Original Paper

Biventricular Pacing Cardiac Contractility Modulation Improves Cardiac Contractile Function via Upregulating SERCA2 and miR-133 in a Rabbit Model of Congestive Heart Failure

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Abstract

Objective: To compare the effects of biventricular electrical pacing and conventional single-ventricular pacing for cardiac contractility modulation (CCM) on cardiac contractile function and to delineate the underlying molecular mechanisms. ***Methods:*** Forty rabbits were divided into four groups before surgery: healthy control, HF sham, HF left ventricular pacing CCM (LVP-CCM), and HF biventricular pacing CCM (BVP-CCM) groups with n=10 for each group. A rabbit model of chronic heart failure was established by ligating ascending aortic root of rabbits. Then electrical stimulations during the absolute refractory period were delivered to the anterior wall of left ventricle in the LVP-CCM group and on the anterior wall of both left and right ventricles in the BVP-CCM group lasting six hours per day for seven days. Changes in ventricular structure, cardiac function and electrocardiogram were monitored before and after CCM stimulation. ***Results:*** Compared with the sham-operated group, heart weight, heart weight index, LV end-systolic diameter (LVESD), LV end-diastolic diameter (LVEDD) in the LVP-CCM and BVP-CCM groups were significantly decreased ($p<0.05$), while LV ejection fraction (LVEF) and fractional shortening fraction (FS) were increased ($p<0.05$). Notably all these changes were consistently found to be greater in BVP-CCM than in LVP-CCM. Moreover, plasma BNP levels were highest in the HF sham-control group, followed by the LVP-CCM group, and lowest in the BVP-CCM group ($p<0.05$). Furthermore, sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) protein levels were upregulated by 1.7 and 2.4 fold, along with simultaneous upregulation of a cardiac-enriched microRNA miR-133 levels by 2.6 and 3.3

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Cellular Physiology and Biochemistry

Cell Physiol Biochem 2014;33:1389-1399

DOI: 10.1159/000358705
Published online: May 05, 2014

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fold, in LVP-CCM and BVP-CCM, respectively, compared to sham. **Conclusions:** Biventricular pacing CCM is superior to conventional monoventricular pacing CCM, producing greater improvement cardiac contractile function. Greater upregulation of SERCA2 and miR-133 may account, at least partially, for the improvement by BVP-CCM.

Introduction

Congestive heart failure (CHF) is a highly prevalent disease in western society characterized by severely impaired cardiac contractile function and is associated with high mortality and morbidity. Increasing myocardial contractility therefore has long been considered a rational goal for therapy of CHF [1]. Drug therapies aimed at this goal, however, have been associated with decreased survival. Recently, a number of short- and mid-term clinical studies have suggested adjuvant or alternative therapies to CHF using modified pacing techniques that is able to either increase cardiac contractility or restore synchrony of contraction. The technique used to increase contractility is termed Cardiac Contractility Modulation (CCM), and the technique for restoring synchrony of contraction is coined cardiac resynchronization therapy (CRT).

CCM has been developed in recent years as a treatment for refractory systolic CHF through electrical stimulation during myocardial absolute refractory period (ARP) to increase myocardial contractility but not to cause cardiac excitability [2-5]. CCM stimulation in patients with CHF enhances regional and global measures of left ventricular (LV) systolic function. CCM pacing is usually delivered to a single ventricular chamber: LV pacing (LVP) or right ventricular pacing (RVP). Only patients with wide but normal QRS complex in the electrocardiogram are eligible for CCM, whilst patients with abnormally wider QRS (> 120 ms) are examined to CRT.

CRT has recently emerged as an adjuvant treatment to limited group of CHF patients with aberrant LV conduction and low ejection fraction [6-8]. This is because dilated CHF due to systolic LV dysfunction frequently leads to intraventricular conduction disturbances with a prolonged QRS complex. The prolonged conduction time in the ventricles causes electrical asynchrony of the ventricles and contributes to a further reduction of systolic function, changes filling and relaxation intervals, and promotes mitral valve insufficiency. CRT is usually delivered to two ventricular chambers (simultaneous stimulation of both the right and left ventricles), and CRT with chronic biventricular pacing (BVP) synchronizes contraction of the ventricles and improve overall ventricular performance.

Despite the fact that CCM and CRT have different applicabilities and utilize different pacing protocols, we thought that it might be advantageous to combine the two techniques to improve the outcome of CCM. In our pilot animal studies for testing this hypothesis, we found that integrating biventricular pacing of the CRT protocol into CCM (BVP-CCM) during ARP of cardiac excitability yielded better results in terms of cardiac contractile function than LV pacing (LVP-CCM). We therefore set up the present study to investigate the modified CCM by comparing the beneficial effects of conventional single-chamber pacing CCM, specifically, LVP-CCM with those of BVP-CCM, and to decipher the potential molecular mechanisms underlying the differences.

