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Chronic CaMKII inhibition reverses cardiac function and cardiac reserve in HF mice

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ABSTRACT

Aims: The present study was to explore the impact of KN93 - a specific inhibitor of CaMKII - on cardiac function and cardiac reserve in HF mice.

Main methods: We have generated pressure-overload HF mice using modified transverse aortic constriction (TAC) method. For acute inhibition (AI) experiment, HF mice were randomly divided into HF group, HF + KN93 AI group and HF + KN92 AI group, using sham mice as control. Mice in HF + KN93 AI group and HF + KN92 AI group were injected with CaMKII inhibitor KN93 or its inactive analogue KN92 on post-TAC day 15, while mice in HF group and Sham group were treated with saline. For chronic inhibition (CI) experiment, mice were injected daily with KN93, KN92 or saline for one week. At baseline and after isoproterenol (Iso) injection, in vivo cardiac function was assessed by echocardiography and left ventricular pressure-volume catheter.

Key findings: Acute inhibition of CaMKII leads to decreased -dP/dtmin, increased EF, FS, longitudinal strain, longitudinal strain rate, ESPVR, dP/dtmax-EDV, PRSW, Tau and EDPVR, and unaltered reactivity to Iso in HF mice. Chronic inhibition results in increased EF, FS, longitudinal strain, longitudinal strain rate, ESPVR, dP/dtmax-EDV and PRSW, without alteration in -dP/dtmin, Tau and EDPVR. In addition, chronic inhibition reverses the effect of Iso on HF mice.

Significance: Although acute CaMKII inhibition can repair systolic function in HF mice, it also exacerbates the diastolic function, whereas chronic inhibition improves both systolic function and cardiac reserve to β-adrenergic stimulation without impairing diastolic function.

1. Introduction

Heart failure (HF) accounts for substantial morbidity, mortality and healthcare expenditure in the developing and developed countries [1]. Despite the advances in heart failure therapy, the prognosis of HF patients remains frustrating. Clearly, novel strategies for therapeutic intervention against HF are needed. Among the plethora of molecular changes that occur during HF development, alterations in protein kinase pathways often play crucial mediator roles since they link upstream pathologic stress signaling with downstream regulatory programs and thus affect both the structural and functional integrity of the heart muscle [2]. One of the notable and well-established protein kinases that are centrally involved in HF remodeling is Ca^{2+}/calmodulin-dependent kinase II (CaMKII).

CaMKII is a multifunctional serine/threonine protein kinase consisting of 8–12 subunits. This kinase has different isoforms in different tissues, including α, β, γ, and δ isoforms. Each isoform is encoded by a separate gene. In heart, the δ isoform is predominant, and the γ isoform is expressed at low levels [3–6]. CaMKIIδ has two splice variants, the cytosolic form CaMKIIδ_c and nuclear form CaMKIIδ_b. The CaMKII subunit is made up of three key domains – regulatory domain, catalytic domain and association domain. Under resting conditions, the catalytic activity is restrained by the binding of regulatory domain. With elevated intracellular Ca^{2+} concentration, Ca^{2+}/calmodulin (Ca^{2+}/CaM) binds to the Ca^{2+}/CaM binding site in regulatory domain, resulting in a conformational change which sets the catalytic domain free and activates CaMKII [7]. In addition, CaMKII activity can also be regulated by posttranslational modifications such as oxidation [8], nitrosylation [9] and glycosylation [10]. Upon activation, CaMKIIδ_c phoshorylates numerous proteins involved in cardiac Ca^{2+} cycling [11], such as L-type Ca^{2+} channel (LTCC), ryanodine receptor (RyR), sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (SERCA) and phospholamban (PLB).

Excessive CaMKII activation seems to be a general feature and the main cause of LTCC current remodeling and SR Ca^{2+} leak in failing

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ventricle [12–15]. Moreover, transgenic CaMKII over-expression causes HF in mice [16,17], suggesting that CaMKII plays a critical role in the pathophysiology of HF and can be an effective therapeutic target. Indeed, a plethora of studies demonstrate that inhibition of CaMKII protects against HF development. However, most of the data provided so far is derived from in vitro cell experiment, and there is little evidence about the effect of CaMKII inhibition on in vivo cardiac function in HF animals and patients. Furthermore, overwhelming majority of the existing CaMKII inhibitory studies actually investigated the preventive effect because CaMKII inhibition started before the onset of HF. Therefore, our study aims to explore the impact of CaMKII inhibition on cardiac function and cardiac reserve in HF mice after maladaptive remodeling has already set in.

2. Methods

2.1. Severe transverse aortic constriction

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Pressure-overload HF was induced by modified transverse aortic constriction (TAC) in mice [18], according to protocols approved by the institution's Animal Care and Use Committee. Briefly, male mice (C57BL6, 6 weeks old) were anesthetized with pentobarbital sodium (60 mg/kg, administered intraperitoneally). Adequate sedation was determined by a lack of toe-pinch reflex. The mice were then orally intubated with 20-gauge tubing and ventilated (Harvard Apparatus Rodent Ventilator) at 120 breaths per minute (0.1-ml tidal volume). A 3-mm left-sided thoracotomy was created at the second intercostal space. The transverse aortic arch was ligated (7–0 Prolene) between the innominate and left common carotid arteries with an overlying 28-gauge needle, and then the needle was removed, leaving a discrete region of stenosis. Successful banding was confirmed with visual confirmation of differential carotid pulsatility. The chest was closed and the left-sided pneumothorax was evacuated. The control mice underwent sham operations at equivalent time points with the same process of anesthesia and chest opening but no aortic banding.

