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Article

Activation of CaMKIIδA promotes Ca^{2+} leak from the sarcoplasmic reticulum in cardiomyocytes of chronic heart failure rats

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Abstract

Activation of the Ca^{2+}/calmodulin-dependent protein kinase II isoform δA (CaMKIIδA) disturbs intracellular Ca^{2+} homeostasis in cardiomyocytes during chronic heart failure (CHF). We hypothesized that upregulation of CaMKIIδA in cardiomyocytes might enhance Ca^{2+} leak from the sarcoplasmic reticulum (SR) via activation of phosphorylated ryanodine receptor type 2 (P-RyR2) and decrease Ca^{2+} uptake by inhibition of SR calcium ATPase 2a (SERCA2a). In this study, CHF was induced in rats by ligation of the left anterior descending coronary artery. We found that CHF caused an increase in the expression of CaMKIIδA and P-RyR2 in the left ventricle (LV). The role of CaMKIIδA in regulation of P-RyR2 was elucidated in cardiomyocytes isolated from neonatal rats *in vitro*. Hypoxia induced upregulation of CaMKIIδA and activation of P-RyR2 in the cardiomyocytes, which both were attenuated by knockdown of CaMKIIδA. Furthermore, we showed that knockdown of CaMKIIδA significantly decreased the Ca^{2+} leak from the SR elicited by hypoxia in the cardiomyocytes. In addition, CHF also induced a downregulation of SERCA2a in the LV of CHF rats. Knockdown of CaMKIIδA normalized hypoxia-induced downregulation of SERCA2a in cardiomyocytes *in vitro*. The results demonstrate that the inhibition of CaMKIIδA may improve cardiac function by preventing SR Ca^{2+} leak through downregulation of P-RyR2 and upregulation of SERCA2a expression in cardiomyocytes in CHF.

Keywords: chronic heart failure; cardiomyocyte; Ca^{2+}/calmodulin-dependent protein kinase II; ryanodine receptors; sarcoplasmic reticulum; sarcoplasmic reticulum calcium ATPase 2a; Ca^{2+} leak

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Introduction

A disturbance in intracellular Ca^{2+} homeostasis has been well known to contribute to the cardiac dysfunction observed in chronic heart failure (CHF)[1]. Ca^{2+}/calmodulin-dependent protein kinase IIδ (CaMKIIδ) is predominantly expressed in the heart[2] and is alternatively spliced to generate three subtypes[3]. These CaMKIIδ isoforms are regulated by calcium-ligand binding calmodulin (Ca^{2+}/CaM)[2]. The CaMKIIδA subtype has been found to be expressed in the heart[4]. The CaMKIIδB subtype is expressed in the heart and aorta, and the CaMKIIδC subtype is expressed in the brain, heart, aorta, liver and intestine under physiological conditions[3, 5]. Additionally, studies have indicated that the expression of CaMKIIδ subtypes is altered during cardiomyocyte differentiation and maturation and in association with the development of CHF[3].

CaMKIIδ regulates calcium homeostasis and cardiac function[6]. Expression of the phosphorylated ryanodine receptor type 2 (P-RyR2), the channel through which Ca^{2+} exits the SR, is upregulated in CaMKIIδC transgenic (TC) mice[7]. Hyperphosphorylation of RyR2 by CaMKIIδC is expected to enhance the leak of Ca^{2+} from the SR because acute inhibition of CaMKII in CaMKIIδC TC myocytes has been shown to decrease SR Ca^{2+} leak[8]. Interestingly, CaMKIIδA expression is also increased in isoproterenol-induced cardiac hypertrophy, and thus, regulation of CaMKIIδ splicing may be relevant to cardiac function[4]. Therefore, regulation of CaMKIIδ splicing may account for altered subtype expression and signaling in both physiological and pathophysiological settings.

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Accelerating diastolic Ca^{2+} release from the sarcoplasmic reticulum (SR) via phosphorylation of RyR2 provokes cardiac dysfunction in CHF[9-12]. Furthermore, CaMKII can also induce protein kinase A (PKA)-independent diastolic SR Ca^{2+} leak[13-15]. SERCA2a expression and activity are downregulated after myocardial ischemia in failing hearts[16, 17]. Decreased SERCA2a was shown to result in impaired SR Ca^{2+} reuptake, which combined with leaky RyR2 to deplete SR Ca^{2+} and lead to impaired cardiac contractility. Therefore, restoration of normal levels of SERCA2a has been targeted as a novel therapeutic for CHF[18, 19]. SERCA2a, similar to RyR2, is also regulated by CaMKII, which mediates calcium activity in the SR of cardiomyocytes[20]. The critical roles of CaMKIIδB and δC in CHF have been well investigated[21-23]. Recently, a study showed that CaMKIIδA, which selectively interferes with the histone deacetylase/myocyte enhancer factor-2 signaling pathway, is also critically involved in cardiac hypertrophy in both neonatal and adult models[24].

A better understanding of the molecular mechanisms underlying the effects of CaMKIIδA on the regulation of intracellular Ca^{2+} homeostasis might facilitate the identification of novel therapeutic targets to improve cardiac function in CHF. Therefore, the purpose of the present study was to determine the molecular signaling mechanisms by which CaMKIIδA activation disturbs cytoplasmic Ca^{2+} homeostasis in cardiomyocytes with CHF.