Materials and Methods

Rabbit model of heart failure

Forty New Zealand white rabbits of both genders, 6 months age, weighing 2.5~3.5 kg, were provided by the Experimental Animal Center of the HeBei Medical University. The animals were randomly divided into four groups before surgery: healthy control, HF sham, HF left ventricular pacing CCM (LVP-CCM), and HF biventricular pacing CCM (BVP-CCM) groups with n=10 for each group. Rabbits were anesthetized with sodium pentobarbital

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2014;33:1389-1399

DOI: 10.1159/000358705
Published online: May 05, 2014

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1ml/kg in 3% solution. The chest was open between intercostals 2 and 4 at the left sternal border area and the heart was exposed. The ascending aorta was dissected free for about 4-5mm at 1.0cm distal to aortic root. Aortic circumference was measured, and occluded to make cerclage constriction to 60% of the original circumference. Heart failure was considered being successfully established if ejection fraction (%, EF) was ≤ 40% of the baseline level prior to aortic coarctation. For LVP-CCM, a pacing electrode was sutured to left ventricular anterior wall, and for BVP-CCM, a pair of pacing electrodes were fixed one on the left ventricular anterior wall and the other on right ventricular anterior wall. The bare wires were ensured to have good contact with myocardial tissue, and were insulated from the chest wall tissue. The other ends of the pacing electrodes were punctured subcutaneously to the neck for later use. Sham group only received cerclage to create aortic coarctation without ventricular pacing. Rabbits that survived and met the criteria of heart failure 12 weeks post-aortic coarctation included 7 in the sham group, 8 in the LVP-CCM group, and 8 in the BVP-CCM group. These 23 rabbits along with 10 normal control rabbits were used for the subsequent experiments and included in the statistical analysis.

Ventricular pacing protocols

The free ends of the pacing electrodes on the neck were connected to an R-wave triggering stimulation instrument (BARD MICROPACE EPS320 Cardiac Electrophysiology Stimulator, Germany) to deliver CCM stimuli. CCM signals were biphasic square-wave pulses with adjustable amplitude, duration, and time delay from sensing of local electric activity. Specifically, electrical stimuli (2ms, 7V, 30ms delay after R-wave sensing) were delivered to the absolute refractory period of cardiac excitations under sinus rhythm 6 hrs per day for consecutive 7 days. ECG was monitored for each animal by Holter recordings.

Noninvasive ultrasound examination of cardiac function

Prosound a10 ultrasonic diagnostic system (Japan ALOKA Company) was to measure cardiac performance. Under M-mode image, Teichholz was adopted to measure LV end-systolic diameter (LVESD), LV end-diastolic diameter (LVEDD), LV shortening fraction (LVFS), interventricular septal thickness (IVS), LV posterior wall thickness (LVPW), and ejection fraction (LVEF). The Doppler sampling probe was placed in between diastolic mitral tip to measure peak flow during early diastolic period of mitral values (E) and peak flow of late diastole (A). The E/A ratio was calculated, and each data point represented the average of three consecutive measurements. All measurements were conducted before and after CCM for comparisons.

At the end of the experiments, the animals were sacrificed and hearts were removed. Hearts were weighted, and heart weight index (HWI) was calculated (heart weight HW to body weight BW ratio).

Measurements of Plasma brain natriuretic peptide (BNP)

Plasma BNP levels were measured at two time points (before and after CCM), using double-antibody sandwich ABC-ELISA kit (Westang Biotech, Shanghai) according to the manufacturer's protocol.

mRNA and miRNA Quantification

Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA), and small RNA-enriched RNA fractions were obtained using the miRNeasy and miRNA cleanup kits (Qiagen, Valencia, CA). SERCA2a mRNA and miR-133 levels were analyzed using the TaqMan quantitative real-time PCR (qRT-PCR) method (10 ng/assay), and quantified with an ABI 7000 Real-Time PCR System (Applied Biosystems, Foster City, CA). TaqMan Primer assays for miRNAs and the reagents for reverse transcriptase and qRT-PCR reactions were obtained from Applied Biosystems. Relative expression was calculated using the comparative cycle threshold (Ct) method ($2^{-\Delta\Delta Ct}$). mRNA levels were normalized to GAPDH, and miRNA levels were normalized to U6 RNA.

