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Increased calcium leak associated with reduced calsequestrin expression in hyperthyroid cardiomyocytes

David R. de Alba-Aguayo[a], Natalia Pavón[b], Martha Mercado-Morales[a], Miyamin Miranda-Saturnino[a], Mavil López-Casamichana[c], Agustin Guerrero-Hernández[a], Angelica Rueda[a,d,*]

[a] Department of Biochemistry, Cinvestav-IPN, Mexico City, Mexico
[b] Department of Pharmacology, Instituto Nacional de Cardiología "Ignacio Chávez", Mexico City, Mexico
[c] Posgrado en Ciencias Genómicas, Universidad Autónoma de la Ciudad de Mexico, Mexico City, Mexico
[d] Center for Arrhythmia Research, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, 48109, USA

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ABSTRACT

Introduction: Calcium (Ca^{2+}) leak during cardiac diastole is chiefly mediated by intracellular Ca^{2+} channel/Ryanodine Receptors. Increased diastolic Ca^{2+} leak has been proposed as the mechanism underlying the appearance of hereditary arrhythmias. However, little is known about alterations in diastolic Ca^{2+} leak and the specific roles played by key intracellular Ca^{2+}-handling proteins in hyperthyroidism, a known arrhythmogenic condition.

Aim: We sought to determine whether there were modifications in diastolic Ca^{2+} leak, based on the recording of Ca^{2+} sparks and Ca^{2+} waves; we also investigated changes in the expression and activity of key Ca^{2+} handling proteins, including ryanodine receptors, Sarco-Endoplasmic Reticulum Ca^{2+} ATPase pump and calsequestrin in isolated left-ventricular cardiomyocytes isolated from hyperthyroid rats.

Materials and methods: Electrocardiography (ECG) recordings were performed in control and hyperthyroid rats. Ca^{2+} sparks, Ca^{2+} waves, and electrically-stimulated Ca^{2+} transients were recorded in Fluo-3-loaded cardiomyocytes from both experimental groups using confocal microscopy. In addition, left-ventricular homogenates and Ryanodine Receptor-enriched membrane fractions were prepared for assessing [^{3}H]-ryanodine binding, hydrolytic ATPase activity of SERCA pump and expression levels of key proteins by Western blot, and cDNA for real-time qPCR.

Results and conclusions: Extrasystoles were observed in hearts of hyperthyroid rats by ECG recordings. Arrhythmogenic activity, high incidence of Ca^{2+} waves, and *de novo* Ca^{2+} wavelets –in the absence of sarcoplasmic reticulum Ca^{2+} overload- were recorded in these cardiomyocytes. The exacerbated diastolic Ca^{2+} leak and arrhythmogenic activities were related to a diminished expression of calsequestrin along with increased SERCA pump activity, which, in effect, promoted a gain-of-function in RyRs without alterations in SR Ca^{2+} load, RyR expression or its Ca^{2+} sensitivity.

1. Introduction

The thyroid hormones thyroxine (T_4) and its main active metabolite, 3,3′5-triiodo-L-thyronine (T_3), are synthesized in the thyroid gland; the hormones have a major role in the regulation of several biological processes, including metabolic rate, oxygen consumption, gene transcription, and protein synthesis [1,2]. Hyperthyroidism results in profound cardiovascular alterations such as increased heart rate, ventricular contractility, and blood ejection volume; the condition also promotes cardiac hypertrophy and cardiac arrhythmias [3,4].

Under physiological conditions, cardiomyocytes translate electrical action potentials into mechanical force through the excitation-contraction coupling (ECC) mechanism [5]. Activation of voltage-dependent L-type Ca^{2+} channels (VDCCs) produces an inward Ca^{2+} current (I_{Ca}) that activates the ryanodine receptors (RyR) through the mechanism known as calcium-induced calcium release (CICR), which raises cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$)

Abbreviations: RyR, Ryanodine Receptor; SERCA, Sarco-Endoplasmic Ca^{2+} ATPase; Csq2, cardiac calsequestrin; T_3, 3,3′5-triiodo-L-thyronine.

* Corresponding author at: Department of Biochemistry, Cinvestav–IPN, Av. IPN 2508. Col. San Pedro Zacatenco, 07360, Mexico City, Mexico.
E-mail address: arueda@cinvestav.mx (A. Rueda).

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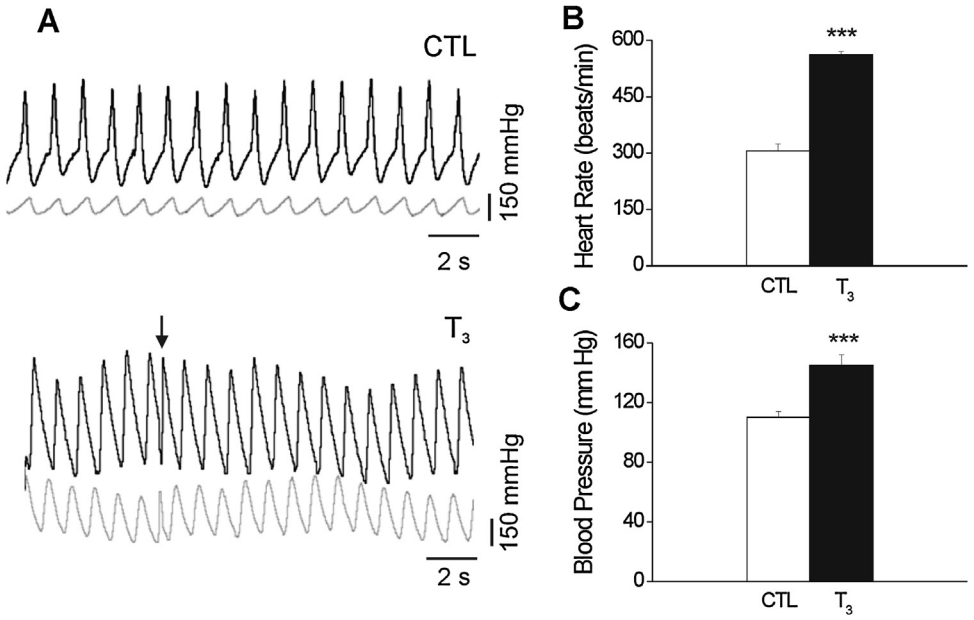