Materials and methods

Animals and cardiomyocyte isolation

Experiments were carried out on 30 male Sprague Dawley (SD) rats weighing 200 to 220 g. The present study was approved by the Nantong University Council on Animal Care and Use and complied with the Guide for the Care and Use of Laboratory Animals. Animals were anesthetized by chloral hydrate (50 mg/kg ip). Artificial ventilation was used with oxygen-enriched room air. Tidal volume was 2.0 to 3.0 mL. After the heart was exposed through a lateral thoracotomy, the left anterior descending (LAD) coronary artery was ligated as we described previously[25]. Acute myocardial infarction was confirmed by the observation that the anterior wall of the left ventricle became cyanotic and through an elevation in the ST segment of the electrocardiogram (ECG, lead II) waveform. Three days later, acute heart failure was confirmed by echocardiography (data not shown here). Rats were randomly assigned into two groups: (1) sham controls, which received a lateral thoracotomy but no ligation of LAD artery, and (2) the CHF group (after 4 weeks of LAD artery ligation). Ventricular cardiomyocytes from 1 to 2-day-old neonatal rat hearts were prepared by trypsin digestion as described previously[26]. After 24 h, these cardiomyocytes were treated with hypoxia and transfected with CaMKIIδA siRNA or scrambled RNA.

Hypoxic culture condition

Hypoxia is used to model cardiovascular diseases because of the insufficient oxygen supply associated with these diseases. Hypoxia is a hazardous consequence of cardiac ischemia, which triggers a wide range of cellular responses[27]. Therefore, in the present study, the culture of neonatal cardiomyocytes under hypoxic conditions was used to mimic the insufficient oxygen supply induced by cardiac ischemia. Hypoxic conditions were performed as previously described[28, 29]. Cells were incubated for 12 h with humidified gas containing 1% O_2, 5% CO_2 and 94% N_2 (tri-gas incubator, Thermo Scientific^{TM}). Control cells were grown in normal oxygen conditions for the same duration.

Intracellular Ca^{2+} imaging

Neonatal cardiomyocytes were plated in a 96-well plate. Cardiomyocytes were loaded with 1 μmol/L Fluo-4 (Invitrogen, USA) in extracellular buffer (in mmol/L, 140 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 Hepes) for 30 min at room temperature. Next, cells were washed with extracellular buffer and kept in this buffer until use. Fluo-4 was used to monitor cytosolic Ca^{2+} in cardiomyocytes. SR Ca^{2+} leak was measured by the tetracaine method as previously described[30, 31]. Briefly, Fluo-4-loaded cardiomyocytes were paced at 0.5 Hz in normal Tyrode's solution (NT, 140 mmol/L NaCl, 4 mmol/L KCl, 2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 10 mmol/L glucose, and 5 mmol/L HEPES, pH 7.4) for 20 s. The solution was then rapidly changed into 0 Na^+, 0 Ca^{2+} NT (NT with no added Ca^{2+}, 10 mmol/L EGTA, and 140 mmol/L LiCl substituted for NaCl, pH 7.4 with 4 mmol/L tetracaine). The tetracaine-induced intracellular Ca^{2+} drop was considered to be an index of SR Ca^{2+} leak[32, 33]. Fluo-4 fluorescence was excited at 488 nm, and data were expressed as normalized changes in background-corrected fluorescence emission (F/F_0). Data were acquired using Leica SP2 confocal software (Leica Microsystems, Germany).

Western blot analysis

Protein lysates were subjected to separation on a 10% SDS-PAGE gel, followed by electrotransfer to nitrocellulose membranes. Blots were individually probed with specific antibodies against RyR2 (phospho S2814) (A010-31, Badrilla), SERCA2a (A010-20, Labome), and GAPDH (G9545, Sigma). Signals were detected by the enhanced chemiluminescence detection method and quantified by densitometry using Quality One software (Bio-Rad).

Quantitative real-time PCR analysis

Total RNA was extracted from the isolated left ventricle (LV) heart tissue from both groups of rats (n=5 for each group) using a total RNA isolation kit (Qiagen, Hilden, Germany). cDNA was prepared with a Superscript kit (Invitrogen, Tokyo, Japan) according to the manufacturer's instructions. Quantitative real-time PCR was performed as we described[32]. The oligonucleotide primer sequences were as follows: CaMKIIδA (sense: 5′-CGAGAAATTTTTCAGCAGCC-3′ and antisense: 5′-ACAGTAGTTTGGGGCTCCAG-3′), CaMKIIδB (sense: 5′-CGAGAAATTTTTCAGCAGCC-3′ and antisense: 5′-GCTCTCAGTTGACTCCATCATC-3′), and CaMKIIδC (sense: 5′-CGAGAAATTTTTCAGCAGCC-3′ and anti-

sense: 5′-CTCAGTTGACTCCTTTACCCC-3′). GAPDH (sense: 5′-CCTCAAGATTGTCAGCAAT-3′ and antisense: 5′-CCATCCACAGTCTTCTGAGT-3′) was used as the internal standard[4]. Small interfering RNAs (siRNAs) targeting the CamkIIδA gene were synthesized by Biomics (Nantong, China), and the effect of the siRNA was identified by RT-PCR. The siRNA was then used in further experiments. The two siRNA sequences used were as follows: 5′-AGCCAACGUGGUAACCAGCTT-3′ and 5′-GCUGGUUACCACGUUGGCUTT-3′[24]. Scrambled RNA oligonucleotides were used as a control. Twenty-four hours prior to transfection, cardiomyocytes were plated onto a 6-well plate (Corning Inc, Corning, NY, USA). For each well, 50 nmol/L of the three oligos were transfected using LipoD293 (SignaGen) according to the manufacturer's instructions. The medium was replaced with DMEM containing 10% fetal bovine serum (FBS) after 6 h.