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2014;33:1389-1399

DOI: 10.1159/000358705
Published online: May 05, 2014

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Table 1. Comparison of changes of heart weight between LVP-CCM and BVP-CCM. Control: non-heart failure (HF) rabbits; Sham: HF rabbits without CCM; LVP-CCM: left ventricular pacing cardiac contractility modulation in HF rabbits; BVP-CCM: biventricular pacing in HF rabbits; BW: body weight; HW: heart weight; HWI: heart weight index (=HW/BW) cardiac contractility modulation; *$P<0.05$ compared to Control; •$P<0.05$ compared to the HF Sham group; ▪$P<0.05$ compared to the LVP-CCM group

	Control (n=10)	Sham (n=7)	LVP-CCM (n=8)	BVP-CCM (n=8)
HW (g)	4.12±0.52	6.72±0.82*	5.23±0.56	4.41±0.36
BW (kg)	3.83±0.36	3.69±0.32	3.71±0.21	3.74±0.21
HWI (g/kg)	1.08±0.12	1.82±0.25*	1.41±0.27•	1.18±0.17•▪

Western Blot Analysis

Total proteins were purified from LV wall. The protein content was determined by BCA protein assay reagent (Pierce, USA). Protein sample (30 μg) was loaded to SDS-PAGE and then transferred to membrane. Membrane was incubated with a 1:1000 dilution of primary antibody, followed by a 1:5000 dilution of horseradish peroxidase–conjugated secondary antibody.

Protein bands were visualized by ECL (GE Healthcare). The primary antibody against SERCA2a (rat polyclonal), phospholamban (L-15; goat polyclonal) and phospho-phospholamban (Ser16; goat polyclonal), and the secondary antibody to GAPDH were all purchased from Santa Cruz Biotech (Santa Cruz, CA, USA), and the phospho-phospholamban (Thr17) was obtained from PhosphoProteinResearch (Bardsey, UK). Mouse monoclonal anti- sodium/calcium exchanger 1 (NCX1) was obtained from Merck Millipore (Billerica, MA, USA). The intensity (area × density) of the individual bands on Western blots was measured by densitometry (model GS-700, Imaging Densitometer; Bio-Rad).

Statistical analysis

Statistical analyses were carried out using SAS8.0 statistical analysis software. Data are presented as mean ± standard deviation (SD). Before and after CCM comparison was analyzed using t-test. Multiple group comparison was conducted using ANOVA F-test followed by SNK-q test. $P<0.05$ is considered statistically significant.

Results

Comparison of cardiac contractile function between BVP-CCM and LVP-CCM

Rabbits with heart failure showed lethargy, increased breathing and heart rate, cyanosis, and reduced weight gain as compared with sham control rabbits. At the time of sacrifice (13 weeks after surgery), HWI in both LVP-CCM and BVP-CCM groups was decreased relative to that in the sham-control group, but the magnitude of decrease was significantly greater in BVP-CCM than in LVP-CCM (Table 1). Mostly notably, QRS complex was significantly widened from 49.4±1.6 ms for the control rabbits to 58.8±1.9 ms for animals with HF (Fig. 1). Both LVP-CCM and BVP-CCM significantly reversed this QRS widening; by comparison, BVP-CCM elicited a greater restoration.

LVESD and LVEDD were markedly decreased, whereas LVEF and LVFS were increased in both LVP-CCM and BVP-CCM; the extent of these changes was greater with BVP-CCM than with LVP-CCM. IVS, IVPW, E, A, and E/A ratio remained indifferent among the three groups (Table 2).

While the plasma BNP level, as a marker of HF, was not significantly different among the three groups before CCM, it was decreased in LVP-CCM and BVP-CCM compared to the sham group, particularly in the BVP-CCM group (Table 3).

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Fig. 1. Cardiac contractility modulation (CCM) restores the widened QRS complex in a rabbit model of heart failure. HF: heart failure; LVP-CCM: left ventricular CCM; BVP-CCM: biventricular CCM. Upper panels: examples of ECG traces recorded under varying conditions; lower panel: mean±SD of QRS complex. Note that HF Sham Control significantly widened QRS complex relative to normal control rabbits, and CCM restored the widening with BVP-CCM producing a greater magnitude of restoration than LVP-CCM. The number of animals for each group is indicated in the bars. *P<0.05 compared to normal control; •P<0.05 compared to the HF Sham group; ■P<0.05 compared to the LVP-CCM group.
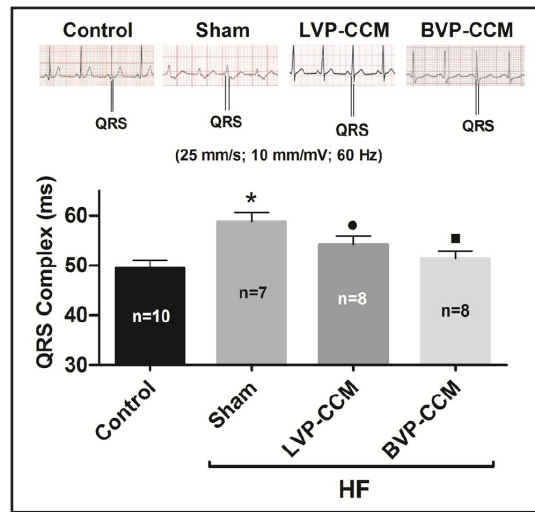

