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Improved systolic function of rat cardiocytes during heart failure by overexpression of SERCA2a

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Abstract. – OBJECTIVE: This study shows that overexpression of SERCA2a can improve the systolic function and reduce the occurrence of arrhythmias in cardiocytes isolated from the heart of a rat model of heart failure.

MATERIALS AND METHODS: An animal model of rats experiencing heart failure was established by a surgical procedure producing abdominal aortic coarctation. Cardiocytes from sacrificed rats were isolated by a collagenase digestion method. The SERCA2a adenovirus vector was transfected into the cells after 48h of culture. Overexpression of SERCA2a in cardiocytes was verified by Western Blot. Measurements were taken using a single cell dynamic edge detection system to evaluate the effects on the myocardiocyte function and calcium homeostasis.

RESULTS: Cardiocytes overexpressing SERCA2a displayed a stronger systolic function and lower occurrence rate of abnormal systolic rhythm than mock-transfected cardiocytes. The contraction rhythm abnormality rate percentage was 5.270 ± 1.566% *vs*. 3.955 ± 1.684% ($p < 0.01$). The time at which they reached the maximum contraction (TTP) was 0.095 ± 0.009s *vs*. 0.114 ± 0.008s ($p < 0.01$). The time at which they reached 50% of the diastolic amplitude (R50) was 0.039 ± 0.008s *vs*. 0.057 ± 0.010s ($p < 0.01$). Finally, the occurrence rate of abnormal systolic rhythm during maximal contraction was 58% *vs*. 81% ($p < 0.01$). These results show that all data were significantly improved in the SERCA2a overexpressing group, and that parameters achieved were similar to those in the sham-operated non-heart failure group.

CONCLUSIONS: The overexpression of SERCA2a in cardiocytes during heart failure significantly improves cell function and arrhythmia occurrence.

Key Words:

SERCA2a, Overexpression, Heart failure, Arrhythmia, Cardiocyte function.

Introduction

Heart failure is a progressive and debilitating disease, characterized by a decrease in systolic function. At present, the clinical treatment of heart failure is mainly aimed at alleviating the symptoms in patients, while the subcellular-mechanism that leads to the underlying pathological myocardial remodeling is not targeted. The dysfunction of calcium uptake in the sarcoplasmic reticulum is a common feature in heart failure of human and animal models, which results in abnormal systolic function and calcium homeostasis. The calcium uptake into the sarcoplasmic reticulum in cardiomyocytes is mediated by a sarcoplasmic reticulum Ca^{+}-ATPase, SERCA2a. The activity of the SERCA2a protein determines the exclusion rate of Ca^{2+} in the cytoplasm and the load of Ca^{2+} in the sarcoplasmic reticulum, a key factor in the process of myocardial relaxation and contraction. Studies in animal models with modified SERCA2a genes have shown that SERCA2a plays an important role in the regulation of calcium homeostasis and cardiac physiological activities. When transgenic mice expressed 1.2 or 1.5 times the normal amount of SERCA2a, the transport of Ca^{2+} in the sarcoplasmic reticulum was increased and relaxation and contraction rates were enhanced[1,2]. In SERCA2a gene knockout rats, the homozygote (SERCA2a -/-) did not express the protein and died at an early developmental stage. The heterozygote (SERCA2a +/-)survived with a protein level of SERCA2a decreased by 35% of the normal, but showed a drop in both myocardial contractility and load level of Ca^{2+} in the sarcoplasmic reticulum[3]. This suggests SERCA2a is essential for the maintenance of normal cardiac function,

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and shows that the heart may tolerate an induced overexpression of SERCA2a. Myocardial infarction animal model experiments *in vivo* showed that the overexpression of SERCA2a by transfecting heart failure cells with adenovirus vector can reduce the prevalence of ventricular arrhythmias[4]. Up until now, there have been no reports about SERCA2a gene therapy *in vitro* for the treatment of heart failure myocardial cells. In this study, we make use of a SERCA2a gene adenovirus carrier vector to transfect cardiomyocytes affected with chronic heart failure and show a resulting improvement in the relaxation and contraction function of single cardiomyocytes and the frequency of arrhythmia occurrence.

Materials and Methods

Experimental animals: 200 SD male rats were provided by the experimental animal center of Xuzhou Medical College, each weighed between 180 and 220g.