2.2. Treatment of experimental animals

The study is composed of two experiments, acute inhibition (AI) and chronic inhibition (CI) of CaMKII. For AI experiment, HF mice were randomly divided into HF group, HF + KN93 AI group and HF + KN92 AI group, using sham mice as control. Mice in HF + KN93 AI group and HF + KN92 AI group were intraperitoneally injected with 20 μmol/kg CaMKII inhibitor KN93 or its inactive analogue KN92 on post-TAC day 15, while mice in HF group and Sham group were treated with isovolumetric saline at the same time. For CI experiment, mice in HF + KN93 CI group and HF + KN92 CI group were injected daily with 10umol/kg KN93 or KN92 from post-TAC day 15 for one week, while mice in HF group and Sham group were treated with isovolumetric saline. At baseline and 10 min after isoproterenol (Iso, 0.5 mg/kg) injection, in vivo cardiac function were assessed by echocardiography and left ventricular pressure-volume (PV) catheter.

2.3. Echocardiography

Transthoracic echocardiography was performed in semiconscious mice (pentobarbital sodium, 30 mg/kg, intraperitoneally) using a high resolution murine echo machine, Visualsonics Vevo 2100 with a 30-MHz mechanical probe [19]. Before collecting data for analysis, all mice are trained twice a day by the operator with a 2 min handling practice and echocardiography performance for 3 consecutive days to reduce mouse stress during data acquisition. A short-axis two-dimensional image-guided M-mode view of the left ventricle (LV) is acquired at the level of papillary muscles, and the following parameters are measured digitally from the M-mode tracings, including LV internal dimensions at end-diastole and end-systole (LVIDd, LVIDs), anterior and posterior wall thickness at end-diastole and end-systole, and heart rate (HR). Based on these measurements, LV volume at end-systole (LV Vols) is calculated as $[(7.0 / (2.4 + \text{LVIDs})]^* \text{LVIDs}^3$; LV volume at end-diastole (LV Vold) is calculated as $[(7.0 / (2.4 + \text{LVDd})]^* \text{LVDd}^3$; fractional shortening (FS) is calculated as [(LVIDd–LVIDs)/LVIDd] × 100%; ejection fraction (EF) is calculated as [(LV Vold–LV VolDs) / LV Vold] × 100%. All measurements will be made as the average from 5 adjacent cardiac cycles. Two-dimensional images from the parasternal long axis also were recorded for measurement of longitudinal strain and longitudinal strain rate.

2.4. Pressure-volume loop analysis

Cardiac function was also assessed by the PV catheter (SPR-839; Millar Instruments Inc., Houston, Tex) [20,21]. Mice were anesthetized and ventilated as 2.1. The PV catheter was inserted via the LV apex and advanced until the proximal electrode on the catheter is just inside the ventricular wall. PV loops were recorded at baseline and during the inferior vena cava occlusions. After catheter calibration, we acquired indices of systolic and diastolic function, including EF, peak rate of pressure rise-end diastolic volume relation (dP/dtmax-EDV), end-systolic pressure-volume relation (ESPVR), stroke work-end diastolic volume relation (preload recruited stroke work, PRSW), peak rate of pressure decline (-dP/dtmin), relaxation time constant (Tau) and end-diastolic pressure-volume relation (EDPVR).

2.5. Western blot

Total protein of ventricle samples were obtained using RIPA lysis buffer (Biyotime) containing protease inhibitors and phosphatase inhibitors. Then, proteins were size-fractionated by SDS–PAGE and transferred to nitrocellulose membranes (Millipore, MA, USA). Target proteins were probed with anti-CaMKII (1:500 v/v, Santa Cruz), anti-phospho-CaMKII (1:500 v/v, Santa Cruz) and anti-GAPDH (1:1000 v/v, Proteintech) primary antibodies, and horseradish peroxidase-conjugated secondary antibody (1:5000 v/v, Proteintech). Blots were developed using ECL reagent (Advansta, CA, USA). Then, the relative protein levels of CaMKII and phospho-CaMKII were quantified with Image-Pro Plus software and normalized to GAPDH.

2.6. Statistical analysis

Continuous variables are presented as mean ± SD, and the normality of the data was checked by Shapiro-Wilk test. Student-*t*-test was performed in paired analyses, and one-way ANOVA was performed between groups. All P values < 0.05 were considered statistically significant.

3. Results

3.1. Generation of pressure-overload HF mouse model

Pressure-overload HF was induced by TAC. About 50% mice died within the first week after surgery, with remaining mice exhibiting signs of a stably developed HF 2 weeks post-TAC. As we reported [14], these HF mice manifested clinical features of HF, including lethargy, impaired mobility, diminished appetite and edema with significant increase in heart weight to body weight ratio, lung weight to body weight ratio, LV end-diastolic volume and reduction in EF (Fig. 1 and Table 1).

3.2. Impact of acute CaMKII inhibition on heart function at baseline

Compared with sham mice, HF mice show decreased EF [(40.44 ± 3.67) vs (80.34 ± 4.32) %, $P < 0.05$], FS[(19.15 ± 2.01)

