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Cellular mechanisms of reduced sarcoplasmic reticulum Ca^{2+} content in *L*-thyroxin-induced rat ventricular hypertrophy[1]

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Key words

left ventricular hypertrophy; sarcoplasmic reticulum; Ca^{2+} release; Ca^{2+}-ATPase

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Abstract

Aim: To examine how the sarcoplasmic reticulum (SR) Ca^{2+} content changes and the underlying mechanism in *L*-thyroxin-induced cardiac hypertrophy. **Methods:** Echocardiography was used to confirm the establishment of the cardiac hypertrophy model. The confocal microscopy and fluorescent indicator Fluo-3 was applied to examine the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), the Ca^{2+} sparks, and the caffeine-induced Ca^{2+} transient in freshly isolated cardiac ventricular myocytes. The activity of sarcolemmal and SR Ca^{2+}-ATPase 2a (SERCA2a) in the ventricular tissue was also measured, respectively. **Results:** *L*-thyroxin (1 mg/kg injection for 10 d) induces left ventricular cardiac hypertrophy with normal myocardial function. The decreased caffeine-induced Ca^{2+} transient in the Ca^{2+}-free solution was detected. The spontaneous Ca^{2+} sparks in hypertrophied myocytes occurred more frequently than in normal cells, with similar duration and spatial spread, but smaller amplitude. Then the basal $[Ca^{2+}]_i$ increase was observed in quiescent left ventricular myocytes from hyperthyroidism rats. The activity of sarcolemmal and SR Ca^{2+}-ATPase was decreased in the hypertrophied ventricle tissue. **Conclusion:** The results suggested that the reduced SR Ca^{2+} content may be associated with an increased Ca^{2+} leak and reduced SERCA2a activity, contributing to abnormal intracellular Ca^{2+} handling during hypertrophy in hyperthyroidism rats.

Introduction

The contraction of cardiac myocytes in the heart is initiated when Ca^{2+} enters the cell via L-type Ca^{2+} channels in the sarcolemma. Ca^{2+} entry then triggers the release of a much larger amount of Ca^{2+} from the sarcoplasmic reticulum (SR)[1,2]. The elementary event of the SR Ca^{2+} release through ryanodine receptor type 2 (RyR2) is the Ca^{2+} spark. The spontaneous Ca^{2+} sparks in quiescent cardiac myocytes reflect the SR Ca^{2+} content, the function of RyR2, and SR Ca^{2+}-ATPase 2a (SERCA2a), as well as the SR Ca^{2+} leak[3]. It is the synchronized activation of many Ca^{2+} sparks triggered by Ca^{2+} entry via L-type Ca^{2+} channels that cause the systolic Ca^{2+} transient and subsequent myocardial contraction[1,2]. There is a re-uptake of released Ca^{2+} from the SR during contraction into the SR through SERCA2a. Given the dependence of the SR Ca^{2+} content on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), SERCA2a function, and spontaneous Ca^{2+} release, the alteration of the SR Ca^{2+} content may contribute to abnormal intracellular Ca^{2+} handling, leading to myocardial dysfunction.

Previous studies have shown that hyperthyroidism causes abnormalities in intracellular Ca^{2+} signaling components, which in turn results in cardiac hypertrophy and arrhythmia[4,5]. For example, the enhanced Ca^{2+} influx through the L-type Ca^{2+} channel could partly account for the prolonged action potential duration and delayed repolarization, and consequently aggravated arrhythmia development during cardiac hypertrophy[6]. The enhanced expression of functional RyR2, increased re-uptake of Ca^{2+} into the SR through SERCA2a, and decreased phospholamban expression have been reported after thyroxin injection, which was suggested to be responsible, at least in part, for the increase in the SR Ca^{2+} release and the Ca^{2+} transient, as well as enhanced myocardial contractility[4,7,8]. In the pressure-overload hypertrophy model, the SR Ca^{2+} content decreased secondary to the reduced SERCA2a-mediated Ca^{2+}

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uptake and increased sarcolemmal-mediated Ca^{2+} efflux from the cell, which caused the smaller Ca^{2+} transient and may contribute to the development of arrhythmias during hypertrophy[9]. However, in the thyroxin-induced cardiac hypertrophy model, the change in the SR Ca^{2+} content and the underlying mechanism have still not been fully understood.