Reagents and instruments: Recombinant adenovirus empty vector (AV.EGFP) and Recombinant adenovirus vector carrying the SERCA2a gene (AV.EGFP.SERCA2a) were all constructed and packaged by Beijing Vector Gene Technology Co., LTD. SDS-PAGE Gel kits were purchased from Beyotime Biotechnology. MW Marker, Anti-Rabbit IgG, Anti-mouse IgG and Anti-SERCA2a monoclonal antibodies were all purchased from Sigma-Aldrich (St. Louis, MO, USA). BCIP/NBT alkaline phosphatase chromogenic reagent kit was purchased from Promega (Madison, WI, USA). Protease Inhibitor Cocktail was purchased from Merck (Kenilworth, NJ, USA). Major equipment used included FibroScan (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), Gel electrophoresis system (Bio-Rad, Hercules, CA, USA), Half-dry transfer system (Bio-Rad, USA) and Single cell dynamic edge detection system (IonOptix, Milton, MA, USA).

Preparation of the rat model of chronic heart failure and determination of echocardiography: A procedure introducing abdominal aorta coarctation was used to induce chronic heart failure in each rat. After the procedure, each rat was fed for 12 weeks before confirming the heart failure by measurements of cardiac function. GE FibroScan was used for diagnosis, a 10s probe (7 MHz frequency) was used to obtain a clear 2D image of the left ventricular standard area taking the long axis view of the left ventricle near the sternum. M-mode echocardiography was used to record left ventricular systolic and diastolic motion curves on the level of the anterior and posterior leaflets of the mitral valve. IVSEDT, IVSEST, LVEDD and LVESD were measured, and the ejection fractions (EF) were calculated. Rats with EF < 50% who exhibited characteristics such as slow action, fluffy gray hair color and tachypnea were chosen for the heart failure group for the next tests. The rats surviving the preparation procedures were divided into four groups: sham-operated group (15 rats), heart failure group (17 rats), heart failure+EGFP group (17 rats), and heart failure+SERCA2a group (19 rats).

Culturing of isolated rat's cardiomyocytes: The isolated heart perfusion II collagenase digestion method was used to isolate rat cardiomyocytes[5]. The cardiomyocytes were diluted with M199 medium and cultured in a CO_2 incubator.

Calculation of the adenovirus transfection rate of cardiomyocytes: adenovirus-containing liquid was added into cultured cardiomyocytes to achieve an MOI (multiplicity of infection) of 100 (MOI). After 48h of transfection, the cells were observed under an inverted microscope. 5 randomly selected visual fields under the 20×objective lens were chosen, and in each, the number of cells that showed green fluorescence and the total number of cells were counted to calculate the transfection rate (number of green fluorescent cells/number of total cells).

Determination of SERCA2a expression: Cardiomyocytes cultured for 48h after transfection were used to detect SERCA2a by Western blot. Results are shown with half quantitative analysis.

Determination of systolic function of single cardiomyocytes: IonOptix single cell dynamic edge detection system was used to record the length-time changing curve of cardiomyocytes. Cardiomyocytes under test were added into the cell perfusion chamber with KH liquid perfusion. The field stimulation frequency was 0.5 Hz. A single rod-shaped cell with stable contraction amplitude was chosen to record response curves of Ca^{2+} concentration. The concentration was kept at 2 mm, 4 mm or 6 mm gradients until the cardiomyocyte's contraction achieved a maximum amplitude or allorhythmia occurred. IonWizard analysis software was used to get the largest percentage of myocardial contraction amplitude (%), the time to peak (TTP), and the 50% diastolic time (R50)[6].

Statistical Analysis

SPSS software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Data were presented by mean ± standard deviation (X ± SD). One-way analysis of variance was used to make comparisons between groups, and the χ^2 test was used to detect the incidence of contraction disorder. $p < 0.05$ was considered as statistically significant.

Results

The Results of Cardiomyocytes Ransfected with SERCA2a Under Fluorescence Microscope

After 48 h of transfection with either SERCA2a-gene or EGFP-empty vectors, the cardiocytes were observed under a fluorescence microscope; the transfection rate was above 85% for both groups of cells (Figure 1).

The Expression of SERCA2a After Transfection with Cardiomyocytes

Compared with cardiocytes in the heart failure group, the expression of SERCA2a in cardiomyocytes transfected with SERCA2a vector showed significantly increased SERCA2a levels ($p < 0.05$). The level of SERCA2a in cardiocytes transfected with SERCA2a vector and in sham-operated healthy cardiocytes were similar. There was no statistically significant difference in the levels of SERCA2a between SERCA2a-vector and EGFP-vector transfected cells (Figure 2).