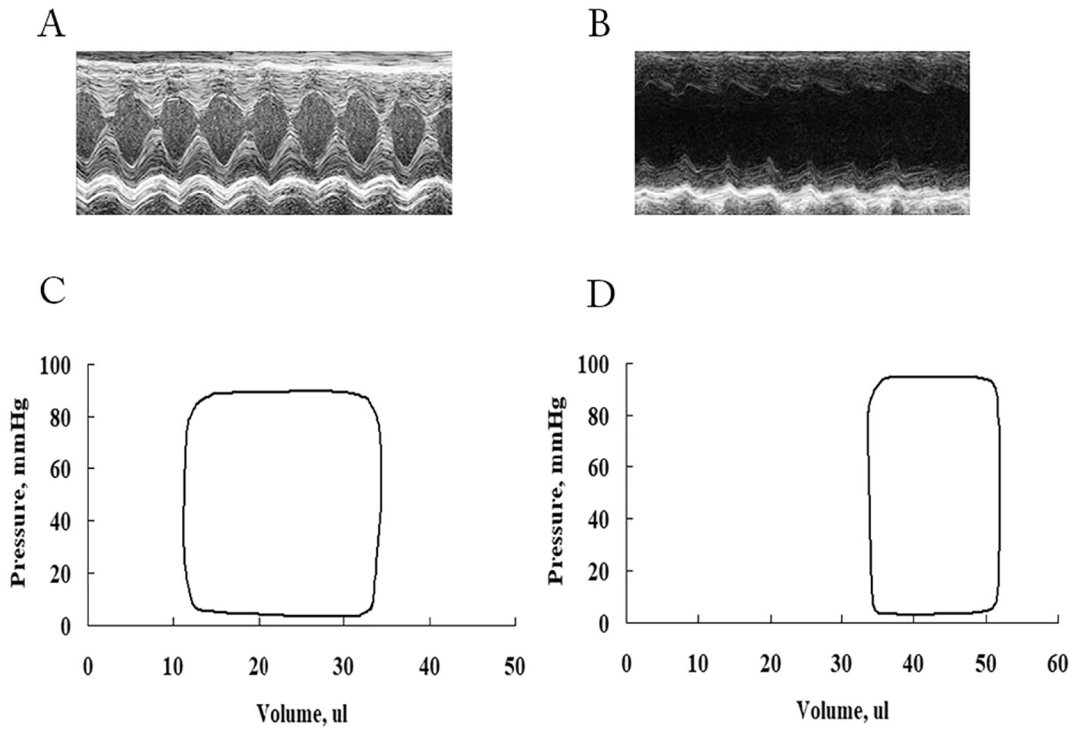

Fig. 1. Generation of pressure-overload HF mouse model. (A) Representative M-mode echocardiograph of sham mice. (B) Representative M-mode echocardiograph of HF mice. (C) Representative PV-loop of sham mice. (D) Representative PV-loop of HF mice.

Table 1
Baseline echocardiographic parameters and HW/BW ratio in sham and HF mice.

Parameter	Sham (n = 12)	HF (n = 10)	P Value
HW/BW (mg/g)	4.71 ± 0.23	10.19 ± 0.81	<0.01
LW/BW (mg/g)	5.69 ± 0.43	14.43 ± 1.35	<0.01
LV Vold (μl)	29.75 ± 3.32	46.01 ± 4.50	<0.01
EF (%)	80.77 ± 4.14	39.53 ± 3.17	<0.01

Values are means ± SD. HW = heart weight; BW = body weight; LW = lung weight; LV Vold = diastolic left ventricular volume; EF = ejection fraction.

vs (47.57 ± 4.84) %, $P < 0.05$], longitudinal strain[(14.27 ± 1.52) vs (21.98 ± 2.49) %, $P < 0.05$] and longitudinal strain rate [(5.30 ± 0.88) vs (9.84 ± 1.32) s^{-1}, $P < 0.05$]. Acute inhibition of CaMKII with KN93 increased EF[(50.45 ± 5.47) vs (40.44 ± 3.67) %, $P < 0.05$], FS[(24.93 ± 3.31) vs (19.15 ± 2.01) %, $P < 0.05$], longitudinal strain[(16.91 ± 1.59) vs (14.27 ± 1.52) %, $P < 0.05$] and longitudinal strain rate[(7.36 ± 1.34) vs (5.30 ± 0.88) s^{-1}, $P < 0.05$] in HF mice, while KN92 failed to improve the cardiac function (Fig. 2 and Table 2).

To further demonstrate the cardiac effect of acute CaMKII inhibition, we measured heart function by the PV catheter. Compared with sham mice, the EF[(36.20 ± 5.88) vs (70.76 ± 5.25) %, $P < 0.05$], ESPVR[(6.29 ± 1.51) vs (9.37 ± 1.48) mmHg/μl, $P < 0.05$], dP/dtmax-EDV[(243.41 ± 25.92) vs (304.61 ± 31.39) mmHg/(s * ml), $P < 0.05$], PRSW[(48.34 ± 5.80) vs (72.09 ± 7.82) mmHg, $P < 0.05$] and -dP/dtmin[(5588.74 ± 610.57) vs (7369.33 ± 749.87) mmHg/s, $P < 0.05$] of HF mice are decreased, while Tau[(14.84 ± 1.69) vs (11.48 ± 1.20) ms, $P < 0.05$] and EDPVR[(0.30 ± 0.03) vs (0.20 ± 0.02) mmHg/μl, $P < 0.05$] increased. Acute inhibition of CaMKII with KN93 increased EF [(45.90 ± 5.26) vs (36.20 ± 5.88) %, $P < 0.05$], ESPVR [(7.82 ± 1.36) vs (6.29 ± 1.51) mmHg/μl, $P < 0.05$], dP/dtmax-EDV[(273.51 ± 27.59) vs (243.41 ± 25.92) mmHg / (s * ml), $P < 0.05$] and PRSW[(55.83 ± 6.05) vs (48.34 ± 5.80) mmHg, $P < 0.05$]. However, it can significantly decrease -dP/dtmin [(4585.82 ± 554.04) vs (5588.74 ± 610.57) mmHg/s, $P < 0.05$] and increase Tau[(16.91 ± 1.80) vs (14.84 ± 1.69) ms, $P < 0.05$] and EDPVR[(0.35 ± 0.04) vs (0.30 ± 0.03) mmHg/μl, $P < 0.05$] (Fig. 2 and Table 2). KN92 had no effect on both systolic and diastolic function. In conclusion, acute inhibition can improve systolic function of HF mice, but further deteriorate the diastolic function.