Ultrasound cardiograph

Echocardiograms were obtained from animals (chloral hydrate, 50 mg/kg, ip) with a Vevo770 system (Visual Sonics Inc, Toronto, Canada) equipped with a 17.5 MHz transducer by an experienced technician who was blinded to the treatment groups. In brief, the rats were anesthetized (chloral hydrate, 50 mg/kg, ip), shaved of chest fur, and placed supine on a special table. M-mode tracings were recorded at baseline. The left ventricular internal diameter at the diastolic phase (LVIDd), LV internal diameter at the systolic phase (LVIDs), posterior wall thickness at the diastolic phase (LVPW), and the ejection fraction (EF%) were measured by the Cardiac Measurements Package of Vevo 770 High-Resolution Imaging System.

Chemicals

Chemicals were purchased from Sigma-Aldrich, Inc (St Louis, MO, USA). Verapamil was dissolved in deionized water (dH_2O) to create a stock solution.

Statistical analysis

Data are presented as the mean±SEM. The results were analyzed using one-way ANOVA followed by Bonferroni *post hoc* test for multi-group comparisons. $P<0.05$ was considered statistically significant.

Results

The expression of CaMKIIδA in CHF and hypoxic cardiomyocytes

A total of 30 rats were randomly assigned to two groups of animals: 1) the sham-operated control rats (n=14); 2) rats with chronic heart failure (CHF, n=16). A representative echocardiography is shown in Figure 1. The ejection fraction (EF) was significantly decreased in the CHF group compared with that in the sham group ($P<0.05$) (Table 1). Left ventricular internal diameter at the diastolic phase (LVIDd) was increased, and the fractional shortening (FS) was decreased in the CHF rats compared to those in the sham group (Table 1). Left ventricular end diastolic dimension pressure (LVEDP) was significantly increased in the CHF groups compared with that in the sham group ($P<0.005$). The dp/dt was significantly decreased in the CHF group compared with that in the sham group ($P<0.05$). Taken together, the increased LVIDd and LVEDP

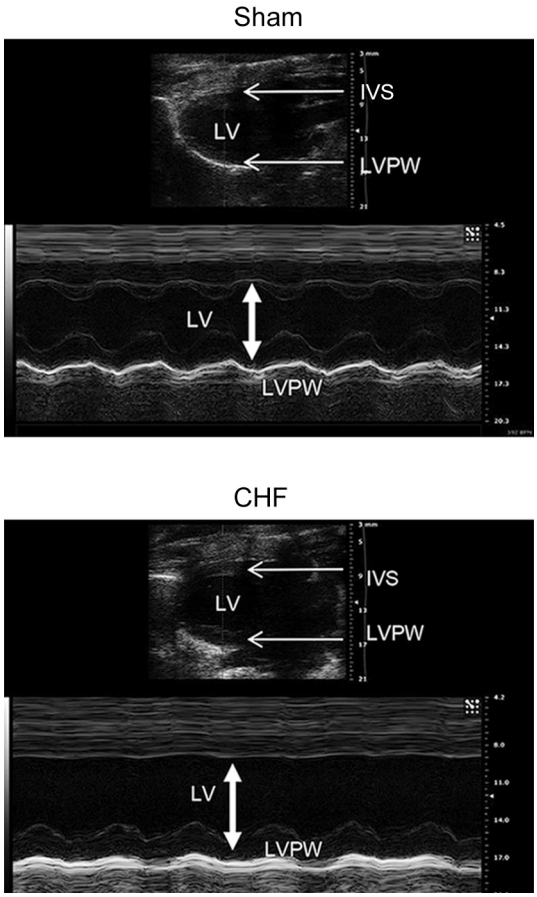

Figure 1. Echocardiographic changes associated with CHF. Echocardiography was performed 4 weeks after sham surgery (control) or ligation of the left anterior descending coronary artery. The representative M-mode echocardiograms were recorded in sham control (up) and CHF animals (bottom). CHF: chronic heart failure; LVPW: left ventricle posterior wall; LV: left ventricle; IVS: interventricular septal.

Table 1. Characteristic of cardiac left ventricular function.

	Sham (n=14)	CHF (n=16)
Ejection fraction (EF), %	83.42±1.91	26.03±1.34*
Fractional shortening (FS), %	53.16±2.55	12.85±1.58*
LVIDd, mm	5.21±0.30	7.07±0.32*
LVEDP, mmHg	5.3±0.8	18.9±1.7*
dp/dt, mmHgs^{-1}	7793±257.7	7042±156.4*

Values are means±SEM; n, number of animals. Echocardiography was performed after 4 weeks of sham surgery (control) or ligation of left anterior descending (LAD) coronary artery. Rats were randomized into the following groups: 1) Sham control; 2) CHF. CHF: chronic heart failure; LV: left ventricle; LVEDP: left ventricular end-diastolic pressure; LVIDd: left ventricular internal diameter at the diastolic. *$P<0.05$ *vs* Sham.

and decreased dp/dt, FS and EF were indicative of cardiac dysfunction in the CHF group. To investigate the alternative splicing of CaMKIIδ in CHF, RNA was extracted and subjected to RT-PCR for the measurement of CaMKIIδ in the left ventricle 4 weeks after LAD artery ligation. We observed CaMKIIδA at the transcription level in the left ventricle of CHF rats (Figure 2A) and in hypoxic neonatal cardiomyocytes (Figure 2B). CaMKIIδA expression was upregulated in the LV of CHF rats compared to that in the LV of the sham controls (P<0.05). CaMKIIδA expression was increased in hypoxic neonatal cardiomyocytes compared to that in controls (P<0.05). The efficiency of the CaMKIIδ siRNA was determined by RT-PCR analysis. The CaMKIIδA siRNAs significantly (P<0.05 *vs* control) decreased the CaMKIIδA mRNA level by ~65% (Figure 2C). CaMKIIδA siRNAs did not change the CaMKIIδB or CaMKIIδC mRNA level in control cells (Figure 2C) (P<0.05 *vs* control).