Fig. 1. Increased heart rate and presence of extrasystoles in hyperthyroid rats. (A) Representative electrocardiogram (upper black line) and blood pressure (lower gray line) recordings of euthyroid (CTL) and hyperthyroid (T_3) rats; the black arrow highlights an extrasystole. Bar graphs of mean values of heart rate (B) and blood pressure (C) for euthyroid (open bars, N = 10) and hyperthyroid (solid bars, N = 10) animals, presented as M ± SE, ***P < 0.001 respect to CTL.

Table 1
Characteristics of hyperthyroid animals.

Group	BW (g)	HW (g)	HW/BW*100	Serum T_3 (pg/mL)	N
CTL	330.00 ± 16.50	1.72 ± 0.04	0.53 ± 0.03	5.40 ± 0.90	11
T_3	280.38 ± 6.52*	2.19 ± 0.10 ***	0.78 ± 0.03***	46.10 ± 10.40***	8

Values are mean ± SE. Euthyroid (CTL) and hyperthyroid (T_3) animals. *$P < 0.05$ and ***$P < 0.001$ *vs.* euthyroid.

and activates the contractile machinery during cardiac systole. Muscle relaxation during ventricular diastole depends upon $[Ca^{2+}]_i$ returning to basal levels. In cardiomyocytes, two proteins are primarily responsible for removing Ca^{2+} from the cytoplasm: the Sarco-Endoplasmic Reticulum Ca^{2+} ATPase (SERCA pump) and the sarcolemmal Na^+/Ca^{2+} exchanger (NCX) [5].

Not all RyRs are closed during diastole, generating a physiological Ca^{2+} leak that acts to offset SERCA pump activity, resulting in a steady SR Ca^{2+} content [6,7]. RyR-mediated Ca^{2+} efflux from the SR during the recovery phase of the cardiac cycle (diastole) is known as *diastolic* Ca^{2+} *leak* [7]. Diverse forms of diastolic Ca^{2+} leak have been described in isolated cardiomyocytes, mainly in the form of Ca^{2+} sparks, Ca^{2+} waves, and "invisible" Ca^{2+} leak [8]. It has been suggested that calsequestrin (Csq2), a low-affinity, high-capacity Ca^{2+} buffer protein found in the lumen of the SR [9,10] has a dual functional role: (1) to provide large amounts of Ca^{2+} to RyR during systole, and (2) to inhibit RyR activity during diastole [9,11], which, in turn, result in efficient SR Ca^{2+} load recovery for the next cardiac cycle. Thus, calsequestrin plays an important role in the reduction of RyR activity during diastole. Pathological diastolic Ca^{2+} leak has been associated with arrhythmogenic activity in the heart, mainly by triggering delayed after depolarizations (DADs) [12–14].

Although there are studies reporting Ca^{2+} handling alterations in cardiomyocytes of hyperthyroid models [15], to our knowledge, no one has yet investigated alterations in diastolic Ca^{2+} leak and the role played by Csq2 in hyperthyroid cardiomyocytes. Thus, the aim of this study is to determine whether the arrhythmogenic cardiac activity associated with hyperthyroidism can be attributed to modifications in diastolic Ca^{2+} leak due to changes in the expression levels or the activity of the primary Ca^{2+} handling proteins (RyR, SERCA pump and Csq2).

2. Materials and methods

2.1. Animal model

Experiments were performed according to the ethical guidelines for the Use of Animals at the Cinvestav-IPN (approved animal protocol No. 500/11). Hyperthyroidism in rats was induced as previously reported [16] with some modifications. Briefly, male Wistar rats (from 250 to 300 g of body weight) were randomly divided in two groups: euthyroid group (CTL) and hyperthyroid group (T_3). The T_3 group received a daily i.p. injection of 3,3′5-triiodo-L-thyronine (Cat #T6397, Sigma-Aldrich Co., 2 mg/kg of body weight) for 12 days, while the CTL group received only vehicle injections. Both groups of animals were fed with standard rat chow (Formulab Diet 5008, *LabDiet*; Forumlab, Albuquerque, NM, USA) and received tap water *ad libitum*. Animals were maintained under a dark-light cycle of 12 h and controlled temperature of 22 ± 2 °C.

2.2. Electrocardiograph and blood pressure recordings

Both electrocardiography (ECG) and blood pressure recordings were done as described previously [16]. Briefly, rats were anaesthetized by sodium pentobarbital injection (50 mg/kg of body weight, i.p.) and maintained under assisted respiration by thoracotomy procedure. One lead-II surface electrocardiograph was used to monitor heart rate while blood pressure was measured with a pressure transducer attached to a femoral cannula.