3.3. Impact of chronic CaMKII inhibition on heart function at baseline

Chronic inhibition of CaMKII with KN93 significantly decreased HW/BW ratio (from 10.24 ± 0.79 to 8.10 ± 0.56 mg/g, $P < 0.05$), LW/BW ratio (from 14.54 ± 1.32 to 11.42 ± 1.32 mg/g, $P < 0.05$), and LV end-diastolic volume (from 46.28 ± 4.83 to 40.40 ± 3.21 μl, $P < 0.05$) in HF mice. As demonstrated by echocardiography, chronic inhibition of CaMKII with KN93 significantly improved EF [(51.03 ± 4.25) vs (40.72 ± 3.61) %, $P < 0.05$], FS[(25.21 ± 2.64) vs (19.27 ± 2.00) %, $P < 0.05$], longitudinal strain[(17.31 ± 2.08) vs (13.88 ± 1.78) %, $P < 0.05$] and longitudinal strain rate [(7.88 ± 1.51) vs (5.35 ± 1.11) s^{-1}, $P < 0.05$] in HF mice (Fig. 3 and Table 3). PV catheter showed similar results. Chronic inhibition increased EF[(47.80 ± 4.42) vs (36.45 ± 3.91) %, $P < 0.05$], ESPVR [(8.39 ± 1.65) vs (6.21 ± 1.52) mmHg/μl, $P < 0.05$], dP/dtmax-EDV[(280.26 ± 29.68) vs (239.06 ± 25.42) mmHg / (s * ml), $P < 0.05$] and PRSW[(61.52 ± 6.85) vs (45.73 ± 5.23) mmHg, $P < 0.05$], while had no obvious influence on -dP/dtmin [(6379.82 ± 653.06) vs (5810.31 ± 571.50) mmHg/s, $P > 0.05$], Tau[(14.98 ± 1.45) vs (15.42 ± 1.64) ms, $P > 0.05$] and EDPVR [(0.29 ± 0.03) vs (0.31 ± 0.03) mmHg/μl, $P > 0.05$] (Fig. 3 and Table 3). Therefore, chronic inhibition can significantly improve systolic function of HF mice, without worsening the diastolic function.

3.4. Impact of acute CaMKII inhibition on heart function after Iso injection

After measurements at baseline, all mice were intraperitoneally injected with Iso at a dosage of 0.5 mg/kg. Heart function will be

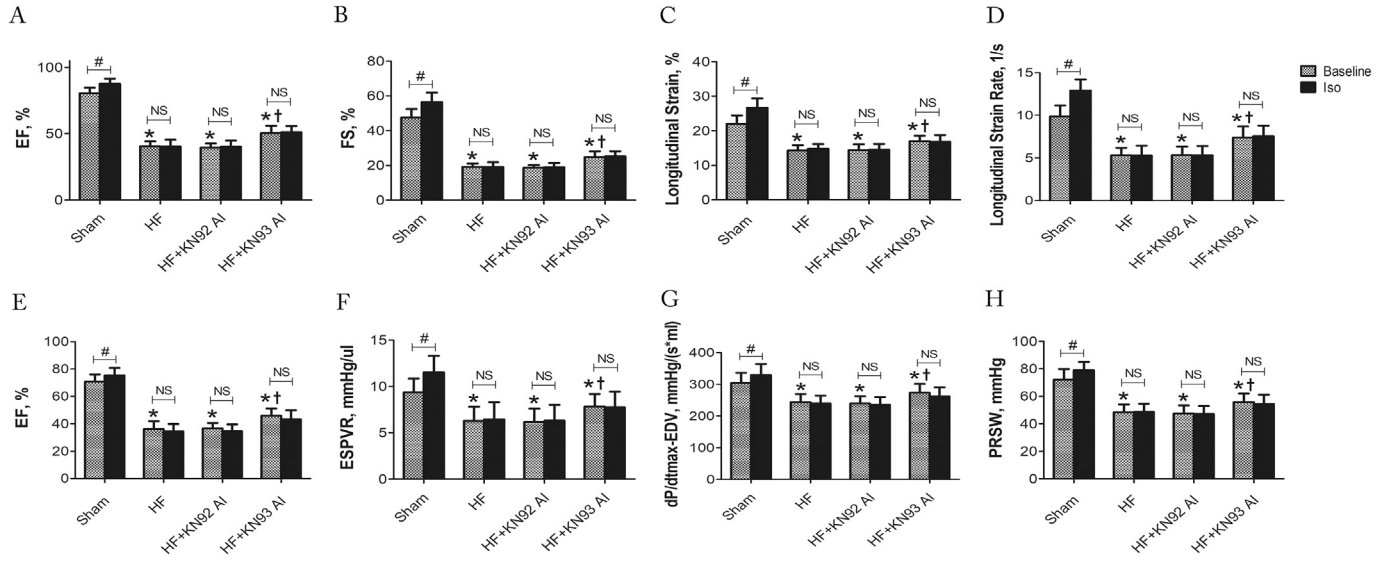

Fig. 2. Impact of acute CaMKII inhibition on heart function at baseline and after isoproterenol injection. EF, ejection fraction; FS, fractional shortening; ESPVR, end-systolic pressure-volume relation; dP/dtmax-EDV, peak rate of pressure rise-end diastolic volume relation; PRSW, preload recruited stroke work, stroke work-end diastolic volume relation. (A) assessed by echocardiography; (E) assessed by pressure-volume catheter. * $P < 0.05$, compared with Sham group; † $P < 0.05$, compared with HF group; $^{\#}$ $P < 0.05$, compared with baseline; NS, no significance compared with baseline. *P*-values denoted with * or † were derived from ANOVA, while *P*-values denoted with $^{\#}$ from *t*-test.

Table 2
Echocardiographic and PV parameters in acute CaMKII inhibition.

