Electrical Properties of Isolated Cardiomyocytes in a Rat Model of Thiamine Deficiency

Artur Santos-Miranda[2], Jader Santos Cruz[2], Danilo Roman-Campos[1]

Universidade Federal de São Paulo[1], São Paulo, SP; Universidade Federal de Minas Gerais[2], Minas Gerais, MG - Brazil

Abstract

In modern society, thiamine deficiency (TD) remains an important medical condition linked to altered cardiac function. There have been contradictory reports about the impact of TD on heart physiology, especially in the context of cardiac excitability. In order to address this particular question, we used a TD rat model and patch-clamp technique to investigate the electrical properties of isolated cardiomyocytes from epicardium and endocardium. Neither cell type showed substantial differences on the action potential waveform and transient outward potassium current. Based on our results we can conclude that TD does not induce major electrical remodeling in isolated cardiac myocytes in either endocardium or epicardium cells.

Introduction

Thiamine is a pivotal cofactor involved in distinct biochemical reactions. Its deprivation causes significant changes in physiology, especially in neurons and cardiac tissue[1]. In the industrialized world, thiamine deficiency (TD) is particularly related to chronic alcohol consumption and administration of loop diuretics, such as furosemide[1]. In both cases, some degree of heart remodeling is reported, such as high-output heart failure, the most common clinical manifestation of TD[2]. In some cases, patients with TD may develop heart failure in association with cardiac electrical remodeling[2, 3]. Based on previous data from the literature and using animal models, our group and others have determined electrical remodeling of cardiomyocytes. However, there are conflicting results in the literature[1]. In the present study, we determined whether TD leads to electrical remodeling of isolated myocytes from the endocardium (ENDO) and epicardium (EPI) of rats.

Methods

Experimental group

Male Wistar rats (250 g) were fed a control (containing thiamine) or a thiamine-free diet for 35 days as previously described by our group[4-6].

Keywords

Myocytes, Cardiac; Electric Stimulation; Thiamine Deficiency/physiopathology; Action Potentials; Rats, Heart Failure.

Mailing Address: Danilo Roman Campos •
Rua Botucatu 862, Edifício de Ciências Biomédicas. Vila Clementino, Postal Code 04023-062. São Paulo, SP – Brazil
Email: drcampos@unifesp.br; drcbio@gmail.com
Manuscript received on October 10, 2014; revised on December 24, 2014, accepted on December 24, 2014.

DOI: 10.5935/abc.20150010

Cell isolation

ENDO and EPI cells of the left ventricle from age-matched control and TD rats were enzymatically dissociated as previously reported[4].

Electrophysiological recordings

All records were obtained using methods and solutions as previously described[5]. Cells were maintained at a holding potential of -80mV. Action potentials (APs) were elicited by short pulses (3-5 ms) of 1nA current at 1 Hz frequency for 3 minutes. During K current records, cells were perfused with a modified Tyrode's solution, replacing NaCl by N-metyl-D-glucamine (NMDG) (to abolish sodium current), and 100μM $CdCl_2$ (to block L-type calcium current). A junction potential of -20mV was measured and should be applied to every tested potential.

Statistical analysis

All results are expressed as mean ± standard error of the mean. For statistical analysis, we used One-way anova followed by Tukey's post-hoc test. $P < 0.05$ was set as significant level.

Results

In the present study we evaluated the electrical properties of isolated myocytes from ENDO and EPI cells. Our results showed that control myocyte have longer action potential duration (APD) in ENDO cells when compared to EPI cells (Figure 1). This phenomenon is connected to larger outward potassium current in EPI when compared to ENDO cells (Figure 2), corroborating previous data from the literature. To our surprise, TD had minor impact on electrical properties of isolated cardiomyocytes. When compared the repolarization time (RT) at 90% of EPI CTR to EPI TD, it was 26.33 ± 1.56 ms, n = 26 vs. 30.35 ± 2.49 ms, n = 22, respectively. ENDO cells showed similar results. For instance, RT at 90% was 38.93 ± 2.96ms, n = 18 vs. 46.40 ± 6.11 ms, n = 20 for CTR vs. TD cells (Figure 1). In line with these results, peak outward and inward potassium current was similar, when comparing EPI CT to EPI TD. For example at +80 mV it was (A/F) 23.01 ± 1.77, n = 18 and 20.62 ± 1.50, n = 15, respectively. Similar profile was observed for ENDO cells (Figure 2). Finally, in line with previous results, TD myocytes showed smaller capacitance when compared to CTR cells (data not shown).