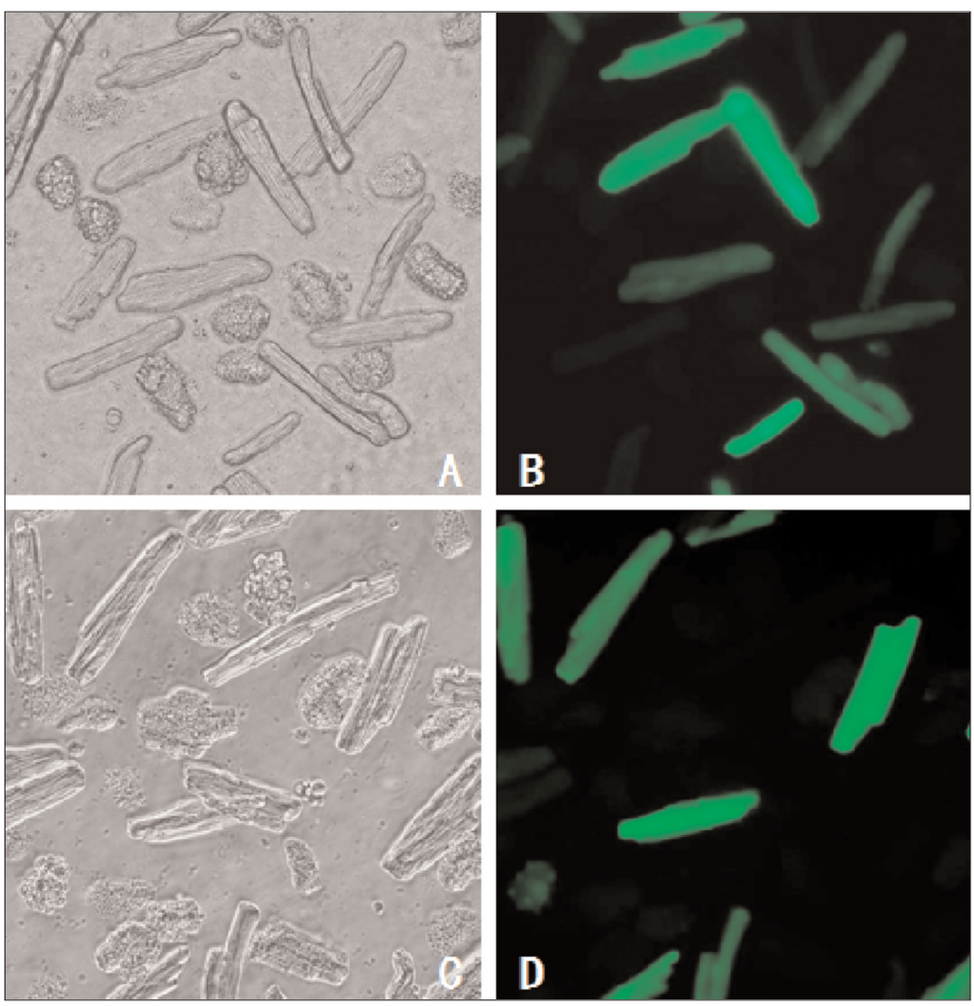

Figure 1. The Expression of Reporter Gene EGFP under 20×Fluorescence Microscope Objective Lens after Cardiomyocytes in Rats with Heart Failure Transfected with Adenovirus Vector for 48h. ***A,*** and ***B,*** the result of heart failure+EGFP group under bright field and fluorescence in the same horizon; ***C,*** and ***D,*** the result of heart failure+SERCA2a group under bright field and fluorescence in the same horizon.

