Evidence of Reduced Cardiac Calcium Channel Activity and the Reversal of Sodium-Calcium Exchanger in Ischemia/Reperfusion Injury by Manganese-Enhanced MRI

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Introduction

Calcium (Ca^{2+}) cycling is central to the excitation-contraction coupling in the heart. Upon the arrival of a cardiac action potential, Ca^{2+} enters the cell via the voltage-sensitive L-type Ca^{2+} channels, which triggers Ca^{2+} release from the sarcoplasmic reticulum. During relaxation, Ca^{2+} is transported out of the cells via the sarcolemmal Na^{+}-Ca^{2+} exchanger (NCX). However, ischemia/reperfusion (IR) injury causes the reversal of the NCX, leading to intracellular Ca^{2+} overload and cell death. Previously, manganese-enhanced MRI (MEMRI) has been shown to be sensitive to both the L-type Ca^{2+} channels and the NCX[1, 2]. In the current study, we used SEA0400, a selective inhibitor of NCX, to evaluate the L-type Ca^{2+} channel and NCX activities in IR injury.

Methods

Heart Perfusion Protocol Male Sprague Dawley rats were anesthetized. The heart was excised, cannulated, and perfused with the Krebs-Henseleit buffer equilibrated with 95% O_2-5% CO_2 at 37°C. A water-filled latex balloon was inserted into the left ventricle and connected to a pressure transducer to record the left ventricular pressure and heart rate. The rate-pressure product (RPP) was used as an index of the workload. The perfusion column was placed in a vertical bore 9.4T Bruker scanner. Once the setup was finished, the heart was perfused with a modified Krebs-Henseleit buffer containing 30 μM $MnCl_2$ for 30 min (the wash-in period), followed by a 30 min washout period without $MnCl_2$. There were four experimental groups: 1) control: normal Mn^{2+} wash-in and washout protocol (CNTL, n=9); 2) ischemia/reperfusion: 20-min no flow ischemia, followed by Mn^{2+} wash-in and washout protocol (IR, n=6); 3) control+SEA0400: normal perfusion with 1 μM SEA0400 during the Mn^{2+} wash-in period (CNTL+SEA, n=6); and 4) ischemia/reperfusion+SEA0400: 20-min no flow ischemia, followed by Mn^{2+} wash-in and washout protocol with 1 μM SEA0400 during the Mn^{2+} wash-in period (IR+SEA, n=9).

MRI Study MR images were acquired with a 20 mm volume coil. A 1-mm thick short-axis slice at the midventricular level was prescribed for imaging. A triggered saturation recovery Look-Locker sequence was used for rapid T_1 mapping during Mn^{2+} perfusion and washout[3]. During the imaging protocol, the heart was paced at 360 beats/min, and the pacing signal was used to trigger the image acquisition. Imaging parameters were: TE, 2 ms; TR, trigger interval (166 ms); flip angle, 10°; matrix size, 128x64; FOV, 2.5x2.5 cm^2. Prior to Mn^{2+} perfusion, two baseline T_1 maps were acquired. To delineate the kinetics of Mn^{2+} induced contrast enhancement, T_1 maps were acquired continuously at 3 min temporal resolution during the wash-in and washout periods. Myocardial tissues were freeze-clamped at the end of washout for the analysis of Mn^{2+} content by flame atomic absorption spectrophotometry.

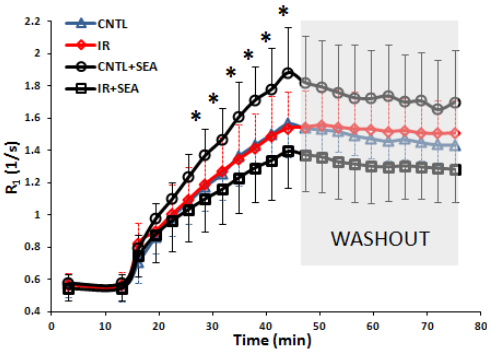

Figure 1. Dynamic changes in relaxation rate.

Results

Changes in relaxation rate (R_1) during the time course of Mn^{2+} perfusion and washout are shown in Fig. 1. All four groups showed progressive increase in R_1 during the washin period. Consistent with our previous observation[2], NCX inhibition led to increased Mn^{2+} uptake in the control hearts. T_1 at the end of Mn^{2+} perfusion was 0.64±0.08 s and 0.54±0.09 s in CNTL and CNTL+SEA hearts, respectively ($p<0.05$). As a result, manganese content in CNTL+SEA group was also significantly higher than that of the CNTL group (9.13±2.5 vs 5.03±0.83 μg/g wet weight, $p<0.001$). These data suggest increased Mn^{2+} retention with NCX inhibition.

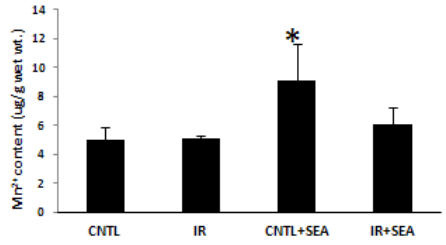

Figure 2. Mn^{2+} content at the end of washout.

Compared to the controls, IR hearts showed decreased Mn^{2+} uptake with NCX inhibition. T_1 at the end of Mn^{2+} wash-in was 0.54±0.09 s and 0.73±0.11 s in CNTL+SEA and IR+SEA hearts, respectively ($p<0.05$). As a result, Mn^{2+} content was also significantly lower in the IR+SEA group (6.09±1.13 μg/g wet weight, $p<0.05$). These data suggest that L-type calcium channel activity was signficant reduced in IR hearts.

Comparison between the two IR group suggests that IR hearts also showed a trend of decreased T_1 reduction with NCX inhibition, which is consistent with the reversal of NCX. T_1 at the end of Mn^{2+} wash-in was 0.66±0.09 s and 0.73±0.11 s in IR and IR+SEA hearts, respectively. However, Mn^{2+} content at the end of washout was not significantly different between IR and IR+SEA hearts (5.07±0.25 vs 6.09±1.13 μg/g wet weight, P=NS). The time course of R_1 in IR hearts was almost identical to that of the controls.

Table 1. Comparison of Mn^{2+} uptake and efflux in control and IR.

	CNTL		IR	
NCX inhibition	-	+	-	+
Mn^{2+} via L_{Ca}	Influx	Influx	Influx ↓	Influx ↓
Mn^{2+} via NCX	Efflux	--	Influx	--
Total Mn^{2+}	Normal	High	Normal	Low

Ventricular function remained constant during image acquisition in the control group, while SEA0400 perfusion induced a small increase in RPP in CNTL+SEA group. There were no statistically significantly different between the CNTL, IR and IR+SEA groups.

Conclusion

IR hearts showed decreased Mn^{2+} uptake via the L-type Ca^{2+} channels, which is compensated by Mn^{2+} uptake via the NCX due to its reversal, leading to the same R_1 dynamics as the controls (Table 1).

Reference

1. Waghorn B. et al., NMR Biomed. **22**:874-881, 2009.
2. Chen Y. et al., ISMRM 2010
3. Li W. et al., Magn Reson Med. **64**:1296-1303, 2010.