Discussion

In previous studies, using a rat model, our group showed that TD is able to induce cardiomyocyte contractility dysfunction[7]. Such changes were attributed to altered

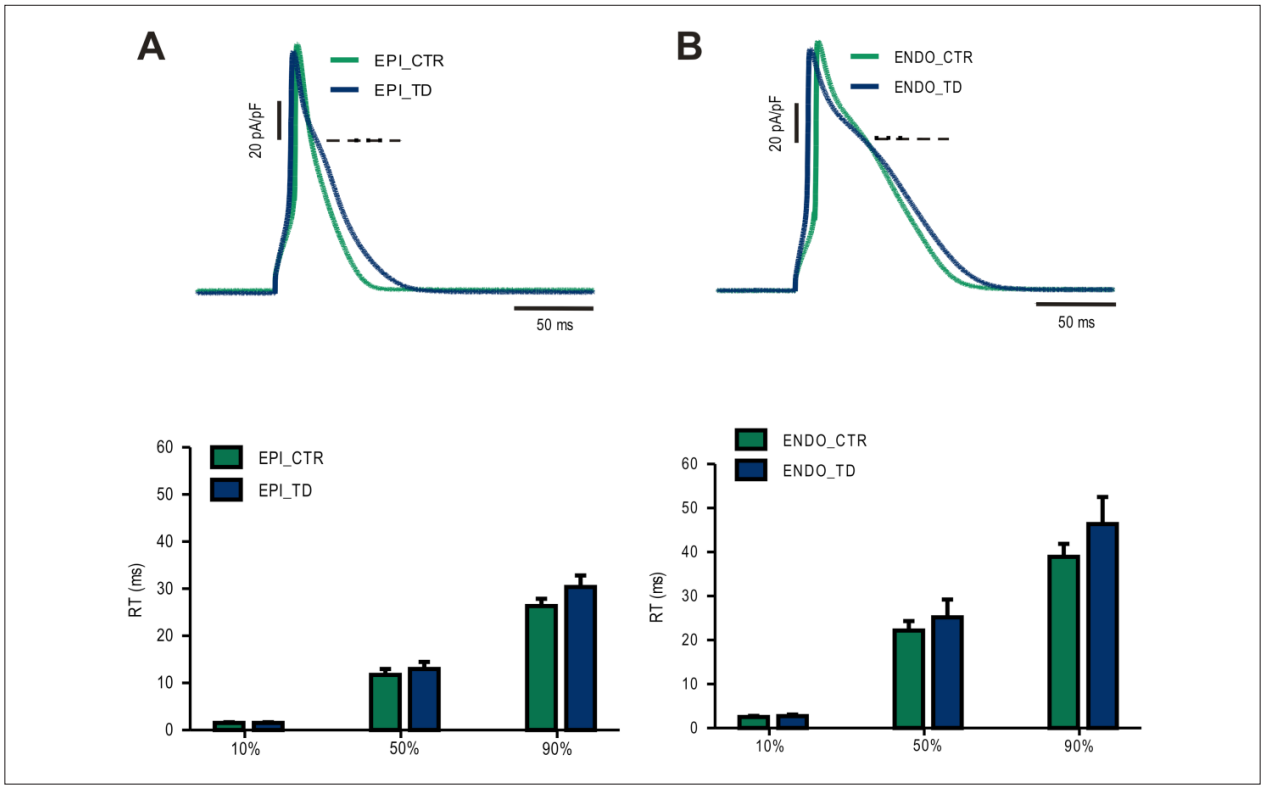

Figure 1 – *Action potentials in isolated cardiac myocytes. Top panels show representative recordings for (A) epicardium (EPI) cells (left) and (B) Endocardium (ENDO) cells (right), considering control (CT) (green line) and thiamine-deficient (TD) (blue line) groups. Bottom panels show average time required for the action potential repolarization to occur at 10%, 50% and 90% of the repolarization in control (green bars) and TD (blue bars) groups for EPI- CT (n = 26), EPI-DT (n = 22) (left) and ENDO-CT (n = 18), ENDO-TD (n = 20) (right).*

calcium handling, with reduced sarcoplasmatic reticulum calcium content[8]. Additionally, an increased production of reactive oxygen species (ROS) was observed, which may have contributed to the reduced heart mass observed in this model[4]. Together, these results are able to explain the reduced heart and myocyte function during TD.

In the context of electrical alteration, it is more difficult to draw a more conclusive idea. There have been many reports in the literature showing distinct alterations in the electrocardiogram of humans and animals during TD, including tachycardia, ST elevation and depression, altered T wave morphology with prolonged QT interval, accompanied by A-V block and QRS prolongation[2, 9]. In the context of animal models, it seems that cardiomyocytes from young rats are more prone to develop electrical disturbances than adult rats (the latter were used in the present study)[1].

However, it is intriguing that TD is not able to induce robust electrical remodeling in cardiomyocytes, especially in the context of increased ROS production[4]. It is well know that ROS is able reduce outward potassium current in myocytes, due to reduced expression of $Kv_{4.3}$, which is responsible for the transient outward potassium current[10]. Thus, it is possible to speculate that there are endogenous systems and/or aging factors that modulate the electrical remodeling of myocytes in the setting of TD.

Conclusion

Thiamine deficiency leads to minor changes in the electrical properties of isolated cardiac myocytes in both endocardium and epicardium cells. This study was supported by FAPEMIG, CNPq and CAPES.