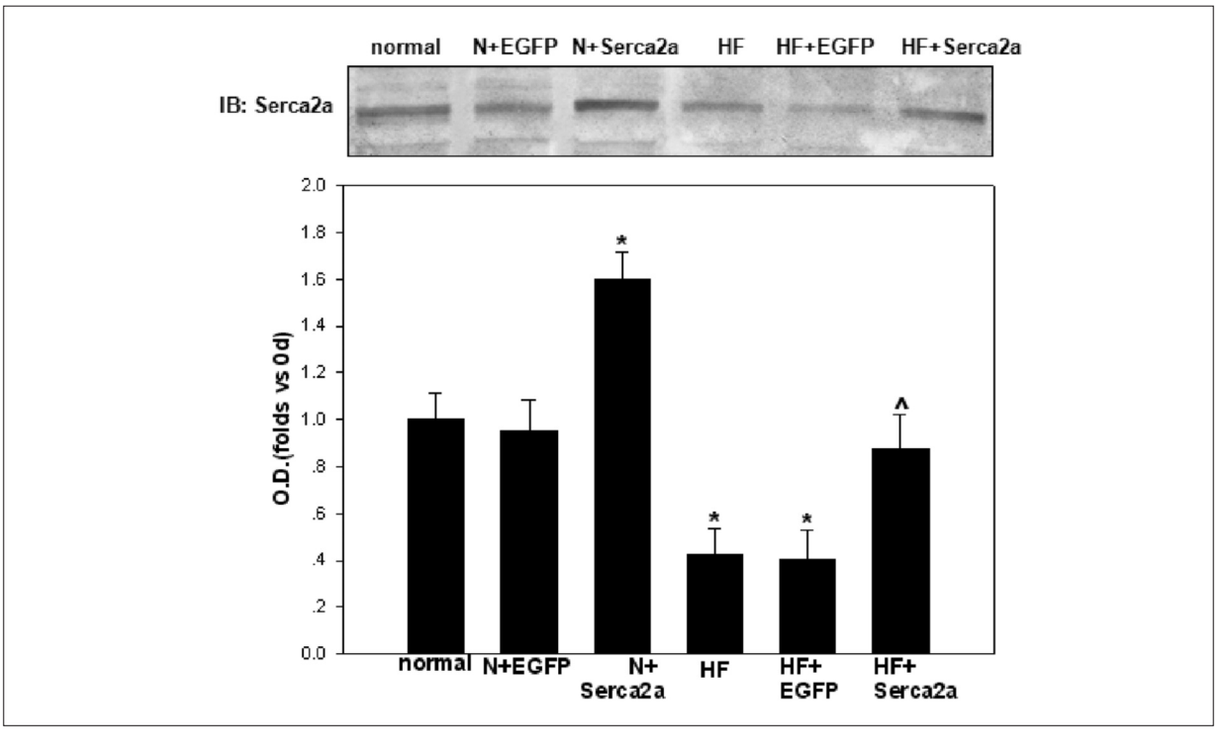

Figure 2. The expression of SERCA2a in cardiomyocytes after transfection.

The effect of SERCA2a Overexpression on Calcium Homeostasis in Cardiomyocytes

The cardiomyocyte's contraction rhythm abnormality rate was 58% in the heart failure+SERCA2a group, which was lower than the same rate of 81% in heart failure group and of 80% in heart failure+EGFR group ($p < 0.01$). Furthermore, the rate in heart failure group was higher than that in sham-operated group ($p < 0.01$). Comparisons between the heart failure+EGFP group and heart failure group, or heart failure+SERCA2a group and sham-operated group yielded results with no statistical differences among them (Figure 3, Table I).

The effect of SERCA2a Transfection on Systolic and Diastolic function of Cardiomyocytes

At the state of base contraction, the percentage of contraction amplitude was 5.270 ± 1.566% in the heart failure+SERCA2a group, which was significantly higher than that in heart failure group (3.955 ± 1.684%) or in the heart failure+EGFR group (3.716 ± 1.631%) ($p < 0.01$). Virtually, no difference was found between the heart failure+SERCA2a group and the sham-operated group (5.472 ± 1.218%) (Figure 4).

The time at which cardiomyocytes reached a maximum contraction amplitude (TTP) in the

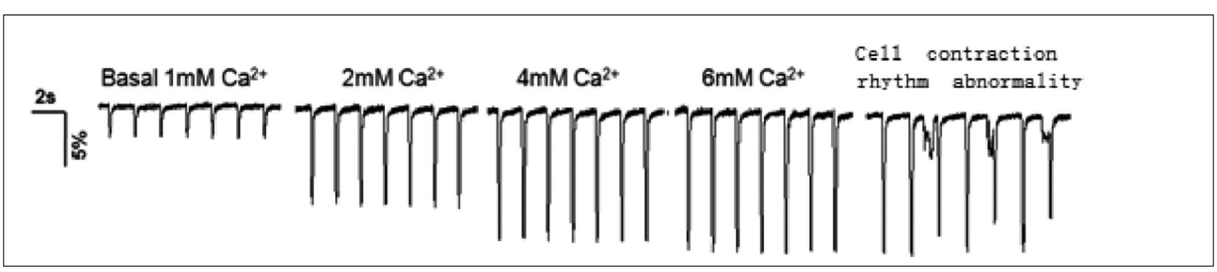

Figure 3. The shrinkage curve of cardiomyocytes in rats with heart failure. With the increase of Ca^{2+} concentration gradients, the contraction amplitude increases, and some cells develop allorhythmia at their maximum contraction.