In this study, we used laser scanning confocal microscopy and Ca^{2+}-sensitive fluorescent indicators to examine and quantitatively analyze the SR Ca^{2+} content, the spontaneous Ca^{2+} sparks, and the basal $[Ca^{2+}]_i$ in quiescent cardiac myocytes from normal rats and *L*-thyroxin-injected rats with left ventricular hypertrophy (LVH). The lower SR Ca^{2+} content was identified in this model. The Ca^{2+} spark recording and analysis demonstrated the increase in the diastolic SR Ca^{2+} leak, which may be due to more occurrences of spontaneous Ca^{2+} sparks and an increase in the basal $[Ca^{2+}]_i$. In addition, we observed the decreased activity of SERCA2a, which may lead to the deteriorated function of SERCA2a, contributing to the elevated $[Ca^{2+}]_i$ and lower SR Ca^{2+} content in hypertrophied cardiac myocytes.

Materials and methods

***L*-thyroxin-induced cardiac hypertrophy** All experimental procedures were approved by the Sun Yat-Sen University Committee for Animal Research (Guangzhou, China) and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The *L*-thyroxin-induced cardiac hypertrophy model was prepared as previously described[4]. Briefly, adult male Sprague–Dawley rats (200±20 g, Experimental Animal Center, Sun Yat-Sen University, China) were randomly divided into 2 groups. The hyperthyroidism group (HT) was injected with *L*-thyroxin (1 mg/kg, intra-peritoneal) for 10 d to produce hypertrophy. The normal-saline group (NS) was injected with normal saline (controls). The Doppler echocardiographic studies were performed at 10 d to assess the development of heart hypertrophy.

Preparation of cardiac myocytes Single rat ventricular myocytes were isolated from rats using a collagenase-based enzymatic digestion technique[10]. Briefly, the animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal). The hearts were quickly removed and perfused in a Langendorff mode. They were first perfused with Ca^{2+}-free Tyrode's solution composed of (in mmol/L) 136 NaOH, 5.4 KCl, 0.33 NaH_2PO_4, 1 $MgCl_2 \cdot 6H_2O$, 10 HEPES, and 10 glucose (pH 7.4) at 37 °C for 10 min, then perfused with Ca^{2+}-free Tyrode's solution containing collagenase (type II, 0.5 mg/mL) for 15 min. The left and right ventricular tissues were removed and myocytes were harvested. The isolated cells were stored in a Krebs–bicarbonate solution containing (in mmol/L) 50 K-glutamate, 20 KOH, 40 KCl, 20 taurine, 20 KH_2PO_4, 3 $MgCl_2 \cdot 6H_2O$, 10 HEPES, 10 glucose, 0.5 ethylene glycol tetraacetic acid (EGTA), and 1% bovine serum albumin (pH 7.4) at room temperature. This procedure yields 50%–70% of Ca^{2+}-tolerant, rod-shaped ventricular myocytes with clear striations. Cells were used within 10 h after isolation.

Line-scan imaging and Ca^{2+} spark analysis Myocytes were loaded with 4 µmol/L Fluo-3 AM (Molecular Probes, Eugene, OR, USA) for 30 min at room temperature. The cells were then placed on a Petri plate coated with poly-lysine and were washed for 10 min to allow the de-esterification of the indicator; quiescent myocytes with a typical rod-shaped form and clear cross-striations were used for experiments. Fluo-3 was excited at 488 nm and the Ca^{2+} fluorescent signal was acquired at 526 nm by confocal microscopy (FV500, Olympus, Tokyo, Japan).

The $[Ca^{2+}]_i$ in quiescent cells was reported as fluorescence intensity (FI). To control the background FI, all parameters of confocal microscopy were fixed when different samples were measured. The SR Ca^{2+} content was assessed by the rapid application of caffeine (20 mmol/L) in Ca^{2+}-free Tyrode's solution. The amplitude of the caffeine-induced Ca^{2+} transient could be an index of the SR Ca^{2+} load[11]. The caffeine-induced Ca^{2+} transient was derived from changes in FI (F) and normalized to basal fluorescence (F_0) and expressed as F/F_0.