The expression of phosphorylated RyR2 (P-RyR2) in CHF and hypoxic cardiomyocytes

P-RyR2 protein levels were significantly (n=6, P<0.05) upregulated in the left ventricle of CHF rats compared to those in the sham controls (n=6) (Figure 3A). These data indicated that P-RyR2 was activated in rats with CHF. To determine the role of CaMKIIδA in mediating the expression of P-RyR2, CaMKIIδA siRNA was transfected into cultured neonatal cardiomyocytes treated with hypoxia. The expression of P-RyR2 was significantly higher (n=3 cultures, P<0.05) in hypoxic cardiomyocytes than in the control group. CaMKIIδA siRNA significantly (n=3 cultures, P<0.05) normalized the increase in the expression of P-RyR2 in the cardiomyocytes treated with hypoxia (Figure 3B). CaMKIIδA siRNA did not significantly change the expression of P-RyR2 in control cardiomyocytes (Figure 3B). These data indicated that activation of CaMKIIδA in cardiomyocytes in CHF contributed to the upregulation of P-RyR2 expression in the sarcoplasmic reticulum (SR).

Effect of CaMKIIδA siRNA on SR Ca^{2+} leak in cardiomyocytes with hypoxia

We then determined the role of CaMKIIδA in the regulation of the SR Ca^{2+} leak in cardiomyocytes. The present study found that the SR Ca^{2+} leak in cells with hypoxic treatment (n=12 cells in 3 cultures) was significantly greater than that in control cells (n=11 cells in 3 cultures) (P<0.05, Figure 4A, 4B). CaMKIIδA siRNA significantly (n=16 cells in 3 cultures) normalized the SR Ca^{2+} leak in cardiomyocytes (P<0.05, Figure 4A, 4B). CaMKIIδA siRNA did not significantly affect the SR Ca^{2+} leak in control cardiomyocytes (Figure 4A, 4B). These data indicated that upregulation of CaMKIIδA in cardiomyocytes may increase SR Ca^{2+} leak via upregulation of P-RyR2 expression, potentially contributing to the molecular mechanisms of cardiovascular function impairment in CHF.

