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Reduced SERCA2a converts sub-lethal myocardial injury to infarction and affects postischemic functional recovery

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Abstract

The goal of the present study was to assess how reduced SERCA2a expression affects *in vivo* myocardial ischemia/reperfusion (I/R) injury. We specifically wanted to determine to what extent hearts with reduced SERCA2a levels are susceptible to *in vivo* I/R injury. Therefore, we examined the effects of different ischemic periods on post-ischemic myocardial injury in wild-type (WT) and SERCA2a heterozygous knockout (SERCA2a^{+/-}) mice expressing lower levels of SERCA2a pump *in vivo*. Following 20-min ischemia and 48-hour reperfusion, SERCA2a^{+/-} mice developed significant myocardial infarction (MI) compared to negligible infarction in WT mice (14 ± 3% vs. 3 ± 1%, *P* < 0.01); whereas following 30-min ischemia, the infarction was significantly larger in SERCA2a^{+/-} mice compared to WT mice (49 ± 5% vs. 37 ± 3%, *P* < 0.05). Further, echocardiographic analysis revealed worsened postischemic contractile function in SERCA2a^{+/-} mice compared to WT mice. Thus, these findings demonstrate that maintaining optimal SERCA2a function is critical for myocardial protection from I/R injury and postischemic functional recovery.

Keywords

Sarcoplasmic reticulum; Ca²⁺-ATPase (SERCA); Ischemia; Reperfusion; Myocardial infarction; Contractile function

1. Introduction

Myocardial infarction (MI) is the single largest cause of cardiac death worldwide. Acute myocardial ischemia/reperfusion (I/R) injury is associated with contractile dysfunction, MI, and sudden death [1]. Patients may die from progressive hemodynamic deterioration due to heart failure (HF) or as a result of a new ischemic event such as recurrent angina or MI [2]. Ventricular remodeling and wall thinning are critically dependent on the infarct size, and thus, the MI size is a major determinant of mortality [3]. Therefore, preventing acute MI or limiting infarct size is critical to improve immediate and long-term outcome of patients with acute coronary syndrome and to avoid the development of HF. Recently, we have

demonstrated in isolated hearts that reduced SERCA2a levels worsen postischemic myocardial recovery with increased intracellular Ca^{2+} (Ca^{2+}_i) overload and infarction [4]. Also it has been documented that mice with reduced SERCA2a exhibited an accelerated onset of HF when subjected to pressure overload [5]. Thus, mice with reduced functional SERCA2a are more prone to stress conditions.

While SERCA2a expression is reduced in the failing heart [2,6], the effect of this on susceptibility to further ischemic injury is unknown. Therefore, it is important to determine the effect of reduced SERCA2a on susceptibility to *in vivo* myocardial I/R.

A major goal of this study was to translate our recent *in vitro* findings [4] under *in vivo* conditions to further validate the role of SERCA2a in myocardial I/R. The present study was, thus, designed to determine the effects of both shorter and longer durations of *in vivo* regional ischemia on subsequent MI size and contractile function in both WT and SERCA2a^{+/-} mice.

2. Methods

2.1. Mice

Generation and characterization of heterozygous SERCA2a knockout (SERCA2a^{+/-}) mice have been described previously [7].

2.2. Western blot analysis

Western blotting with cardiac homogenates was performed as described previously [7,8].

2.3. *in vivo* myocardial ischemia/reperfusion (I/R)

in vivo myocardial I/R was performed in male littermates (16–20 weeks) as described [8]. Briefly, mice were anesthetized with intraperitoneal ketamine and xylazine, intubated, ventilated, and chest was opened. The left anterior descending (LAD) coronary artery was ligated by a 7-0 silk ligature. After 20- or 30-min LAD artery occlusion, the knot was released to start reperfusion.

2.4. Echocardiography

In vivo cardiac function was determined 4 days before and 48 hours after reperfusion by M-mode echocardiography as described [4]. Briefly, two-dimensional short-axis view was used, and LV fractional shortening (FS) was calculated as $\text{FS (\%)} = (\text{LVEDD} - \text{LVESD}) / \text{LVEDD} \times 100$.

2.5. Myocardial infarct size measurement

MI was measured after 48 hours reperfusion as described previously with slight modification [8]. Each myocardial slice was photographed, and area at risk (AAR), infarct area (IA) and non risk area were determined by computerized planimetry.

2.6. Data analysis

All results are expressed as the mean $\pm$ SEM. Data were analyzed either by two-tailed Student's *t*-test for paired data from the same experiment or unpaired data from different experiments. Values of $P < 0.05$ were considered statistically significant.