The spontaneous Ca^{2+} sparks were captured in Ca^{2+}(1.5 mmol/L)-containing Tyrode's solution over the entire cell with the confocal microscope operating in x-t imaging mode. The amplitude of spontaneous Ca^{2+} sparks (F/F_0, where F_0 refers to the background of the Fluo-3/AM signal), duration (full-duration-half-maximum [FDHM]), width, spatial size (full-width-half-maximum [FWHM]), and Ca^{2+} spark frequency (CaSpF) were measured in a line-scan mode using a 60× water immersion objective by an algorithm coded in IDL 5.4[12] and self-developed program with Matlab 6.5 (Mathworks, Natick, MA, USA). All experiments were performed at room temperature.

Preparation of SR membrane The SR was prepared as previously described[13,14]. Briefly, the isolated ventricle was frozen and homogenated in ice-cold homogenizing medium containing (in mmol/L) 10 $NaHCO_3$ and 5 NaN_3, pH 7.0 using Polytron PT-20 (Brinkmann Instruments, Westbury, NY, USA). The homogenate was centrifuged at 14 000×*g* for 20 min at 4 °C. The pellet was resuspended in 5 volumes of ice-cold buffer and centrifuged as before. The supernatant from the second spin was sedimented at 45 000×*g* for 30 min and the pellet was resuspended in 25 mL of 0.6 mmol/L KCl

and 30 mmol/L histidine, pH 7.0, and centrifuged again. The pellet consisting of the SR was resuspended in the solution containing (in mmol/L) 30 histidine and 250 sucrose, pH 7.4, and was stored at –80 °C.

Preparation of sarcolemma from rat hearts Sarcolemma was prepared from rat ventricles as per the kit manual (Jiancheng, Nanjing, China). Briefly, the left and right ventricles were minced in 9 volumes of ice-cold (0–4 °C) homogenizing medium containing reagent I separately and filtrated by double-deck gauze. The filtrate was sedimented at 10 750×*g* for 20 min, and the pellet obtained was washed twice by reagent I. The pellet was then suspended with 10 mL reagent II and placed at 0 ºC. After 48 h, the sediment was centrifuged for 20 min at 10 750×*g* and then washed twice by reagent III and preserved in reagent IV at 0 °C; the activity of Ca^{2+}-ATPase was measured within 48 h.

Measurement of Ca^{2+}-ATPase activity The activity of Ca^{2+}-ATPase was determined as per the kit manual (Jiancheng, China) by measuring the inorganic phosphate liberated from ATP hydrolysis[14]. Briefly, Ca^{2+}-ATPase activity was assayed in a medium containing (in mmol/L) 50 histidine, 3 $MgCl_2$, 100 KCl, 5 sodium azide, 3 ATP, and 0.05 $CaCl_2$, pH 7.0. The cardiac SR membrane was added to the reaction mixture at a final concentration of 25 µg of protein/mL, pre-incubated for 10 min at 37 °C, and the reaction was initiated by the addition of ATP. The ATP hydrolysis that occurred in the absence of Ca^{2+} (1 mmol/L EGTA) was subtracted to determine the activity of Ca^{2+}-stimulated ATPase. Ouabain was added freshly to a final concentration of 1 mmol/L in the media, which remained unchanged throughout the incubation. Mitochondrial contamination was excluded by determining the activity of azide-sensitive ATPase[15].

Statistics All data were expressed as mean±SEM. The differences between the groups were analyzed by paired *t*-test or ANOVA, $P<0.05$ was considered significant.