Table I. The statistical table of allorhythmia occurrence when cardiomyocytes reach the maximum contraction in rats with heart failure.

	Sham-operated group	Heart failure group	Heart failure+ EGFP group	Heart failure + SERCA2a group
Total number of cardiomyocytes (n)	137	151	148	180
Number of cases with allorhythmia (n)	86	122	115	104
Number of cases without allorhythmia (n)	51	29	33	76
Percentage of allorhythmia	63%	81%**	78%**	58%##

Note: $^{**}p < 0.01$ vs. sham-operated group, $^{\#\#}p < 0.01$ *vs*. heart failure group.

heart failure+SERCA2a group was 0.095 ± 0.009s, which was lower than that in the heart failure group (0.114 ± 0.008s), in the heart failure+EGFR group (0.117 ± 0.010s) or the sham-operated group (0.105 ± 0.009s) ($p < 0.01$). While the TTP for the heart failure group was higher than that of the sham-operated group ($p < 0.01$) (Figure 5).

The 50% diastole time (R50) of cardiomyocytes in the heart failure+SERCA2a group was 0.039 ± 0.008s, which was significantly lower than that in heart failure group (0.057 ± 0.010s), the heart failure+EGFR group (0.054 ± 0.011s) or the sham-operated group (0.048 ± 0.010s) ($p < 0.01$). While the RD50 of the heart failure group was higher than that of sham-operated group ($p < 0.01$) (Figure 6).

When comparing the indexes of the heart failure+EGFR group with those of heart failure group, there was no statistical difference among them (Figures 4-6).

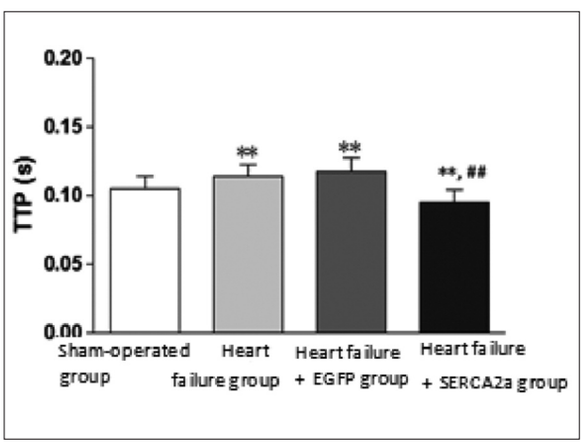

Figure 5. The time that cardiomyocytes reached maximum contraction amplitude (TTP). $^{**}p < 0.01$ *vs*. sham-operated group, $^{\#\#}p < 0.01$ *vs*. heart failure group, $^{\#}p < 0.05$ *vs*. heart failure group.

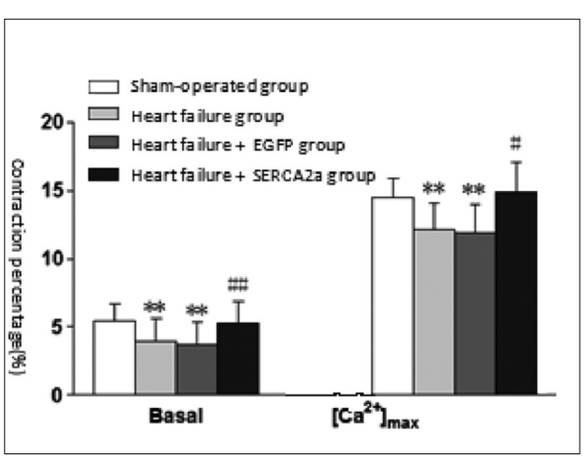

Figure 4. The percentage of contraction amplitude of cardiomyocytes in rats with heart failure. $^{**}p < 0.01$ *vs*. sham-operated group, $^{\#\#}p < 0.01$ *vs*. heart failure group, $^{\#}p < 0.05$ *vs*. heart failure group.