Parameter	Sham (n = 8)		HF (n = 8)		HF + KN92 AI (n = 8)		HF + KN93 AI (n = 8)	
	Baseline	Iso	Baseline	Iso	Baseline	Iso	Baseline	Iso
HR	561 ± 12	580 ± 12$^{\#}$	552 ± 11	554 ± 12	549 ± 14	553 ± 14	554 ± 14	553 ± 12
EF[a], %	80.34 ± 4.32	87.76 ± 3.85$^{\#}$	40.44 ± 3.67*	40.39 ± 5.01	39.47 ± 3.11*	40.11 ± 4.72	50.45 ± 5.47*†	51.05 ± 4.75
FS, %	47.57 ± 4.84	56.44 ± 5.47$^{\#}$	19.15 ± 2.01*	19.14 ± 2.75	18.63 ± 1.66*	18.99 ± 2.54	24.93 ± 3.31*†	25.26 ± 2.97
Longitudinal strain, %	21.98 ± 2.49	26.67 ± 2.71$^{\#}$	14.27 ± 1.52*	14.78 ± 1.37	14.38 ± 1.69*	14.51 ± 1.68	16.91 ± 1.59*†	16.77 ± 1.96
Longitudinal strain rate, s^{-1}	9.84 ± 1.32	12.92 ± 1.29$^{\#}$	5.30 ± 0.88*	5.28 ± 1.14	5.33 ± 0.99*	5.31 ± 1.08	7.36 ± 1.34*†	7.54 ± 1.24
EF[b], %	70.76 ± 5.25	75.35 ± 5.45$^{\#}$	36.20 ± 5.88*	34.41 ± 5.46	36.79 ± 3.89*	34.57 ± 5.03	45.90 ± 5.26*†	43.27 ± 6.57
ESPVR, mmHg/μl	9.37 ± 1.48	11.52 ± 1.78$^{\#}$	6.29 ± 1.51*	6.46 ± 1.84	6.15 ± 1.44*	6.31 ± 1.69	7.82 ± 1.36*†	7.74 ± 1.69
dP/dtmax-EDV, mmHg / (s ∗ ml)	304.61 ± 31.39	329.47 ± 34.83$^{\#}$	243.41 ± 25.92*	239.83 ± 24.07	239.56 ± 23.01*	235.05 ± 24.20	273.51 ± 27.59*†	261.69 ± 28.76
PRSW, mmHg	72.09 ± 7.82	78.96 ± 6.06$^{\#}$	48.34 ± 5.80*	48.66 ± 5.96	47.56 ± 5.82*	47.15 ± 5.82	55.83 ± 6.05*†	54.42 ± 6.74
-dP/dtmin, mmHg/s	7369.33 ± 749.87		5588.74 ± 610.57*		5417.09 ± 627.45*		4585.82 ± 554.04*†	
Tau, ms	11.48 ± 1.20		14.84 ± 1.69*		14.70 ± 1.46*		16.91 ± 1.80*†	
EDPVR, mmHg/μl	0.20 ± 0.02		0.30 ± 0.03*		0.29 ± 0.02*		0.35 ± 0.04*†	

Values are means ± SD. EF = ejection fraction; FS = fractional shortening; dP/dtmax-EDV = peak rate of pressure rise-end diastolic volume relation; ESPVR = end-systolic pressure-volume relation; PRSW = preload recruited stroke work, stroke work-end diastolic volume relation; -dP/dtmin = peak rate of pressure decline; Tau = relaxation time constant; EDPVR = end-diastolic pressure-volume relation. a = assessed by echocardiography; b = assessed by pressure-volume catheter. * $P < 0.05$, compared with Sham group; † $P < 0.05$, compared with HF group; $^{\#}$ $P < 0.05$, compared with baseline. *P*-values denoted with * or † were derived from ANOVA, while *P*-values denoted with $^{\#}$ from *t*-test.

measured again 10 min after Iso injection, and the changes are defined as cardiac reserve. Sham mice showed positive response to β-adrenergic stimulation. After Iso injection, the systolic function was greatly enhanced, with increased EF[(87.76 ± 3.85) vs (80.34 ± 4.32) %, $P < 0.05$], FS[(56.44 ± 5.47) vs (47.57 ± 4.84) %, $P < 0.05$], longitudinal strain[(26.67 ± 2.71) vs (21.98 ± 2.49) %, $P < 0.05$], longitudinal strain rate[(12.92 ± 1.29) vs (9.84 ± 1.32) s^{-1}, $P < 0.05$], ESPVR[(11.52 ± 1.78) vs (9.37 ± 1.48) mmHg/μl, $P < 0.05$], dP/dtmax-EDV[(329.47 ± 34.83) vs (304.61 ± 31.39) mmHg / (s ∗ ml), $P < 0.05$] and PRSW[(78.96 ± 6.06) vs (72.09 ± 7.82) mmHg, $P < 0.05$]. As predicted, reduced cardiac responsiveness to β-adrenergic stimulation was observed in HF mice, while acute inhibition of CaMKII cannot improve the responsiveness. Iso injection had no significant effect on EF[(51.05 ± 4.75) vs (50.45 ± 5.47) %, $P > 0.05$], FS[(25.26 ± 2.97) vs (24.93 ± 3.31) %, $P > 0.05$], longitudinal strain[(16.77 ± 1.96) vs (16.91 ± 1.59) %, $P > 0.05$], longitudinal strain rate[(7.54 ± 1.24) vs (7.36 ± 1.34) s^{-1}, $P > 0.05$], ESPVR[(7.74 ± 1.69) vs (7.82 ± 1.36) mmHg/μl, $P > 0.05$], dP/dtmax-EDV [(261.69 ± 28.76) vs (273.51 ± 27.59) mmHg / (s ∗ ml), $P > 0.05$] and PRSW[(54.42 ± 6.74) vs (55.83 ± 6.05) mmHg, $P > 0.05$] in HF + KN93 AI mice (Fig. 2 and Table 2).