Results

***L*-thyroxin injection created LVH** All of the rats were killed 10 d after the injection to examine the gross indexes of hypertrophy. Compared with normal controls, the ratios of heart weight to brain weight (HW/BW) and left ventricular weight to BW (LVW/BW) in the HT group were increased significantly by ≈20% and ≈33%, respectively, whereas the ratio of right ventricular weight to BW (RVW/BW) showed no significant difference between the control and HT rats (Table 1). Doppler echocardiography demonstrated that the HT group had increased interventricular septum end-diastolic thickness (IVSd) and interventricular septum end-systolic thickness (IVSs; $P<0.05$; Table 1), but left ventricle end-diastolic dimension (LVDd), left ventricle end-systolic dimension (LVDs), end-diastolic posterior wall thickness (PWd), and end-systolic posterior wall thickness (PWs) were normal and similar in the HT and NS groups. Left systolic ventricular function was assessed by ejection fraction (EF) and left ventricular fractional shortening (LVFS); both were not significantly different from the NS group ($P>0.05$; Table 1).The increased LVW/BW and preserved left ventricular function in the HT group suggested that the exposure to *L*-thyroxin produced compensated LVH.

Table 1. Echocardiographic analysis of *L*-thyroxin-injected rats. Values are mean±SEM. $n=6$ per group. $^bP<0.05$, $^cP<0.01$, compared with age-matched NS controls.

Parameters	NS	HT
IVSd (mm)	1.25±0.04	1.67±0.1^b
IVSs (mm)	2.19±0.02	2.73±0.12^c
LVDd (mm)	4.70±0.35	4.09±0.25
LVDs (mm)	2.04±0.27	1.81±0.21
PWd (mm)	1.65±0.13	1.81±0.15
PWs (mm)	2.66±0.21	2.75±0.19
EF (%)	60.5±1.7	58.6±1.66
LVFS (%)	57.3±3.2	55.1±6.2
HW/BW	0.45±0.02	0.54±0.02^b
LVW/BW	0.34±0.01	0.43±0.01^b
RVW/BW	0.11±0.01	0.11±0.01

SR Ca^{2+} content In the Ca^{2+}-free medium, the difference in the caffeine-induced Ca^{2+} transient reflects the change in the SR Ca^{2+} content. Figure 1A and 1B shows the representative recordings of the Ca^{2+} transient upon the application of 20 mmol/L caffeine in the left and right ventricular myocytes from the NS and HT groups, Figure 1C compares the amplitude of the caffeine-induced Ca^{2+} transient ($\Delta F/F_0$) between different groups. In the left ventricular myocytes, the mean values of $\Delta F/F_0$ in the HT group was significantly lower than in the NS (0.42±0.06, $n=21$ *vs* 0.65±0.08, $n=27$; $P<0.05$). However, the regional difference in the SR Ca^{2+} content was absent in right ventricular myocytes.

Spontaneous Ca^{2+} sparks Confocal microscopy was applied to directly quantify the Ca^{2+} spark (Figure 2A,2B). The Ca^{2+} spark is the basic Ca^{2+} release event from the SR, and it is a local, discrete elevation in myoplasmic $[Ca^{2+}]_i$ due to the opening of the RyR2[18]. Figure 2C–2F summarizes the characteristics of spontaneous Ca^{2+} sparks in the NS and HT groups. The CaSpF was higher in the HT animals than in the NS animals (Figure 2C; 6.86±0.74 *vs* 2.89±0.32 sparks/s*100