Table 2. Comparison of cardiac function parameters between LVP-CCM and BVP-CCM. Control: non-heart failure (HF) rabbits; Sham: HF rabbits without CCM; LVP-CCM: left ventricular pacing cardiac contractility modulation in HF rabbits; BVP-CCM: biventricular pacing in HF rabbits; LVSDD: left ventricular end systolic diameter; LVEDD: left ventricular end diastolic diameter; EF: ejection fraction; FS: shortening fraction; IVS: interventricular septal thickness; LVPW: LV posterior wall thickness; E: peak flow during early diastolic period of mitral values; A: peak flow of late diastole; *P<0.05 compared to Control; •P<0.05 compared to the HF Sham group; ■P<0.05 compared to the LVP-CCM group

		LVEDD (mm)	LVESD (mm)	EF (%)	FS (%)	IVS (mm)	LVPW (mm)	E (cm/s)	A (cm/s)	E/A
Control	12w	11.89±0.64	8.41±0.37	52.04±4.03	29.05±2.01	2.02±0.22	2.11±0.15	77.93±5.84	62.32±5.50	1.25±0.21
	13w	11.72±0.61	8.60±0.39	57.00±4.14	31.87±2.19	2.04±0.23	2.14±0.21	78.45±5.46	61.50±4.86	1.24±0.15
Heart Failure										
Sham	12w	14.49±0.72*	11.71±0.57	37.14±3.13*	19.00±2.21*	2.05±0.30	2.00±0.19	69.86±6.24*	63.29±4.50	1.09±0.23*
	13w	14.32±0.71*	11.76±0.49	37.00±3.10*	17.90±1.69*	2.08±0.27	2.04±0.31	71.29±6.16*	63.43±5.26	1.14±0.18
LVP-CCM	12w	14.36±0.83	11.66±0.62	38.12±3.87	19.26±2.54	2.06±0.34	2.05±0.24	73.43±5.52	65.21±5.12	1.20±0.64
	13w	13.54±0.53•	10.26±0.49•	46.50±4.31•	24.21±2.94•	2.01±0.32	2.03±0.33	3.48±5.89	60.17±4.86	1.16±0.21
BVP-CCM	12w	14.25±0.78	11.52±0.72	37.67±4.12	19.85±3.15	2.11±0.41	2.10±0.36	72.95±5.15	60.11±6.58	1.23±0.34
	13w	12.50±0.71•■	8.95±0.41•■	55.75±4.53•■	28.20±2.67•■	2.07±0.37	2.08±0.29	75.14±4.60	63.88±6.12	1.18±0.64

Table 3. Comparison of serum BNP levels before and after CCM between LVP-CCM and BVP-CCM ($\overline{x}$ ±s; ng/ml). BNP: brain natriuretic peptide; Control: non-heart failure (HF) rabbits; Sham: HF rabbits without CCM; LVP-CCM: left ventricular pacing cardiac contractility modulation in HF rabbits; BVP-CCM: biventricular pacing in HF rabbits; *P<0.05 compared to Control; •P<0.05 compared to the HF Sham group; ■P<0.05 compared to the LVP-CCM group

	Control (n=10)	Sham (n=11)	LVP-CCM (n=17)	BVP-CCM (n=14)
Before CCM	33.10±9.03	63.54±12.01*	62.74±12.77	66.32±13.53
After CCM	35.15±10.21	65.21±12.11*	51.37±13.01•	39.44±11.7•■

