12], and eventually leads to chamber remodeling. The extensive cellular remodeling in Hypo could include alterations in the transverse tubules (T-Tubules), the highly specialized system of sarcolemmal invaginations that contain most of the L-type Ca^{2+} channels (LCC), essential for the excitation-contraction coupling (ECC) in ventricular myocytes [18]. T-Tubules are prone to disarrange or dedifferentiate in uncompensated hypertrophy and HF [19–23], affecting local ECC and causing inhomogeneous and blunted Ca^{2+} transients and CD [22–24]. Furthermore, it has recently been suggested that T_3, in combination with glucocorticoids, is essential for T-tubules development and proper ECC in cardiac myocytes differentiated from human pluripotent stem cells [25]. Whether T-Tubules remodeling occurs in Hypo atrophied ventricular myocytes is unknown, however, if present, it could alter the spatial and temporal properties of SR Ca^{2+} release and contribute to CD. Therefore, assessing possible T-Tubule remodeling in Hypo ventricular myocytes was a primary aim of this work.

On the other hand, TH play an important role in controlling myocardial aerobic metabolism and mitochondrial biogenesis. TH upregulate expression of mitochondrial enzymes of the oxidative metabolism (e. g., citrate synthase; CS, respiratory complexes I to IV, etc.), as well as transcriptional controllers involved in mitochondrial biogenesis (e.g. peroxisome proliferator-activated receptor α; PPARα, co-activator-1α of peroxisome proliferator activated receptor gamma; PGC-1α, the nuclear respiratory factors 1 and 2; NFR-1 and −2, mitochondrial transcription factor; mtTFA, etc.) [11,26,27]. Therefore, Hypo might have the opposite effect on mitochondria energetic metabolism and biogenesis. A decrease in oxidative capacity and mitochondrial density would diminish cellular ATP supply in Hypo myocytes, as it occurs in HF, impairing contractility [18,28] and SR Ca^{2+} pumping [28–30]. Nevertheless, although myocardial aerobic metabolism and oxidative capacity decrease in Hypo [3,11,16], this change is, apparently, smaller than that of the mechanical performance [3,16]. Furthermore, it is unclear whether mitochondrial biogenesis is affected by Hypo [16]. Therefore, the relative contribution of mitochondrial dysfunction, biogenesis and energy supply to Ca^{2+} cycling and contractility in Hypo, is unclear.

Therefore, our main objectives were: A) Provide high spatial and temporal resolution of systolic and diastolic Ca^{2+} cycling dynamics in ventricular myocytes from a murine model of Hypo, induced with a treatment with 6-propyl-2-thiouracil (PTU) for 3 weeks. B) Determine whether T-Tubules remodeling and/or decreased mitochondrial density and energy metabolites availability, contribute to altered Ca^{2+} dynamics and CD.

2. Materials and methods

2.1. Ethical approval

The animal protocols were approved by the Internal Committee for Care and Handling of Laboratory Animals of the School of Medicine of the Tecnologico de Monterrey (Protocol 2012-RE-017), and were performed according to the National Institutes of Health guidelines.

2.2. Murine model of hypothyroidism

Hypothyroidism (Hypo) was induced in male Wistar rats of 270–340 g by PTU (0.05%) administration in the drinking water as previously described [12,31]. All experiments were performed after 3 weeks of PTU treatment. Age-matched control rats were kept under similar conditions but did not received PTU and were used as control (Ctrl).

2.3. Reagents

All chemical reagents used were obtained from Sigma-Aldrich, St. Louis, MO, unless indicated otherwise, and fluorophores were obtained from Life Technologies, Carlsbad, CA.

2.4. Thyroid hormone profile characterization

To assess TH status of the animals, blood samples were taken from the caudal lateral vein; one sample before starting the treatment and a second sample at the end of the 3-week treatment. The rats were weighed and anesthetized with pentobarbital (30 mg/kg body weight, I.P.). Animals were kept warm by wrapping them in a cloth and placed on a heating plate at 37 °C. A 26 G catheter impregnated with heparin was used to collect ~2 ml blood samples and placed in heparinized micro centrifuge tubes. Blood samples were processed with the Architect FT4 and FT3 chemiluminescent microparticle immunoassays (Abbott Laboratories, Abbott Park, IL), for the quantitative determination of free thyroxine (T_4) and free T_3, respectively, according to the manufacturer's instructions. The T_4 and T_3 assays have analytical sensitivities of ≤0.4 ng/dl and ≤0.25 ng/dl, respectively.

2.5. Ex vivo heart performance

Rats were anaesthetized with pentobarbital sodium (80 mg/kg body weight, I.P.), hearts were quickly excised and mounted on a Langendorff apparatus. The heart was perfused retrogradely (12 ml/min) with a saline solution of the following composition (in mM): 125 NaCl, 5.4 KCl, 1 $MgCl_2$, 0.5 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$, 11 glucose, and 0.0001 octanoate, and was bubbled with 95% O_2/5% CO_2 and kept at 37 °C [32]. A latex balloon connected to a pressure transducer was inserted into the left ventricle and filled with saline solution. All recordings were performed in Ctrl and Hypo hearts during a period of 30 min under basal conditions. Data were acquired and processed with a Data-Trax acquisition system (World Precision Instruments, Sarasota, FL). The Myocardium Performance Index (MPI) was assessed as the product of Left Ventricular Developed Pressure (LVDP) × Heart Rate (mmHg × number of heart beats × min^{-1} × 1000) in steady state. The maximum and minimum rate of rise of intraventricular pressure (+dLVP/dt and −dLVP/dt; mmHg × s^{-1} × 100) were automatically assessed online from the contraction and relaxation velocity, respectively. Data were recorded during 30 min of constant perfusion and the mean values assessed for that period were reported. Upon completion of