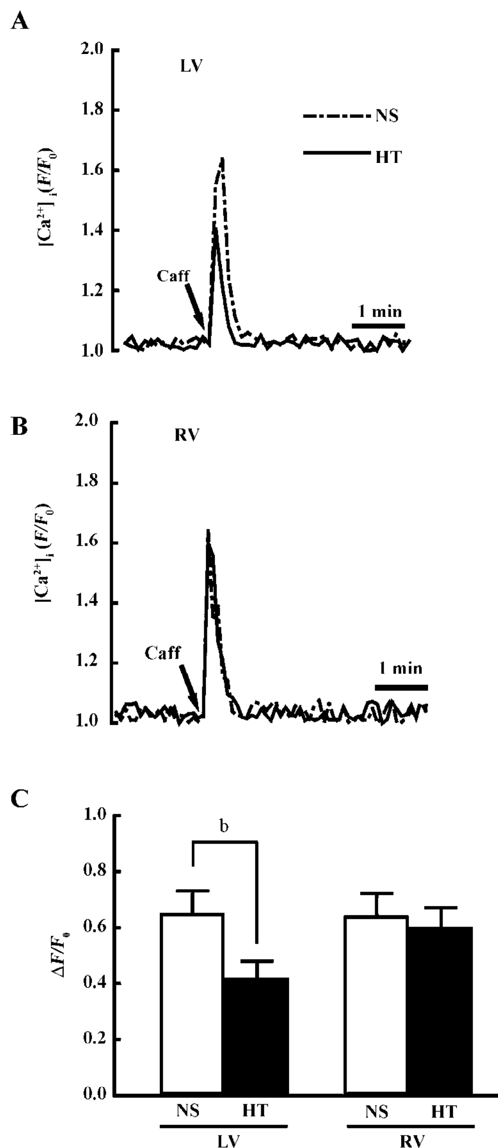

Figure 1. Caffeine-induced Ca^{2+} transient was decreased in LVH myocytes. Superimposed traces of $[Ca^{2+}]_i$ were recorded under Ca^{2+}-free and subsequent restoration of 1.5 mmol/L Ca^{2+} conditions in left (A) and right (B) ventricular myocytes. (C) mean $\Delta[Ca^{2+}]_i$ for caffeine-induced Ca^{2+} transients measured in left ventricular and right ventricular myocytes from the NS and HT groups. Left ventricle: 21 cells from 6 NS rats and 27 cells from HT rats. Right ventricle: 39 cells from 6 NS rats and 56 cells from HT rats; $^{b}P<0.05$ *vs* corresponding controls.

μm; $P<0.01$). The mean amplitude of the Ca^{2+} spark was lower in HT group than in NS group (Figure 2D; 2.84±0.28 *vs* 3.87±0.34, F/F_0; $P<0.01$), consistent with the lower SR Ca^{2+} content. The width and duration of the Ca^{2+} sparks were not significantly changed in the HT group compared with those in the NS group (Figure 2E,2F; FWHM: 1.4±0.15 *vs* 1.29±0.1 μm; FDHM: 25.7±2.14 *vs* 24.6±1.8 ms; $P>0.05$). The diastolic SR Ca^{2+} leak was found to be related to the product CaSpF×amplitude×FDHM×FWHM[16], which was 1.5 times higher in the HT group than the NS group.

Because of the decreased SR content and increased diastolic SR Ca^{2+} leak, we further compared the basal $[Ca^{2+}]_i$ between the NS and HT groups. The basal $[Ca^{2+}]_i$ was significantly elevated in quiescent left ventricular myocytes from the HT group compared with that from the control group (1432±153, n=38 *vs* 1143±144, n=32; $P<0.05$), whereas the basal $[Ca^{2+}]_i$ was unchanged in the right ventricle myocytes from the hypertrophied heart (1110±123, n=40 *vs* 1150±130, n=63; $P>0.05$).

Ca^{2+}-ATPase activity SERCA2a is the key Ca^{2+}-transport protein that re-uptakes Ca^{2+} into the SR during relaxation. The activity of SERCA2a in left ventricular myocytes from the HT group was significantly lower than in the NS group (4.34±0.44 *vs* 6.15±0.41 μmol·h^{-1}·mg^{-1} protein, n=6, $P<0.01$; Figure 3A), whereas there was no obvious change in the right ventricle in both groups (n=6, $P>0.05$). The activity of sarcolemmal Ca^{2+}-ATPase in the hypertrophied left ventricle decreased significantly compared with the NS controls (1.49±0.12 *vs* 3.09±0.18 μmol·h^{-1}·mg^{-1} protein, n=6, $P<0.01$; Figure 3B), whereas there was no obvious change in the right ventricle (n=6, $P>0.05$).

Discussion

In the present study, it was demonstrated that the ventricular myocytes from the *L*-thyroxin-induced hypertrophy model decreased the caffeine-induced Ca^{2+} transient in the Ca^{2+}-free solution. The smaller caffeine-induced Ca^{2+} transient could be explained by the lower SR Ca^{2+} content. We also observed the increased Ca^{2+} leak, reduced SERCA activity, and increased basal $[Ca^{2+}]_i$ in hypertrophied ventricular myocytes in hyperthyroidism rats, which may be involved in the possible mechanisms for the lower SR Ca^{2+} content in ventricular myocytes in this hypertrophy model.