Comparison of SERCA2a, PLB and NCX1 expression between BVP-CCM and LVP-CCM
While the above results indicate that BVP-CCM produces more favorable effects on rescuing cardiac function than LVP-CCM, we wanted to get an insight into the molecular
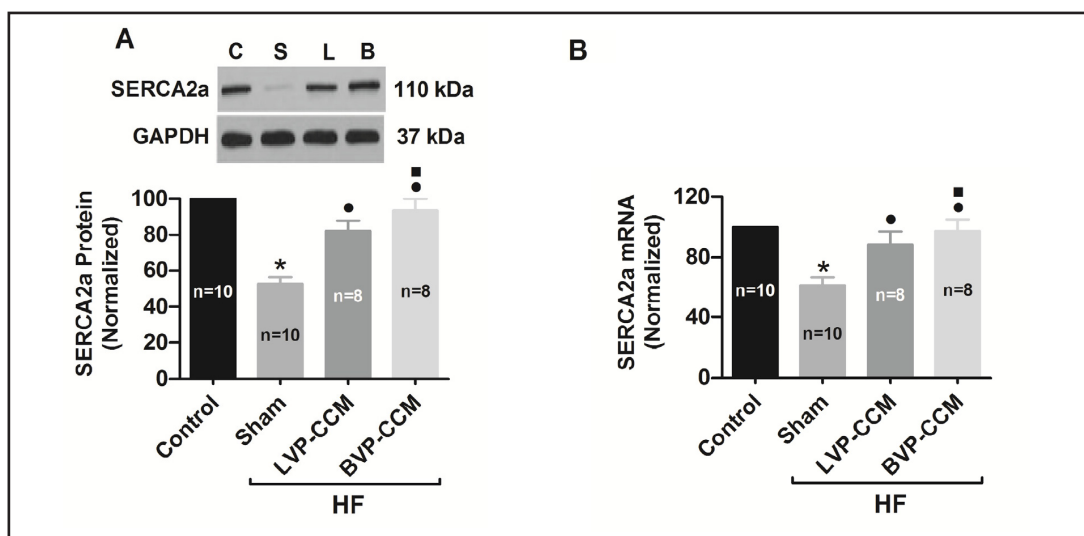
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Cellular Physiology and Biochemistry

Cell Physiol Biochem 2014;33:1389-1399

DOI: 10.1159/000358705
Published online: May 05, 2014

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Fig. 2. CCM restores the downregulated SERCA2a protein and mRNA levels in a rabbit model of heart failure (HF). *A*, SERCA2a protein level was determined by Western blot analysis. Upper panel shows a typical example of immunoblotting bands and lower panel represent a plot of averaged values of band density under various conditions. C: non-HF control; S: HF-sham animal; L: HF LVP-CCM; B: HF BVP-CCM. Note that HF Sham Control significantly decreased SERCA2a protein level relative to normal control rabbits, and CCM restored the downregulation with BVP-CCM producing a greater magnitude of restoration than LVP-CCM. *B*, SERCA2a mRNA level was determined by real-time RT-PCR. The number of animals for each group is indicated in the bars. *$P<0.05$ compared to normal control; •$P<0.05$ compared to the HF Sham group; ■$P<0.05$ compared to the LVP-CCM group.

Fig. 3. Effects of CCM on the phospholamban (PLB) protein levels in a rabbit model of heart failure (HF). Upper panel shows a typical example of immunoblotting bands and lower panel represent a plot of averaged values of band density under various conditions. t-PLB: total PLB protein; p-Ser16: Ser16-phosphorylated PLB; p-Thr17: Thr17-phosphorylated PLB; C: non-HF control; S: HF-sham animal; L: HF LVP-CCM; B: HF BVP-CCM. *$P<0.05$ compared to normal control; •$P<0.05$ compared to the HF Sham group; n=4.
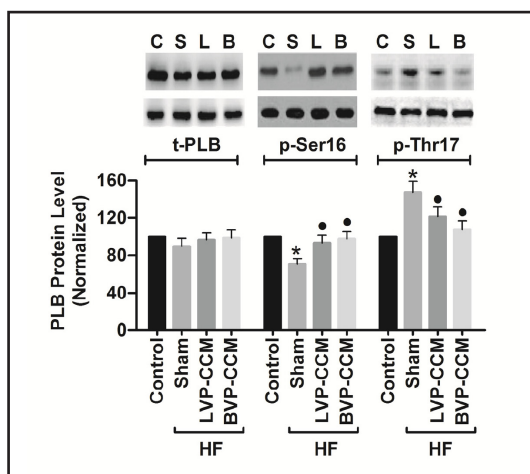
mechanisms underlying the difference. SERCA2a, the cardiac sarco/endoplasmic reticulum Ca^{2+}-ATPase, or SR Ca^{2+}-ATPase, is a calcium ATPase-type P-ATPase which plays a critical role in cardiac intracellular Ca^{2+} handling. Expression of SERCA2a is substantially downregulated in heart failure and on the other hand, defects in SERCA2a contribute importantly to heart failure [9-12]. SERCA2a replacement has been acclaimed as a promising gene therapy for heart failure. We sought to see if CCM ameliorates SERCA2a defects and if yes, which of LVP-CCM and BVP-CCM gives more favorable effects. As illustrated in Figure 2A, SERCA2a protein level was significantly decreased in rabbits with HF compared to the sham control animals, and this downregulation was restored by CCM with BVP-CCM producing greater magnitude
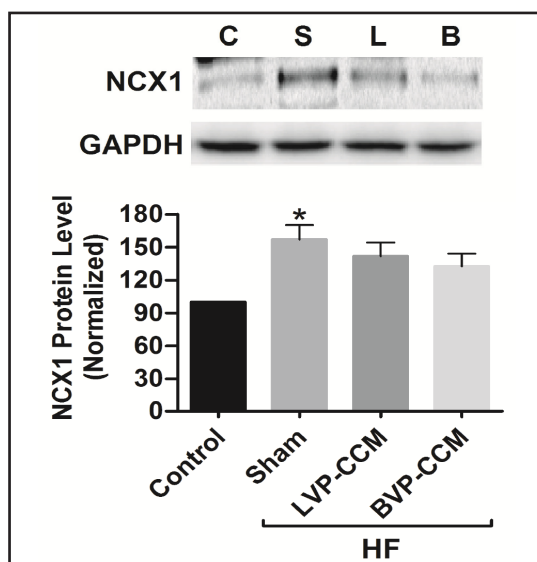
Cellular Physiology and Biochemistry