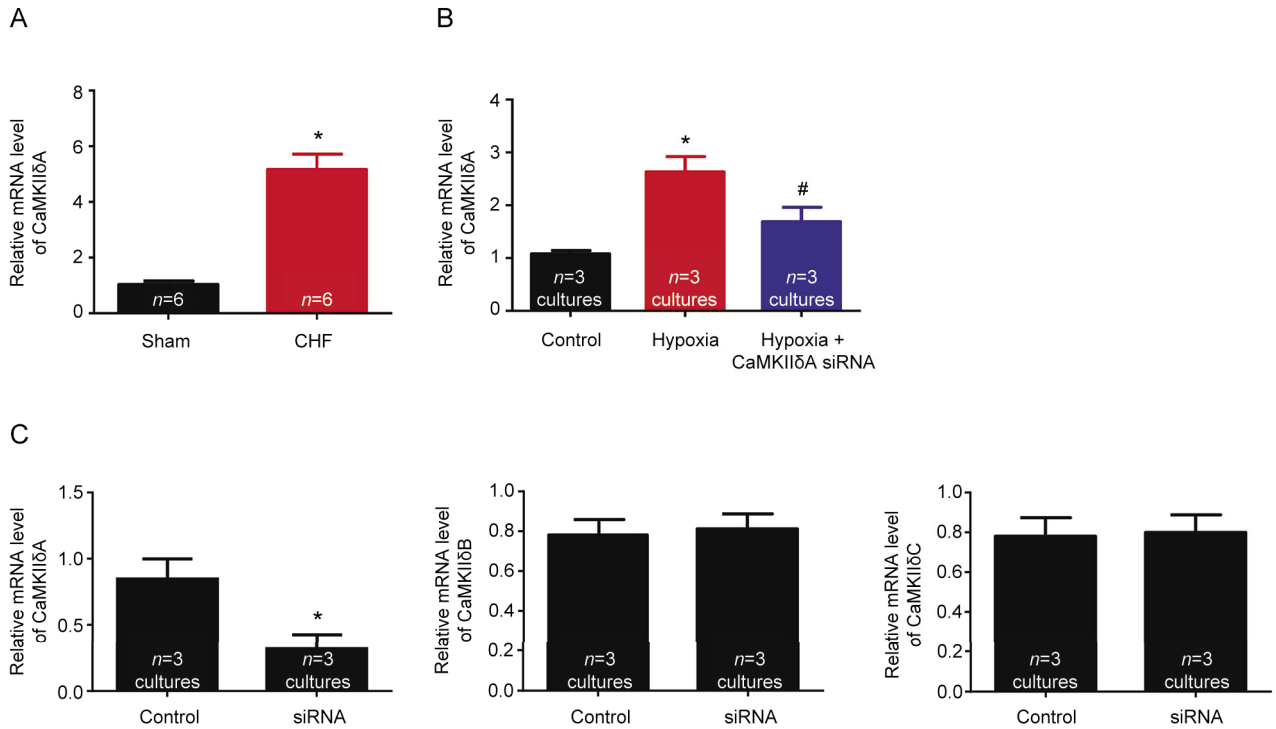

Figure 2. Expression of CaMKIIδA in CHF and hypoxic cardiomyocytes. CaMKIIδA mRNA was detected by using RT-PCR in the isolated LV of the heart and neonatal cardiomyocytes. CaMKIIδA mRNA expression was upregulated in CHF animals (A, $^{*}P$<0.05 *vs* the sham group) and cardiomyocytes with hypoxia (B). CaMKIIδA siRNA transfection significantly attenuated the increase in hypoxic cardiomyocytes ($^{*}P$<0.05 *vs* control; $^{\#}P$<0.05 *vs* the hypoxia group). CaMKIIδ siRNA efficiency was determined by RT-PCR analysis. CaMKIIδA siRNAs significantly decreased CaMKIIδA mRNA expression ($^{*}P$<0.05 *vs* control). CaMKIIδA siRNA did not change CaMKIIδB or CaMKIIδC mRNA expression in control cardiomyocytes (C).

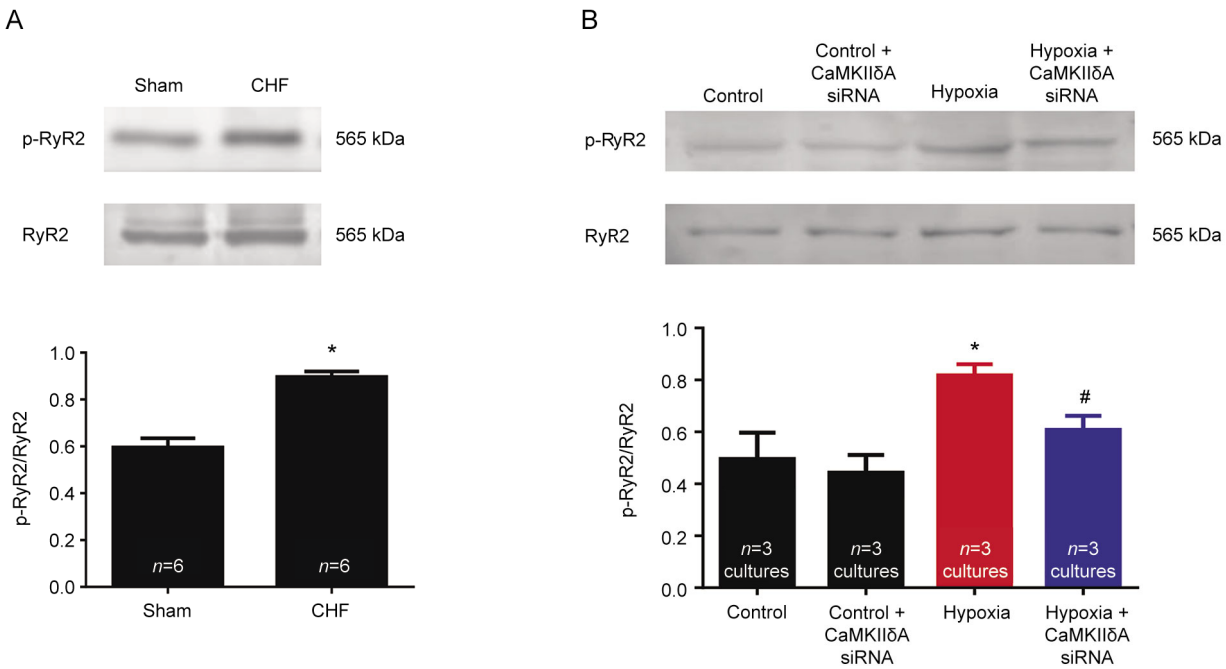

Figure 3. Effect of CaMKIIδA siRNA on the expression of phosphorylated ryanodine receptor type 2 (p-RyR2). P-RyR2 protein was detected in the LV of the heart *in vivo* and in neonatal cardiomyocytes *in vitro*. (A) p-RyR2 was upregulated in the LV of the heart in the CHF group. $^{*}P<0.05$ *vs* the sham group. (B) p-RyR2 protein expression was increased in hypoxic cardiomyocytes compared to that in control cardiomyocytes. CaMKIIδA siRNA transfection significantly reduced the increase in the expression of p-RyR2 in cardiomyocytes. CaMKIIδA siRNA transfection did not significantly change the expression of p-RyR2 in control cardiomyocytes. $^{*}P<0.05$ *vs* control cardiomyocytes; $^{\#}P<0.05$ *vs* cardiomyocytes with hypoxia treatment.