The SR Ca^{2+} content in cardiac cells reflects the balance between Ca^{2+} release through RyR and Ca^{2+} uptake into the SR via SERCA2a. The basic Ca^{2+} release event was quantitatively detected and analyzed by a Ca^{2+} spark recording and analysis. Since the SR Ca^{2+} content mostly consists of the Ca^{2+} transient (approximately 92%) leading to the initiation of extracellular Ca^{2+} entry and subsequent myocardial contraction in adult hearts[17], the decrease in the amplitude of Ca^{2+} sparks indicates the lower SR Ca^{2+} content.

Previous studies have shown the frequency of Ca^{2+} sparks increases as the SR Ca^{2+} load elevates, and the smaller

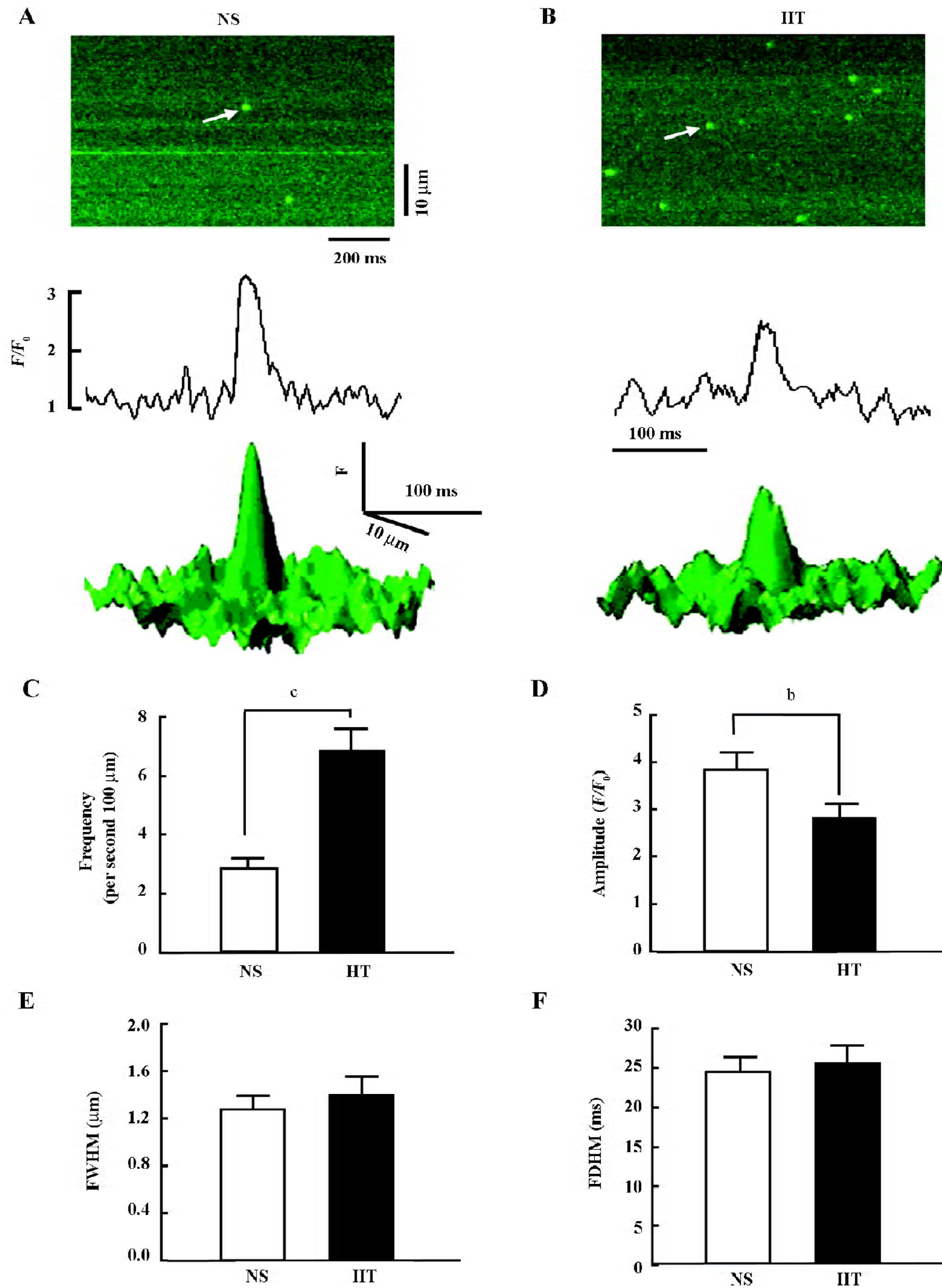

Figure 2. Spontaneous Ca^{2+} sparks in left ventricular myocytes from the NS (A) and HT (B) groups. Representative longitudinal line-scan images of Ca^{2+} sparks from NS and HT are shown at the top; line plots of $[Ca^{2+}]_i$ (measured as white arrows) are in the middle; 3-D surface plots of averaged signals from Ca^{2+} sparks (marked by white arrow) are in the lower panel. Characteristics of spontaneous Ca^{2+} spark in left ventricular myocytes from the HT and NS groups are shown in C,D,E,F. (C) Ca^{2+} sparks in resting LVH myocytes occur more frequently than in control myocytes. (D) Ca^{2+} spark amplitude in LVH myocytes was smaller than that recorded in control myocytes. Spatial width (E) and duration (F) showed no difference in both groups. Results are based on 190 sparks in 13 left ventricular myocytes from 7 NS rats, as well as 244 sparks in 7 left ventricular myocytes from 7 HT rats. $^bP<0.05$, $^cP<0.01$ *vs* corresponding controls.