Cell Physiol Biochem 2014;33:1389-1399

DOI: 10.1159/000358705
Published online: May 05, 2014

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Fig. 4. Effects of CCM the upregulated Na^+/Ca^{2+} exchanger 1 (NCX1) protein levels in a rabbit model of heart failure (HF). Upper panel shows a typical example of immunoblotting bands and lower panel represent a plot of averaged values of band density under various conditions. C: non-HF control; S: HF-sham animal; L: HF LVP-CCM; B: HF BVP-CCM. *$P<0.05$ compared to normal control; n=4.
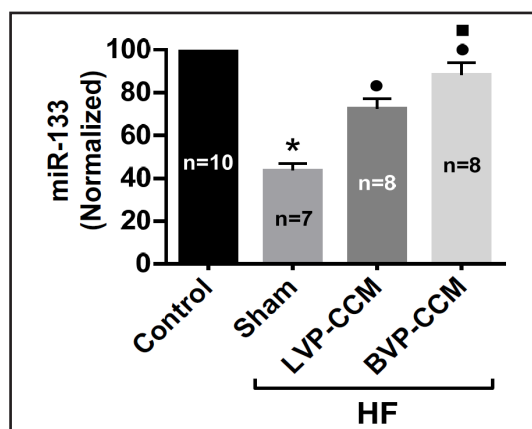

Fig. 5. CCM restores the decreased cardiac miR-133 in a rabbit model of heart failure. miR-133 level was determined by real-time RT-PCR. Note that HF Sham Control significantly downregulated miR-133 relative to normal control rabbits, and CCM restored the decrease with BVP-CCM producing a greater magnitude of restoration than LVP-CCM. The number of animals for each group is indicated in the bars. C: non-HF control; S: HF-sham animal; L: HF LVP-CCM; B: HF BVP-CCM. *$P<0.05$ compared to normal control; •$P<0.05$ compared to the HF Sham group; ■$P<0.05$ compared to the LVP-CCM group.

of restoration than LVP-CCM. Similar changes of SERCA2a mRNA levels were observed (Fig. 2B).

Phospholamban (PLB), a SERCA2a regulatory protein, is a small, plasma membrane-associated phospho-protein assembled into a pentamer in the sarcoplasmic reticulum of cardiac muscle. PLB regulates cardiac contractility and Ca^{2+} affinity for cardiac SERCA2a: non-phosphorylated PLB associates with SERCA2a to inhibit its function whereas PLB phosphorylation at Ser16 and/or Thr17 causes dissociation from SERCA2a to increase in the rate of Ca^{2+} reuptake [13-15]. Our Western blot detected the 25-kDa pentamer of PLB. The expression level of PLB (total PLB) was only slightly reduced (Fig. 3). The Ser16-phosphorylated form of PLB (p-Ser16) was significantly reduced in rabbits with heart failure; in sharp contrast, Thr17-phosphorylated PLB (p-Thr17) was significantly enhanced in failing heart relative to non-heart failure control. Both BVP-CCM and LVP-CCM restored the aberrantly altered phosphorylation status of PLB, and no significant differences between BVP-CCM (Fig. 3) and LVP-CCM were observed in terms of PLB expression and PLB phosphorylation.