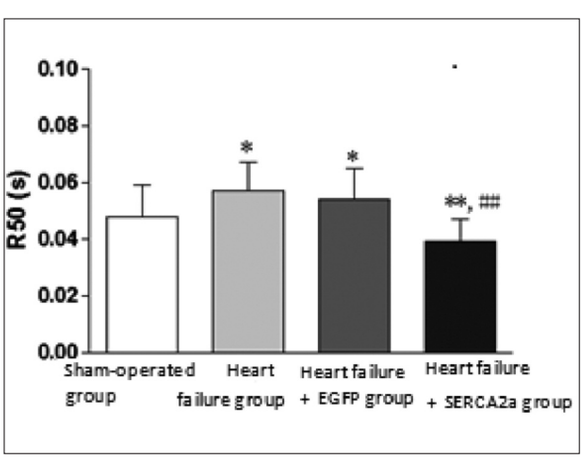

Figure 6. The 50% diastole time (R50) of cardiomyocytes in rats with heart failure. $^{**}p < 0.01$ vs. sham-operated group, $^{\#\#}p < 0.01$ *vs*. heart failure group, $^{\#}p < 0.05$ *vs*. heart failure group.

Finally, under Ca^{2+}, stimulation, the TTP of the heart failure+SERCA2a group was 14.893 ± 2.140%, which was higher than that in the heart failure group (12.136 ± 1.948%) and the heart failure+EGFP group (11.885 ± 2.055%) ($p < 0.05$). And no statistical difference was found when comparing the heart failure+SERCA2a group to the sham-operated group (14.489 ± 1.432%). There was also no statistical difference when comparing the heart failure+EGFR group with the heart failure group (Figure 4).

Discussion

With increasing aging populations, the mortality and morbidity rates of chronic congestive heart failure (CHF) are increasing. The prognosis of a terminal stage is poor; the treatment of choice is limited. At present, treatments mainly target the neuroendocrine pathway to improve the survival rates. However, these methods are flawed in that they cannot really prevent the development of heart failure. There are many studies exploring new targets that should improve processes of excitation-contraction coupling that are affected in heart failure[7,8]. In this study, systolic and diastolic function of cardiomyocytes of rats with heart failure were significantly altered: contraction amplitudes declined, the TTP of reaction rate of systole and diastole, and the R50 were extended, and the expression of SERCA2a was significantly down-regulated, a finding consistent with the performance of the late stages of heart failure.

The expressions of SERCA2a protein, indexes of cell systolic and diastolic amplitude (TTP and R50) were not improved in cardiomyocytes that had only been transfected with EGFR. However, in the cardiomyocytes overexpressing SERCA2a, the expression of SERCA2a protein was increased, the contraction amplitude was enhanced and the time of TTP and R50 were shortened, which indicated that the activity of SERCA2a was enhanced.

After showing that overexpression of SERCA2a can improve the systolic and diastolic function of cardiomyocytes with heart failure, there remained another question to answer – whether it resulted in an increase in the frequency of cardiac arrhythmias. Under heart failure conditions, the spontaneous release of Ca^{2+} during diastole is increased in the sarcoplasmic reticulum of the ventricular muscle. And there is also the possibility that the ryanodine receptor (RyR2) is increased, resulting in a RyR2-mediated Ca^{2+} leak during diastole, which further increases the risk of triggering arrhythmias[9]. Moreover, the activation of β1 adrenergic receptors and the resulting positive inotropic effects should increase the load of Ca^{2+} in the sarcoplasmic reticulum, and increase the risk of calcium leakage mediated by RyR2[4]. The destruction of calcium homeostasis in cardiomyocytes leads to cardiac arrhythmias, which are expressed as contraction rhythm abnormalities in isolated single cardiomyocytes. In this study, it was observed that once the maximal contraction in cardiomyocytes was achieved, the allorhythmia occurrence in cells from rats with heart failure was significantly higher than that in cardiomyocytes from rats in the sham-operated group suggesting that the risk of cardiac arrhythmia was increased in the former group. The occurrence of allorhythmia in cardiomyocytes from the heart failure+EGFP group showed no significant differences compared with those in the heart failure group. But more importantly, the occurrence of allorhythmias in the group that overexpressed SERCA2a was lower than that in heart failure group, which indicates that SERCA2a exerts anti-arrhythmic effects.

Conclusions

Even though the physiological regulation of Ca^{2+} in the myocardium of rodents (such as rats) is different from that in human beings, showing stronger SERCA2a dependence and sensitivity to calcium-dependent cardiac arrhythmias[4], we showed that in cardiomyocytes from heart failure rats, the overexpression of SERCA2a had a beneficial effect on cardiomyocyte function and did not result in an increase in the occurrence of arrhythmias. Together, these results validate SERCA2a as a strong candidate for further studies aimed at generating an effective gene therapy for heart failure in humans.

Sources of Funding

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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