Author contributions

Conception and design of the research: Santos-Miranda A, Cruz JS, Roman-Campos D. Acquisition of data: Santos-Miranda A. Analysis and interpretation of the data: Santos-Miranda A, Roman-Campos D. Statistical analysis: Santos-Miranda A. Obtaining financing:Cruz JS, Roman-Campos D. Writing of the manuscript: Santos-Miranda A, Cruz JS, Roman-Campos D. Critical revision of the manuscript for intellectual content: Santos-Miranda A, Roman-Campos D.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by CNPq, Capes e FAPEMIG.

Study Association

This study is not associated with any thesis or dissertation work.

Brief Communication

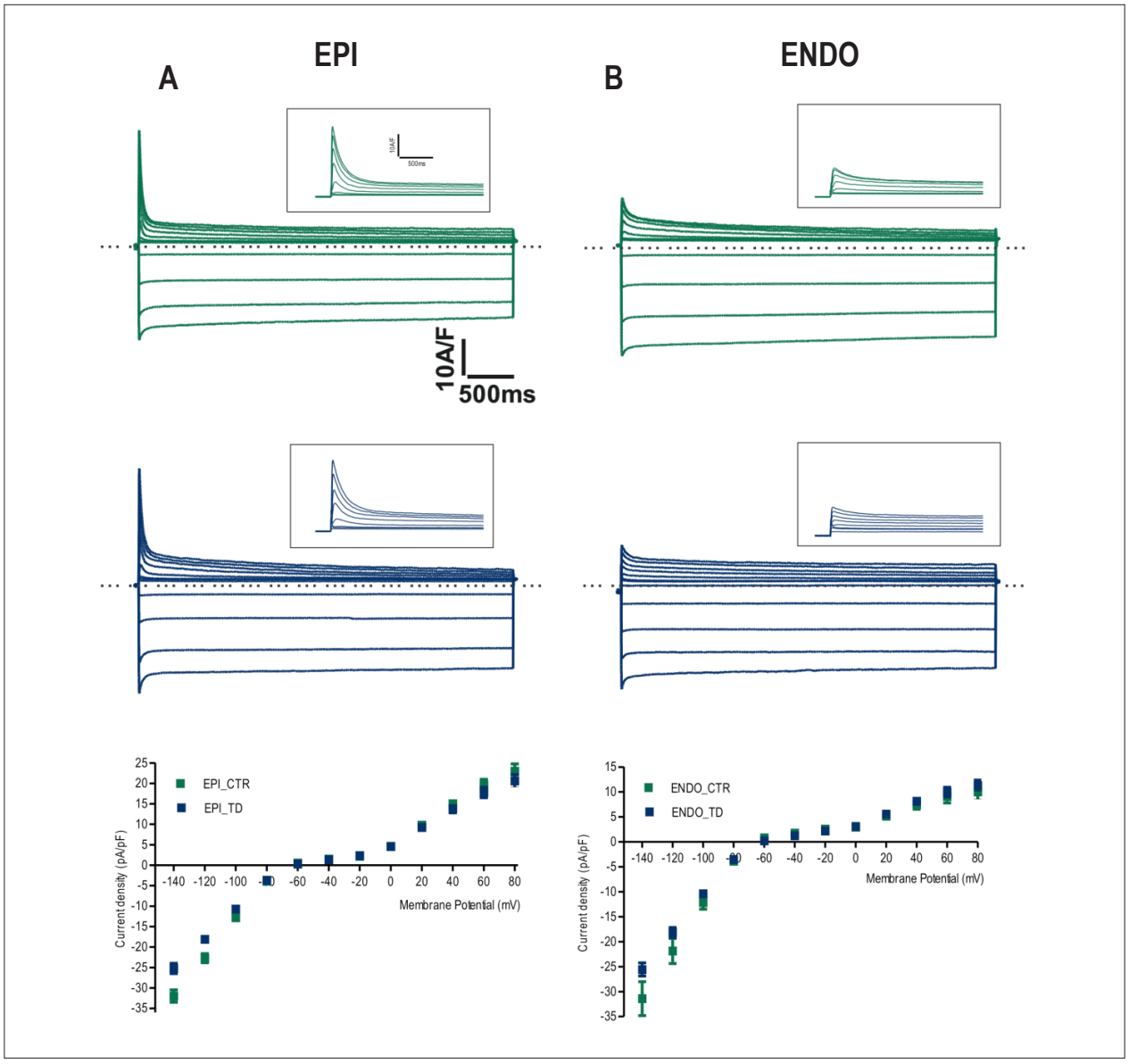

Figure 2 – *Whole-cell potassium current. Currents were elicited upon stimulation steps ranging from -140 to +80mV (for 4s) from a holding potential of -70 mV in steps of 20 mV, every 15s. Top (CTR) and middle (TD) panels show representative recordings for (A) epicardium (EPI) and (B) endocardium (ENDO) cells. Insets represent the initial 500 ms of recordings. Bottom panels represent the current x voltage for maximum potassium current. Green and blue squares represent CTR and TD groups, for EPI (A) and ENDO (B) cells.*

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