On the other hand, Na^+/Ca^{2+} exchanger (NCX1) is an important plasma membrane transport protein that regulates cellular Ca^{2+} homeostasis in cardiac muscle. NCX1 is believed as the dominant Ca^{2+} efflux mechanism during diastole and has been shown to be upregulated in heart failure [16-18]. We thus investigated if CCM exerts any effects on NCX1 expression. As shown in Figure 4, NCX1 protein level was significantly elevated in failing heart. Both BVP-CCM and LVP-CCM tended to reduce this upregulation but the effects did not reach statistical significance.

Comparison of miR-133 levels between BVP-CCM and LVP-CCM

miR-133 is a muscle-specific and cardiac-enriched miRNA, and its expression is found markedly repressed in heart failure in both animal models and human subjects [19-22]. Our

Cellular Physiology and Biochemistry

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results presented in Figure 5 confirmed downregulation of miR-133 in cardiac tissues. Of note, both LVP-CCM and BVP-CCM corrected the aberrant downregulation and the effects appeared to be in the same range in the two groups.

Discussion

CHF is a terminal status consequently to a variety of cardiovascular diseases, representing one of the most difficult clinical problems in medical practice. Pharmacological therapy aiming to restore weakened cardiac contractility is highly encouraged; however, response to drug treatment has been poor in HF patients. Researchers and clinicians are actively seeking non-drug approaches of HF treatment [23]. CCM and CRT are two examples of such efforts. These new technologies are developed as a treatment for refractory systolic heart failure of, through electrical stimulation of small, undetectable or sub-threshold electrical impulses to increase and/or synchronize myocardial contractility. Numerous studies have demonstrated the usefulness of CCM to evoke cardiac inotropic effect [24-26]. The ability of CCM to improve cardiac function is believed mainly due to enhanced local contraction force and electrical stimulation in different parts of the myocardium may have different efficacies on cardiac function. It is not yet clear and is even still confusing as to what parts of the myocardium stimuli should be applied to achieve the best effects [25, 26]. In this study, we compared left ventricle anterior wall stimulation (LVP-CCM) as a mono-ventricular CCM with bi-ventricular CCM. Our results revealed that BVP-CCM is superior to LVP-CCM in improving cardiac contractile function as reflected by the decreased LVESD and LVEDD and increased LVEF and LVFS, in improving cardiac conduction as indicated by the shortened QRS complex, and in improving overall cardiac function as evidenced by the lowered BNP level. Our data further demonstrated that CCM, particularly BVP-CCM, elevated SERCA2a protein level and upregulated miR-133 expression, which is deemed to benefit cardiac contractile function, as SERCA2a is a key regulator of intracellular Ca^{2+} handling [10-12] and miR-133 regulates a number of genes crucial for cardiac function [19, 27, 28]. On the basis of our findings, we concluded that BVP-CCM is superior to LVP-CCM in improving cardiac contractile function which is likely attributable to the greater efficacy of BVP-CCM in elevating SERCA2a and miR-133 expression.

It is generally believed that CCM restores cardiac contractile function by improving Ca^{2+} handling through promoting intracellular Ca^{2+} flow. In animal studies, CCM was found to prolong action potential duration so as to increase Ca^{2+} influx and cardiac contractility [29]. In general, ventricular pacing can evoke Ca^{2+} influx through several pathways: L-type Ca^{2+} channel, Na^+/Ca^{2+} exchanger, and sarcoplasmic reticulum Ca^{2+} release. Such changes can result in a persistent elevation of cardiac cytoplasmic free Ca^{2+} concentration thereby an increase in cardiac contraction. However, how BVP-CCM produces better effects than mono-ventricular pacing remained uncertain. Multiple mechanisms may be involved. One possible explanation is that BVP-CCM can elicit more intracellular Ca^{2+} influx through simultaneous stimulation of two sites than mono-ventricular pacing if it is true that CCM improves overall cardiac contractile function through enhancing regional myocardial contractility. The second explanation may be related to synchronization of cardiac electrical and mechanical activities as dyssynchrony is a highly detrimental factor in heart failure, which can tremendously increase the risk of heart failure and worsen the prognosis of patients with heart failure [30]. This is why synchrony treatment using CRT technology is effective in improving cardiac function in HF subjects [31]. Similar to CRT, BVP-CCM may also be able to improve biventricular systolic synchrony so as to increase cardiac contraction. This point is supported by our finding that BVP-CCM better alleviated the widening of QRS complex in failing heart than LVP-CCM did. Finally, our results show that at the molecular level, BVP-CCM upregulated SERCA2a and miR-133 to greater extends than single ventricular pacing. This property of BVP-CCM is expected to better improve intracellular Ca^{2+} handling and cardiac