SR Ca^{2+} content is accompanied by fewer Ca^{2+} spark rates[18,19]. However, we observed a higher Ca^{2+} spark frequency with a decrease in SR Ca^{2+} content in this hypertrophy model. The paradoxical observation was also found in a severe but com-

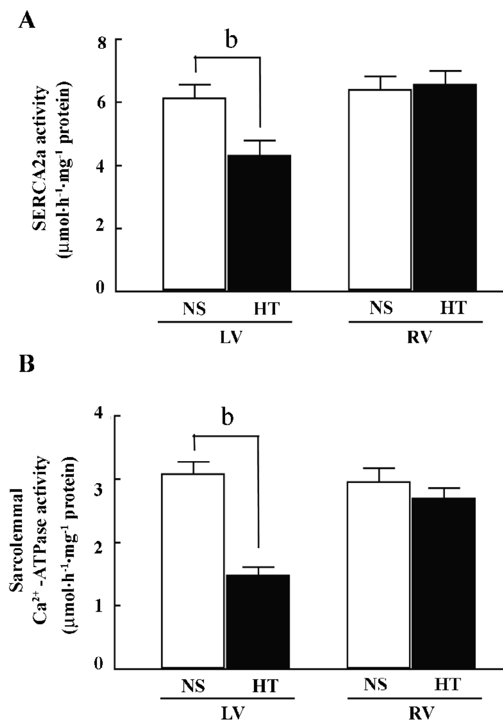

Figure 3. Activity of Ca^{2+}-ATPase in the SR and sarcolemma from NS (n=6) and HT (n=6). (A) SR; (B) sarcolemma. $^{b}P<0.05$ *vs* corresponding controls.

pensated canine LVH model, where the hyperphosphorylation of RyR2 was demonstrated to cause pathological hypersensitivity of RyR2 and release more Ca^{2+} in the diastolic period, leading to an increased occurrence of Ca^{2+} spark frequency[3]. In the same *L*-thyroxin-induced hypertrophy model, the increased expression of RyR2 has been reported in the hypertrophied heart tissue[4,7]. Therefore, more RyR2 in the SR and/or its hypersensitivity may be able to evoke more Ca^{2+} sparks. Another possibility for increased occurrences of spontaneous Ca^{2+} sparks may be due to the firing of Ca^{2+} release from some "Ca^{2+}-overloaded" subcellular regions in hypertrophied myocytes[3]. However, this possibility requires further examination in the *L*-thyroxin induced hypertrophy model.