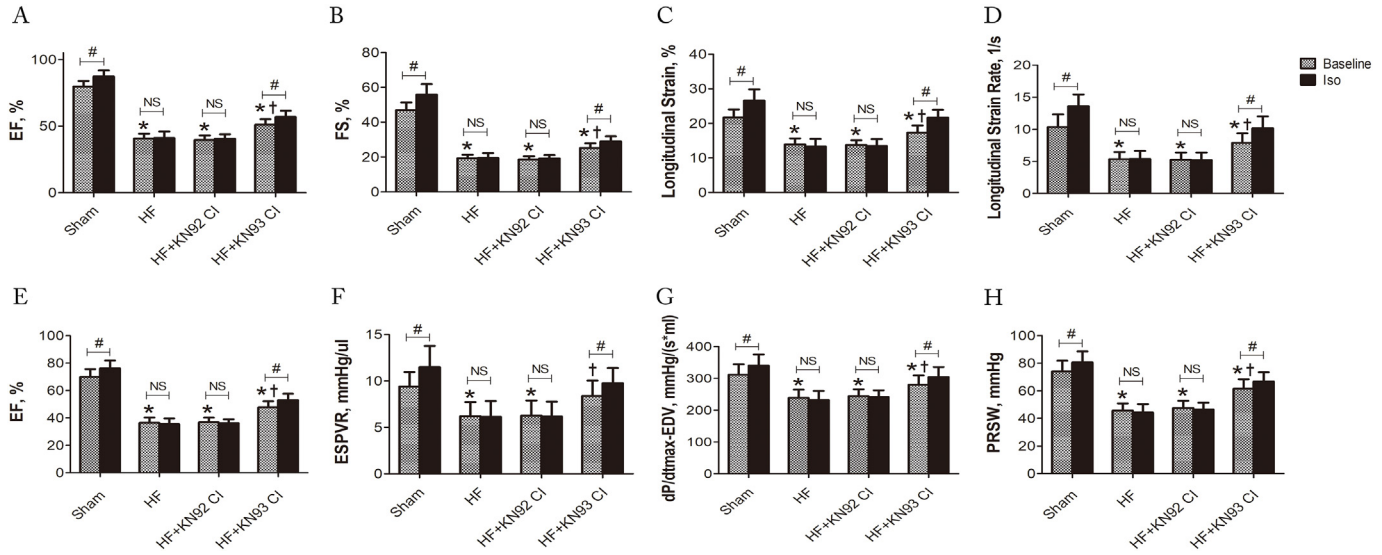

Fig. 3. Impact of chronic CaMKII inhibition on heart function at baseline and after isoproterenol injection. EF, ejection fraction; FS, fractional shortening; ESPVR, end-systolic pressure-volume relation; dP/dtmax-EDV, peak rate of pressure rise-end diastolic volume relation; PRSW, preload recruited stroke work, stroke work-end diastolic volume relation. (A) assessed by echocardiography; (E) assessed by pressure-volume catheter. * $P < 0.05$, compared with Sham group; † $P < 0.05$, compared with HF group; $^{\#}$ $P < 0.05$, compared with baseline; NS, no significance compared with baseline. *P*-values denoted with * or † were derived from ANOVA, while *P*-values denoted with $^{\#}$ from *t*-test.

3.5. Impact of chronic CaMKII inhibition on heart function after Iso injection

As reported above, sham mice showed positive response to β-adrenergic stimulation, while HF reduced this cardiac responsiveness. Nevertheless, chronic CaMKII inhibition with KN93 improved the response of HF mice to Iso, with EF[(56.81 ± 4.70) vs (51.03 ± 4.25) %, $P < 0.05$], FS[(28.87 ± 3.10) vs (25.21 ± 2.64) %, $P < 0.05$], longitudinal strain[(21.64 ± 2.27) vs (17.31 ± 2.08) %, $P < 0.05$], longitudinal strain rate[(10.18 ± 1.82) vs (7.88 ± 1.51) s^{-1}, $P < 0.05$], ESPVR[(9.74 ± 1.66) vs (8.39 ± 1.65) mmHg/μl, $P < 0.05$], dP/dtmax-EDV[(304.85 ± 31.22) vs (280.26 ± 29.68) mmHg / (s ∗ ml), $P < 0.05$] and PRSW[(66.73 ± 6.74) vs (61.52 ± 6.85) mmHg, $P < 0.05$] increasing (Fig. 3 and Table 3).

3.6. Expression of total CaMKII and phosphorylated CaMKII

To verify the efficacy of acute and chronic KN93 administration, we have assessed total CaMKII and phosphorylated CaMKII in sham mice, HF mice, HF + KN93 AI mice and HF + KN93 CI mice by Western blot analysis. As shown in Fig. 4, severe TAC increased both total CaMKII and phosphorylated CaMKII in HF mice, with the phosphorylated to total CaMKII ratio increased > 3-fold ($P < 0.05$). Compared with HF mice, the phosphorylation of CaMKII was reduced in HF + KN93 AI mice and HF + KN93 CI mice by 37% and 48%, respectively ($P < 0.05$).

4. Discussion

Previous studies have already revealed that mice with genetic deletion of CaMKIIδ were resistant to the development of hypertrophy [22] and HF [23] induced by transverse aortic constriction. These studies did not, however, actually examine the “therapeutic” effect of CaMKII inhibition on HF. In this work, we demonstrated for the first time that although acute inhibition of elevated CaMKII levels with KN93 can repair systolic function in HF mice, it also exacerbates the diastolic function, while chronic inhibition significantly improves both systolic function and cardiac reserve to β-adrenergic stimulation without impairing diastolic function.