3. Results and discussion

In this study, we test the hypothesis that hearts with reduced SERCA2a (Fig. 1A) are more susceptible to I/R injury *in vivo*. In order to examine this question, we used SERCA2a^{+/-} mice [7] in which expression was ~35% lower than wild type hearts (Fig. 1A). The most striking finding is that when SERCA2a^{+/-} mice were subjected to a sub-lethal I/R, the hearts developed significant MI compared to negligible infarction in WT (Figs. 1B, C). In addition, SERCA2a^{+/-} hearts exhibited significantly larger MI following lethal I/R compared to WT mice (Figs. 2B, C). Importantly, in contrast to WT, echocardiography revealed markedly reduced post-MI fractional shortening in SERCA2a^{+/-} mice from respective pre-MI value (Fig. 2A). These results further confirm our published results with *in vitro* I/R [4]. It has been reported that Ca²⁺_i overload with reduced expression and/or activity of SERCA2a plays a pivotal role in I/R injury [9], and thus, SERCA2a mediated Ca²⁺ transport is of crucial importance in the transition from reversible to irreversible injury during I/R. We reported that SERCA2a^{+/-} hearts had markedly higher Ca²⁺_i at baseline, after ischemia and during early reperfusion [4]. This Ca²⁺ overload was likely due to decreased cytosolic Ca²⁺ removal by SR, because SERCA2a^{+/-} hearts have a lower Ca²⁺ uptake activity than WT hearts [7]. Consistent with a Ca²⁺ overload mechanism, we also observed that isolated SERCA2a^{+/-} hearts had higher incidence of persistent arrhythmias during reperfusion than WT hearts (5/6 vs. 3/6 hearts). In contrast, SERCA overexpression with its attendant efficient Ca²⁺ removal strongly protected the heart against I/R injury, reperfusion arrhythmias and improved diastolic function [8]. Thus, the present *in vivo* study demonstrates for the first time that decreased SERCA2a pump activity could exacerbate a sublethal injury into a lethal injury, and further increase MI following an acute lethal I/R.

These findings have important clinical implications because SERCA2a^{+/-} mice [7] are an ideal model to explore how decreased SERCA2a pump expression can compromise cardiac function during disease states. It was previously shown that SERCA2a^{+/-} mice exhibit accelerated HF when subjected to pressure overload [5]. In the clinical setting of I/R, reduced SERCA2a would be expected to lead to HF with greater morbidity and mortality [2]. Importantly, 1 in 5 patients experiences recurrent angina within a year of hospitalization for CABG surgery, baseline angina, and cardiac revascularization [10]. Thus, patients with prior MI are at greater risk of recurrent MI and exacerbated HF [2,10]. There is now growing evidence that reduced Ca²⁺ transients and contractile dysfunction in human HF and in animal models are due to depressed SR function [2,6,11,12]. Reduced SR Ca²⁺ uptake has also been reported in human myocardium following reversible I/R [13]. Recently, we [8] and others [14,15] have shown that increasing SR Ca²⁺ uptake function by SERCA overexpression strongly protects the heart against I/R injury with efficient Ca²⁺ removal, improved contractile function, and reduced arrhythmias and myocardial infarction. Of note, efficient SR Ca²⁺ uptake and subsequent improved postischemic myocardial function might impede the consequences of Ca²⁺ overload-induced cellular and molecular changes. Recently, DNA micro-array data in rat MI showed that SERCA2 gene transfer increases cardioprotective gene expression and lowers the expression of genes that are known to exacerbate heart failure [15]. Therefore, future studies incorporating DNA microarray, biochemical, morphological and functional data in clinically relevant models will be able to verify these findings and establish the mechanisms of SERCA2a-induced cardioprotection.

In conclusion, the present study provides convincing data to suggest that reduced myocardial SERCA2a activity renders the heart more susceptible to I/R injury, and further reaffirms the critical role and need of optimal SERCA2a function in MI.

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Fig. 1. Myocardial infarction and function in hearts subjected to 20-min LAD ligation and 48-hour reperfusion. (A) Immunoblots and bar graphs for SERCA2a protein levels in WT and SERCA2a^{+/-} hearts, calsequestrin as internal control (N = 3/group). (B) Representative sections of hearts stained with phthalo blue and 2,3,5-triphenyltetrazolium chloride after I/R. (C) Percentage of area at risk (AAR) over left ventricle (LV) and infarct area (IA) over AAR. All values are means $\pm$ SEM. N = 5–6/group; * P < 0.05 vs. WT; ** P < 0.01 vs. WT.

Fig. 2. Myocardial function and infarction in hearts subjected to 30-min LAD ligation and 48-hour reperfusion. (A) FS (%) before (pre-MI) and after MI (post-MI). (B, C) Please see Fig. 1 for description. $N = 5-7/\text{group}$. [†] $P < 0.05$ vs. Pre-MI; ^{††} $P < 0.01$ vs. Pre-MI; ^{*} $P < 0.05$ vs. WT.