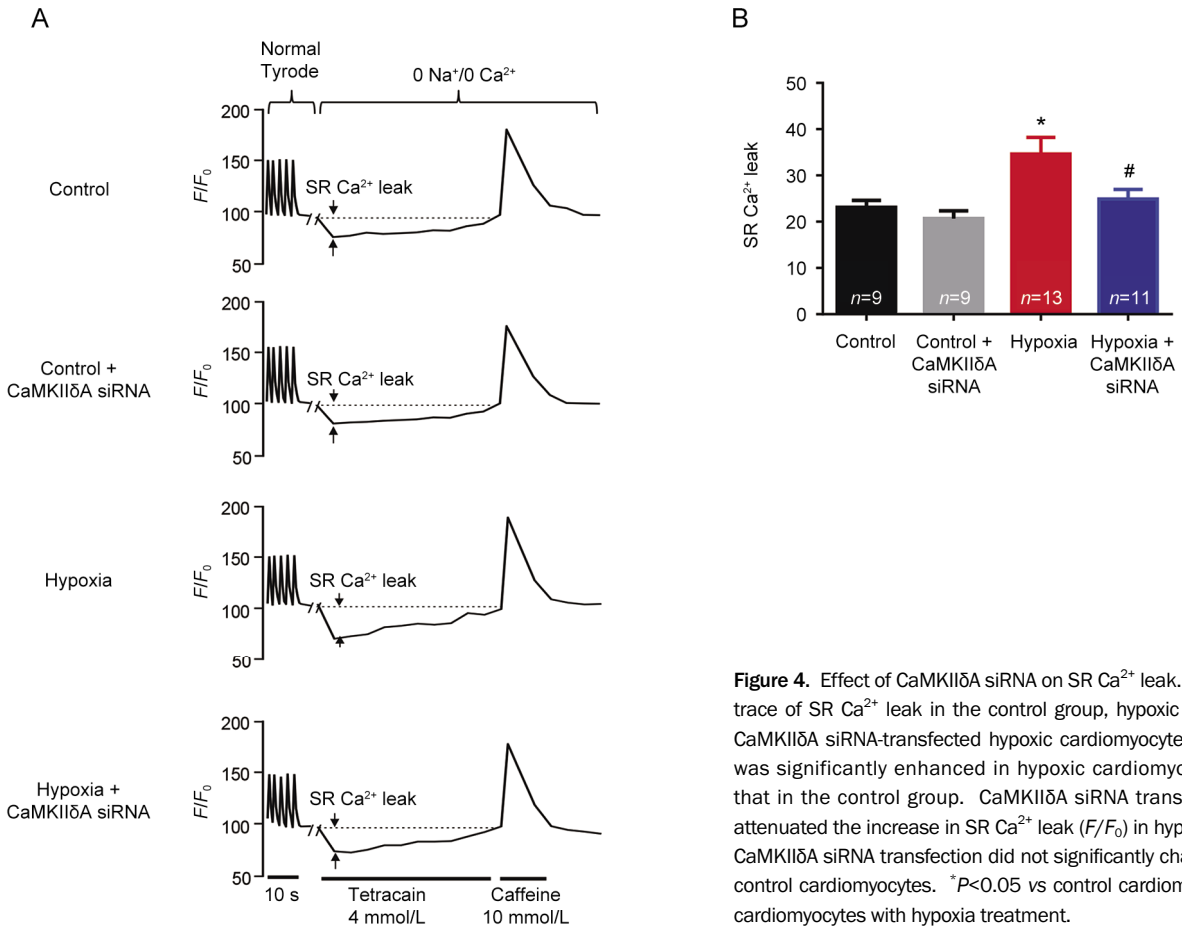

Figure 4. Effect of CaMKIIδA siRNA on SR Ca^{2+} leak. (A) A representative trace of SR Ca^{2+} leak in the control group, hypoxic cardiomyocytes and CaMKIIδA siRNA-transfected hypoxic cardiomyocytes. (B) SR Ca^{2+} leak was significantly enhanced in hypoxic cardiomyocytes compared to that in the control group. CaMKIIδA siRNA transfection significantly attenuated the increase in SR Ca^{2+} leak (F/F_0) in hypoxic cardiomyocytes. CaMKIIδA siRNA transfection did not significantly change SR Ca^{2+} leak in control cardiomyocytes. $^{*}P<0.05$ *vs* control cardiomyocytes; $^{\#}P<0.05$ *vs* cardiomyocytes with hypoxia treatment.

The expression of SERCA2a in CHF and hypoxic cardiomyocytes

SERCA2a protein levels were significantly (n=6, P<0.05) downregulated in the left ventricle of CHF rats compared to the levels in the left ventricle of the sham control animals (n=6) (Figure 5A). These data indicated that SERCA2a was inhibited in rats with CHF. To understand the role of CaMKIIδA in the regulation of SERCA2a in cardiomyocytes, we determined the effect of CaMKIIδA siRNA on SERCA2a expression and tested whether CaMKIIδA siRNA was able to normalize the alteration in SERCA2a expression in hypoxic cardiomyocytes. The present study found that SERCA2a protein levels were significantly decreased by hypoxia treatment in cardiomyocytes (n=3 cultures, P<0.05). This reduction was significantly attenuated in CaMKIIδA siRNA-transfected cells (n=3 cultures, P<0.05) (Figure 5B). CaMKIIδA siRNA did not significantly change the expression of SERCA2a in control cardiomyocytes (Figure 5B). These data indicated that CaMKIIδA upregulation in cardiomyocytes may decrease SR Ca^{2+} uptake via the downregulation of SERCA2a, potentially contributing to cardiac dysfunction in CHF.

Discussion

The major findings of this study were as follows: (1) the expression of CaMKIIδA and P-RyR2 was upregulated in the left ventricle of rats with CHF and cardiomyocytes with hypoxia. (2) Downregulation of CaMKIIδA attenuated the activation of P-RyR2 in hypoxic cardiomyocytes. (3) Downregulation of CaMKIIδA reduced SR calcium leak in hypoxic cardiomyocytes. (4) Downregulation of CaMKIIδA normalized the decrease in the expression of SERCA2a in hypoxic cardiomyocytes. These results suggest that inhibition of CaMKIIδA reduces the increase in SR Ca^{2+} leak elicited by activation of P-RYR2 and reduction in SERCA2a expression in cardiomyocytes with hypoxia.