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2014;33:1389-1399

DOI: 10.1159/000358705
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contractile function given the fact that SERCA2a and miR-133 both are key determinants of Ca^{2+} handling process in cardiac cells [10-12, 19, 27, 28] and are both downregulated in failing heart [9-12, 19-22]. SERCA2a is a critical ATPase responsible for Ca^{2+} re-uptake during excitation-contraction coupling, and impaired Ca^{2+} uptake consequent to decreased expression and activities of SERCA2a is a hallmark of heart failure characterized by severe contractile dysfunction both *in vitro* and *in vivo*. Normalization of SERCA2a expression has been shown to restore cardiac functions and ameliorate associated symptoms in pre-clinical as well as clinical studies. Upregulation of SERCA2a by CCM has also been previously documented [32]. Our finding that BVP-CCM resulted in greater SERCA2a expression restoration than LVP-CCM suggests a molecular mechanism for the superiority of BVP-CCM to LVP-CCM. Moreover, our results showed that both BVP-CCM and LVP-CCM partially corrected the abnormal downregulation of the phosphorylated form of phospholamban. This effect is expected to aid enhancing the function of SERCA2a. However, no difference in the strength of effect between BVP- and LVP-CCM was found. Furthermore, consistent with the general view, NCX1 expression was significantly increased in HF and neither BVP- nor LVP-CCM was able to normalize this anomaly. These findings indicate that among these Ca^{2+} handling proteins, BVP-CCM mainly acts on SERCA2a to produce superior improvement of cardiac function.

PLB can generally be phosphorylated at Ser16 by PKA and at Thr17 by Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) [33]. It is known that in failing heart, beta 1-adrenergic receptors are down-regulated accompanied by decreased activity of PKA while intracellular Ca^{2+} is increased accompanied by enhanced activities of CaMKII [34, 35]. These changes are expected to promote Ser16 phosphorylation and diminish Thr17 phosphorylation. We found that both BVP- and LVP-CCM reversed the reduced Ser16 phosphorylation and enhanced Thr17 phosphorylation in our model. These results would indicate that CCM is able to normalize the decreased PKA activity and increased intracellular Ca^{2+} concentration in failing heart, leading to reduced Ser16 phosphorylation and enhanced Thr17 phosphorylation. The opposite changes of phosphorylation between Ser16 and Thr17 sites would likely result in a minimal net change of total PLB phosphorylation thereby of PLB activation. This result indicates that PLB activation has minimal contribution to the improved cardiac contractile function afforded by CCM in our model.

On the other hand, downregulation of miR-133 in failing heart has also been consistently observed by several laboratories [20-22, 27, 28]. Though how this downregulation contributes to heart failure remains incompletely understood, it is known that miR-133 produces cardioprotective effects against myocardial injuries under various conditions via targeting genes related to cardiac contractile function and fibrosis [19, 20, 26, 28, 36-41], except for its potential arrhythmogenic effect [42]. It is therefore likely that correction of miR-133 downregulation in failing heart should give rise to beneficial effects. Nonetheless, it should be noted that upregulation of SERCA2a and miR-133 is merely one of the multiple factors for CCM to produce positive effects on cardiac function in failing heart. Full understanding of the mechanisms of CCM awaits thorough studies in the future.

In summary, our results indicate that electrical stimulation of myocardial during absolute refractory period can increase myocardial contractility and reverse remodeling of chronic heart failure with alleviation of QRS widening. Biventricular stimulation produces greater beneficial effects than single-ventricular stimulation, as the former integrates the mechanisms for both CCM and CRT. This approach may be applied for the treatment of refractory systolic CHF to increase myocardial contractility but not to cause cardiac excitability. However, more rigorous experimentations are definitely required before this approach can be used in clinical practice. Moreover, normalization of SERCA2a/phospholamban and miR-133 expression by correcting the abnormal downregulation of these molecules may contribute to the cardioprotective action of CCM. Yet it is quite conceivable that in addition to SERCA2a other proteins related to intracellular Ca^{2+} handling all have the potential to participate in the cardioprotection afforded by CCM. Future studies should be directed to widen our understanding of these alternative mechanisms.

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2014;33:1389-1399

DOI: 10.1159/000358705
Published online: May 05, 2014

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KARGER

Errata

In the article by Ning et al., entitled "Biventricular Pacing Cardiac Contractility Modulation Improves Cardiac Contractile Function via Upregulating SERCA2 and miR-133 in a Rabbit Model of Congestive Heart Failure" [Cell Physiol Biochem 2014;33:1389-1399 (DOI: 10.1159/000358705)], is a printing error in the affiliations. The corrected authors and their affiliations are stated correctly here.

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