The spontaneous Ca^{2+} leak occurs as the loss of Ca^{2+} from the SR under resting conditions, which also plays a role in the diastolic removal of Ca^{2+} from the SR Ca^{2+} content[20]. The enhanced SR Ca^{2+} leak was reported in the hypertrophy model induced by the Calcium/calmodulin-dependent protein kinase type II delta (CaMKIIδ) overexpression [16]. This model also points out that the enhanced expression of RyR2 with hypersensitivity may contribute to the increased SR Ca^{2+} leak. The increased Ca^{2+} leak and higher basal $[Ca^{2+}]_i$ that we observed in the present study may therefore explain the arrhythmogenesis in the hyperthyroid heart. In addition, the SR Ca^{2+} release channels are activated as $[Ca^{2+}]_i$ elevates. Therefore, the increased Ca^{2+} spark frequency during hypertrophy may also be secondary to an increase in $[Ca^{2+}]_i$.

The size of the SR Ca^{2+} content is dependent on the Ca^{2+} re-uptake through SERCA2a. The smaller SR Ca^{2+} content may be associated with reduced SERCA2a function. In the *L*-thyroxin-induced cardiac hypertrophy model, enhanced RyR2 and the SERCA2a mRNA level was observed and was associated with Ca^{2+} overload contributing to arrhythmogenesis during hypertrophy[4]. The expression of SERCA2a RNA and protein has been observed in hypertrophy models[4,21], but whether the activity of SERCA2a was altered had not previously been examined. In the present study, we observed a marked decrease in the activity of SERCA2a in the hypertrophied heart, which seemed to result in decreased SERCA2a function and may have contributed to the decreased SR Ca^{2+} content and increased basal $[Ca^{2+}]_i$. However, another possibility could not be excluded. The augmented expression level with the decreased activity of SERCA2a may not only cause more Ca^{2+} re-uptake back into Ca^{2+} store, but also cause oxygen wastage, which is consistent with increased oxygen consumption in the hyperthyroid heart.

In addition, there is controversy over whether there is change in the SERCA2a expression in the hyperthyroid heart. Takeuchi *et al* reported that there was no change in the SERCA2a protein expression in the hyperthyroid heart and proposed that the SERCA2a activity would be enhanced to lead to metabolic derangement[22], but they did not examine the SERCA2a activity in their study. It should be noted that the activity of ATPase (Na^+/K^+-ATPase and K^+/Ca^{2+}-ATPase) at the sarcolemma, SR, and mitochondria might be differently modified in the process of hypertrophy[23]. Because we facilitated the procedure to examine the activity of ATPase in subcelluar populations enriched in the SR and sarcolemma, respectively, the possible contamination from mitochondria was excluded. Although we did not further examine how SERCA2a function is altered and how myocardial contraction changes, the present study raises decreased SERCA2a activity as a potential mechanism for decreased SR Ca^{2+} content.

In the present study, we found that the elevated basal $[Ca^{2+}]_i$ could be due to the increased Ca^{2+} leak and reduced SERCA2a activity. In diastolic Ca^{2+} removal from the cytosol in the rat heart, the contribution of SERCA2a has been demonstrated to be predominant than that of the Na^+–Ca^{2+}

exchange[24]. However, it is noteworthy that acute exposure to the thyroid hormone stimulated the activity of reverse mode Na^+–Ca^{2+} exchange in cat atrial myocytes and increased $[Ca^{2+}]_i$, which was suggested to be involved in Ca^{2+}-mediated arrhythmic activity[25]. Because this mode of Ca^{2+} influx may also account for the increased basal $[Ca^{2+}]_i$ in the hyperthyroid ventricle[25], the role of Na^+–Ca^{2+} exchange in rat hypertrophied ventricular myocytes needs further investigation.

In summary, the results of our present study suggest that the increased Ca^{2+} leak and reduced SERCA2a activity may contribute to decreased SR Ca^{2+} content and increased basal $[Ca^{2+}]_i$ in ventricular myocytes in the *L*-thyroxin-induced hypertrophy model.

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