4.1. Excessive CaMKII activation in HF

Diverse mechanisms contribute to excessive CaMKII activation in HF. For instance, Ca^{2+} overload and oxidative stress are general features of failing myocardium, which activate CaMKII by Ca^{2+}/CaM-dependent and oxidation-dependent pathways. Meanwhile, cardiac transient outward potassium channel (I_{to}) plays an important role in regulating CaMKII activity. I_{to} reduction is a consistent finding in HF, which leads to prolonged action potential duration and an increase in intercellular Ca^{2+} concentration, thus increasing the total intracellular CaMKII activity. We have demonstrated a physical coupling between I_{to} channel subunit Kv4.3 and the inactive CaMKII proteins, in which Kv4.3 acts as an endogenous CaMKII inhibitor. Kv4.3 mainly affects CaMKII activity in the sarcolemmal compartment due to their membrane localization in myocytes [24]. Downregulation of Kv4.3 in HF causes the release of membrane associated inactive CaMKII into the cytoplasm and its subsequent activation. Furthermore, in HF, sustained stimulation of β-adrenergic receptor (β-AR) can also activate CaMKII by several mechanisms. During β-adrenergic stimulation, there are concomitant increases in Ca^{2+} entry through LTCC and SR Ca^{2+} release from RyRs, which could promote localized CaMKII activation [25,26]. Mangmool and colleagues also suggest beta-Arrestin-dependent activation of CaMKII after β-AR stimulation [27].

4.2. CaMKII inhibition in the failing myocardium

In left ventricular myocytes of a rabbit HF model [13], KN93 reduced the diastolic SR Ca^{2+} leak. Consistent with this, results from end-stage HF human cardiomyocytes demonstrated that KN93 and AIP (autocamtide-2-related inhibitory peptide, another CaMKII inhibitor) both led to reduction of the diastolic Ca^{2+} spark frequency and duration as a result of decreased RyR2 phosphorylation [28,29]. An orally bioavailable but less potent CaMKII inhibitor SMP-114 was also shown to significantly diminish SR Ca^{2+} leak in human failing left ventricular cardiomyocytes [30]. In line with this, another novel high-affinity ATP-competitive pyrimidine-based CaMKII inhibitor AS105 effectively reduced diastolic SR Ca^{2+} leak, enhanced the ability of SR to accumulate Ca^{2+}, and improved systolic Ca^{2+}-transient amplitudes and contractility during basal stimulation in ventricular myocytes from

Table 3
Echocardiographic and PV parameters in chronic CaMKII inhibition.

Parameter	Sham (n = 8)		HF (n = 8)		HF + KN92 CI (n = 8)		HF + KN93 CI (n = 8)	
	Baseline	Iso	Baseline	Iso	Baseline	Iso	Baseline	Iso
HR	559 ± 12	577 ± 13$^{\#}$	554 ± 14	550 ± 12	553 ± 13	551 ± 11	556 ± 13	569 ± 14$^{\#}$
EF[a], %	79.75 ± 4.21	87.28 ± 4.48$^{\#}$	40.72 ± 3.61*	41.08 ± 4.87	39.54 ± 3.48*	40.46 ± 3.50	51.03 ± 4.25*†	56.81 ± 4.70$^{\#}$
FS, %	46.91 ± 4.37	55.86 ± 5.96$^{\#}$	19.27 ± 2.00*	19.52 ± 2.71	18.62 ± 1.89*	19.16 ± 1.97	25.21 ± 2.64*†	28.87 ± 3.10$^{\#}$
Longitudinal strain, %	21.71 ± 2.32	26.53 ± 3.30$^{\#}$	13.88 ± 1.78*	13.32 ± 2.16	13.70 ± 1.40*	13.45 ± 1.96	17.31 ± 2.08*†	21.64 ± 2.27$^{\#}$
Longitudinal strain rate, s^{-1}	10.36 ± 1.97	13.59 ± 1.87$^{\#}$	5.35 ± 1.11*	5.38 ± 1.25	5.26 ± 1.11*	5.21 ± 1.16	7.88 ± 1.51*†	10.18 ± 1.82$^{\#}$
EF[b], %	70.08 ± 5.59	76.18 ± 5.70$^{\#}$	36.45 ± 3.91*	35.46 ± 4.05	36.77 ± 3.51*	36.21 ± 2.83	47.80 ± 4.42*†	53.05 ± 4.59$^{\#}$
ESPVR, mmHg/μl	9.40 ± 1.56	11.49 ± 2.28$^{\#}$	6.21 ± 1.52*	6.15 ± 1.68	6.27 ± 1.62*	6.18 ± 1.60	8.39 ± 1.65†	9.74 ± 1.66$^{\#}$
dP/dtmax-EDV, mmHg / (s ∗ ml)	312.07 ± 32.53	340.39 ± 34.87$^{\#}$	239.06 ± 25.42*	232.94 ± 27.60	244.58 ± 21.07*	241.53 ± 21.20	280.26 ± 29.68*†	304.85 ± 31.22$^{\#}$
PRSW, mmHg	74.09 ± 7.68	80.64 ± 7.87$^{\#}$	45.73 ± 5.23*	44.26 ± 6.20	47.65 ± 5.15*	46.66 ± 4.73	61.52 ± 6.85*†	66.73 ± 6.74$^{\#}$
-dP/dtmin, mmHg/s	7422.17 ± 770.51		5810.31 ± 571.50*		5749.54 ± 496.48*		6379.82 ± 653.06*	
Tau, ms	10.25 ± 1.14		15.42 ± 1.64*		15.33 ± 1.67*		14.98 ± 1.45*	
EDPVR, mmHg/μl	0.19 ± 0.02		0.31 ± 0.03*		0.31 ± 0.03*		0.29 ± 0.03*	

Values are means ± SD. EF = ejection fraction; FS = fractional shortening; dP/dtmax-EDV = peak rate of pressure rise-end diastolic volume relation; ESPVR = end-systolic pressure-volume relation; PRSW = preload recruited stroke work, stroke work-end diastolic volume relation; -dP/dtmin = peak rate of pressure decline; Tau = relaxation time constant; EDPVR = end-diastolic pressure-volume relation. a = assessed by echocardiography; b = assessed by pressure-volume catheter. * $P < 0.05$, compared with Sham group; † $P < 0.05$, compared with HF group; $^{\#}$ $P < 0.05$, compared with baseline. P-values denoted with * or † were derived from ANOVA, while P-values denoted with $^{\#}$ from t-test.