Upregulation of CaMKII has been suggested to mediate ischemic myocardial injury and cardiac remodeling[34]. CaMKIIδ is predominantly expressed in the heart and is involved in the process of cardiac hypertrophy and remodeling after pressure overload[35]. In the present study, we found that CaMKIIδA expression was upregulated in the left ventricle (LV) of CHF animals, consistent with a previous report[36]. Moreover, the expression of CaMKIIδA was also found to be elevated in ventricular myocytes in rat hypertrophy[24]. Interestingly, CaMKIIδB was found to be downregulated in abdominal aorta constriction-induced hypertrophy in the rat heart[37], and overexpression of CaMKIIδC in transgenic mice has been reported to contribute to the development of cardiac hypertrophy and heart failure[38]. Therefore, alternative splicing of CaMKIIδ

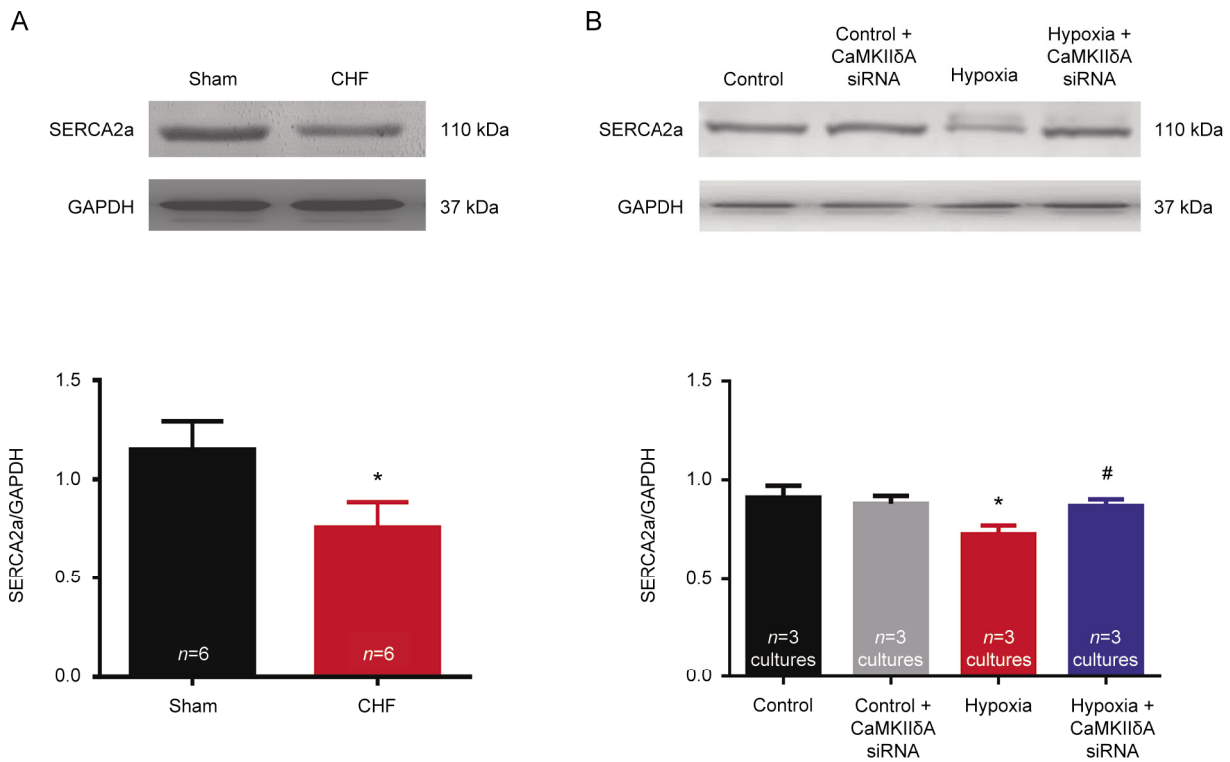

Figure 5. Effect of CaMKIIδA siRNA on SERCA2a expression and activity in cardiomyocytes. SERCA2a protein was detected in the LV of the heart *in vivo* and in neonatal cardiomyocytes *in vitro*. (A) SERCA2a protein was detected in the LV of the heart. SERCA2a protein expression was downregulated in the LV of the heart in the CHF group. $^{*}P$<0.05 *vs* the sham group. (B) SERCA2a protein was detected in neonatal cardiomyocytes. SERCA2a protein expression was significantly reduced in hypoxic cardiomyocytes compared to that in control cells. CaMKIIδA siRNA transfection significantly attenuated the downregulation in SERCA2a protein expression in hypoxic cardiomyocytes. CaMKIIδA siRNA transfection did not significantly change SERCA2a expression in control cardiomyocytes. $^{*}P$<0.05 *vs* control cardiomyocytes; $^{\#}P$<0.05 *vs* cardiomyocytes with hypoxia treatment.

may play an important role in the pathological process of myocardial ischemia.

RyR2 is one of the major downstream targets of CaMKIIδ in cardiac myocytes and plays an important role in regulating Ca^{2+} release from the SR[39]. An increased diastolic SR Ca^{2+} leak is regarded as an important pathological mechanism for the development of cardiac pump failure[40]. In the present study, the data showed that upregulation of P-RyR2 was not only found in the LV of CHF animals *in vivo* but also in the hypoxic cardiomyocytes *in vitro* (Figure 3). In addition, downregulation of CaMKIIδA decreased the Ca^{2+} leak from the SR in hypoxic cardiomyocytes (Figure 4). These findings are consistent with previous reports showing that inhibition of CaMKII was associated with decreased SR Ca^{2+} leak[41].