CaMKIIδ$_C$-overexpressing mice with HF [31]. As for the overall cardiac function, the results are still pending. Sossalla et al. revealed improved contractility in human failing right ventricular trabeculae with unaltered diastolic tension and relaxation kinetics [28]. In HF ventricular myocytes of CaMKIIδ knockout mice [32], we found an improved cellular contraction but slowed sarcomere relaxation. However, most of these results are derived from experiments on the cell-level, and there is little literature about the effect of CaMKII inhibition on in vivo cardiac function in HF animal. To our knowledge, this is the first study to actually examine the overall cardiac effects of CaMKII inhibition on HF and demonstrate that chronic inhibition of elevated CaMKII levels with KN93 is sufficient to attenuate or even reverse maladaptive cardiac remodeling in HF mice.

4.3. Possible mechanisms underlying the effect of CaMKII inhibition on cardiac function and cardiac reserve in HF mice

The present study reveals that although acute inhibition of elevated CaMKII levels with KN93 can repair systolic function in HF mice, it also exacerbates the diastolic function, while chronic inhibition significantly improves both systolic function and cardiac reserve to β-adrenergic stimulation without impairing diastolic function. We propose that this is likely resulted from the reduced diastolic SR Ca^{2+} leak, increased systolic SR Ca^{2+} release, reduced SRECA-to-unphosphorylated PLB ratio, and increased myofilament sensitivity to Ca^{2+} in HF + KN93 AI mice. However, the decrease of ventricular fibrosis may underlie part of the preservation in diastolic function in HF + KN93 CI mice. In addition, it is sufficient for HF + KN93 CI mice to resensitize the β_1-AR.

Despite sustained activation of sympathetic nerve in HF, the cardiac responsiveness to β-adrenergic stimulation is attenuated, which is related to decreased membrane number of β_1-AR, a process termed β_1-AR desensitization [33]. Our recent experiments found that knockout of the predominant CaMKIIδ in HF ventricular myocytes abrogates β_1-AR down-regulation [32], indicating an important role of CaMKII in β_1-AR desensitization. Based on these findings, we propose that CaMKII is not only a downstream target of β_1-ARs but also a mediator of β_1-AR signaling. The possible relationship between CaMKII activity and β_1-AR sensitivity are as follows: CaMKII is activated in response to sustained β_1-AR stimulation in HF, as a feedback, excessively activated CaMKII desensitizes β_1-AR by reducing synthesis, increasing degradation, or promoting internalization. However, the precise interaction is unknown, and further studies are clearly needed to verify the underlying mechanism.

5. Limitations of the study

Numerous strategies for inhibition of CaMKII activity have been described. Considering genetic deletion of CaMKII actually investigate the preventive effect of CaMKII inhibition because it started before the onset of HF, we employed the most widely used pharmacological inhibitor KN93. In the meanwhile, KN92 was applied as control to rule out the off-target effects. However, KN93 blocks the nonphosphorylated CaMKII only. Furthermore, due to the ubiquitous distribution and high homology of CaMKII, KN93 can also affect other CaMKII isoforms in addition to CaMKIIδ, such as CaMKIIα, which is important for neuronal function and cognitive memory. Thus, the effort has to be taken to develop more potent, CaMKIIδ-specific, orally administrable, non-CNS penetrating small compound inhibitors. It has to be stated that the precise molecular mechanisms underlying the different effects of acute and chronic CaMKII inhibition on cardiac function and cardiac reserve in HF mice may be complex and will require further investigations. Despite these limitations, our work shows convincingly that inhibition of CaMKII is a promising therapy for HF patients.

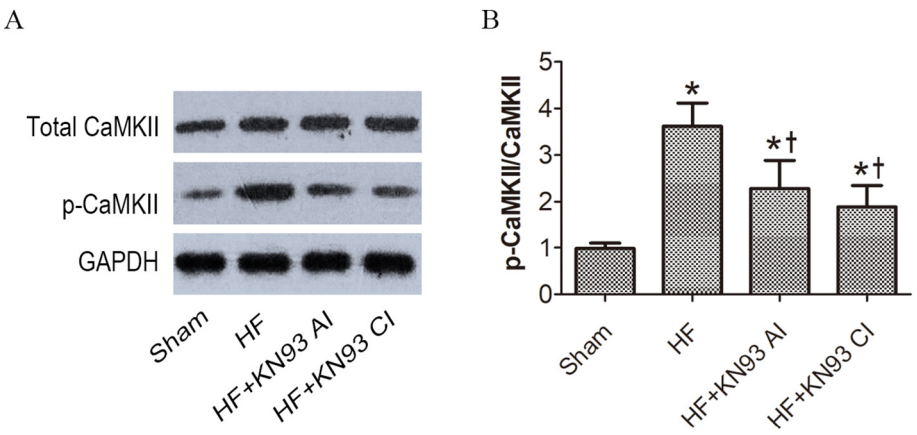

Fig. 4. The efficacy of acute and chronic KN93 administration. (A) Representative Western blots of total and phosphorylated CaMKII; (B) ratio of phosphorylated to total CaMKII in sham mice, HF mice, HF + KN93 AI mice and HF + KN93 CI mice ($n = 3$ per group). * $P < 0.05$, compared with Sham group; † $P < 0.05$, compared with HF group.

6. Conclusion

In summary, we demonstrated for the first time that although acute inhibition of elevated CaMKII levels with KN93 can repair systolic function in HF mice, it also exacerbates the diastolic function, while chronic inhibition significantly improves both systolic function and cardiac reserve to β-adrenergic stimulation without impairing diastolic function.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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