Furthermore, our study also revealed that the activation of P-RyR2 induced by hypoxia was effectively blocked by CaMKIIδA siRNA in cardiomyocytes *in vitro*. These results further suggest that activation of CaMKIIδA induces an increase in SR Ca^{2+} leak through activation of P-RYR2. The prevention of the increased expression of both CaMKIIδA and P-RyR2 in the cardiomyocytes may contribute to the protective effect on cardiac function.

Activation of RyR2 expressed in the SR of cardiomyocytes contributes to the Ca^{2+} leak and SERCA2a downregulation in heart failure[42, 43]. Because SERCA2a pumps Ca^{2+} back to the SR, luminal SR Ca^{2+} levels are increased and provide the electrochemical driving force for SR Ca^{2+} release[44]. Restoration of SERCA2a function might increase spontaneous Ca^{2+} release and the risk of RyR2-mediated diastolic leak[45]. In contrast, Lyon AR and colleagues reported that SERCA2a gene therapy can reduce the total SR Ca^{2+} leak despite the normalized SR Ca^{2+} load[46]. Because CaMKIIδA is activated in heart failure, the downregulation of SERCA2a activity in cardiomyocytes may be reasonably thought to be mediated through the upregulation of CaMKIIδA. This speculation was supported by the present study showing that CaMKIIδA siRNA dramatically attenuated the downregulation of SERCA2a expression in neonatal cardiomyocytes induced by hypoxia (Figure 5). Therefore, CaMKIIδA contributed to SR Ca^{2+} leak through the activation of RyR2 and inhibition of SERCA2a in cardiomyocytes with myocardial ischemia.

CaMKIIδ expression and activity have been demonstrated to be increased in CHF, potentially contributing to cardiac hypertrophy and further worsening CHF[47]. Whether activation of CaMKIIδA is involved in myocardial ischemia and cardiac remodeling through the activation of RyR2 and downregulation of SERCA2a is not clear. In the present study, elevation of P-RyR2 and decreased expression of SERCA2a induced by hypoxia was effectively attenuated by CaMKIIδA siRNA. Therefore, the therapeutic effect on CHF might involve the deactivation of P-RyR2 and upregulation of SERCA2a through the downregulation of CaMKIIδA.

Conclusion

In summary, our study elucidated the potential molecular mechanisms of activation of CaMKIIδA-mediated cardiac dysfunction in CHF (Figure 6). The data from the present study suggested that the inhibition of CaMKIIδA with siRNA may improve cardiac function by preventing SR Ca^{2+} leak through downregulation of RyR2 and upregulation of SERCA2a expression in cardiomyocytes in CHF. We expect that CaMKIIδA might be a potential target for the prevention and treatment of the cardiac dysfunction associated with CHF in the future.

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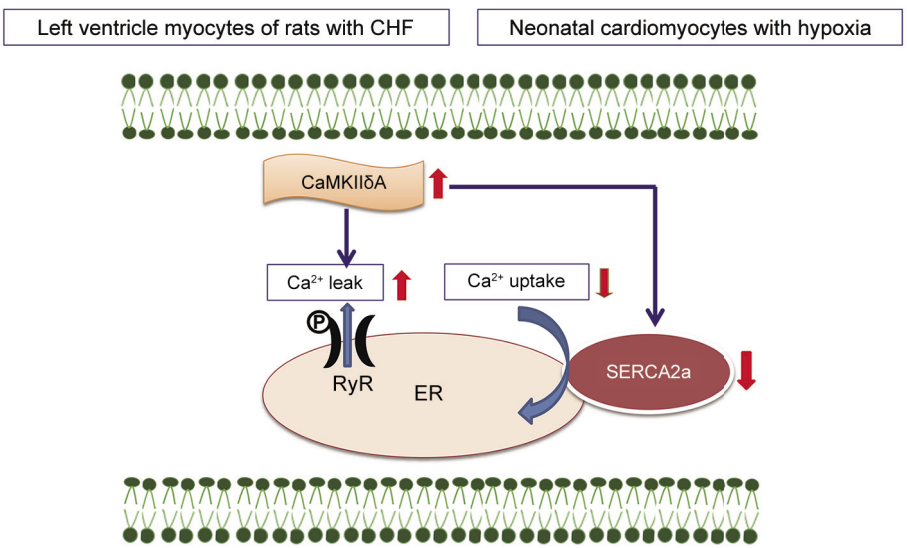

Figure 6. Schematic representation of our working hypothesis: upregulation of CaMKIIδA enhances the SR Ca^{2+} leak elicited by the activation of p-RyR2 and the reduction in SERCA2a expression in cardiomyocytes with CHF and hypoxia. CaMKIIδA, calmodulin-dependent kinase II isoform δA; CHF, chronic heart failure; RyR, ryanodine receptor; SERCA2a, Ca^{2+}-ATPase; SR, sarcoplasmic reticulum.

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Author contribution

Le GUI, Zhe ZHANG, Xin GUO, Hui XU, and Ya-wei JI performed the experiments; Le GUI, Xin GUO, and Ren-jun WANG analyzed the data; Le GUI, Xin GUO, and Ren-jun WANG prepared the figures; Le GUI, Ren-jun WANG, and Qing-hui CHEN drafted the manuscript; Le GUI, Ren-jun WANG, Jiang-hua ZHU, and Qing-hui CHEN interpreted the results of the experiments; Le GUI, Ren-jun WANG, Jiang-hua ZHU, and Qing-hui CHEN edited and revised the manuscript; Le GUI, Ren-jun WANG, Jiang-hua ZHU, and Qing-hui CHEN approved manuscript.

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