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 (Ca²⁺) cycling is central to the excitation-contraction coupling in the heart. Upon the arrival of a cardiac action potential, Ca²⁺ enters the cell via the voltage-sensitive L-type Ca²⁺ channels, which triggers Ca²⁺ release from the sarcoplasmic reticulum. During relaxation, Ca²⁺ is transported out of the cells via the sarcolemmal Na⁺-Ca²⁺ exchanger (NCX). However, ischemia/reperfusion (IR) injury causes the reversal of the NCX, leading to intracellular Ca²⁺ overload and cell death. Previously, manganese-enhanced MRI (MEMRI) has been shown to be sensitive to both the L-type Ca²⁺ channels and the NCX. In the current study, we used SEA0400, a selective inhibitor of NCX, to evaluate the L-type Ca²⁺ 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% O₂-5% CO₂ 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 MnCl₂ for 30 min (the wash-in period), followed by a 30 min washout period without MnCl₂. There were four experimental groups: 1) control: normal Mn²⁺ wash-in and washout protocol (CNTL, n=9); 2) ischemia/reperfusion: 20-min no flow ischemia, followed by Mn²⁺ wash-in and washout protocol (IR, n=6); 3) control+SEA0400: normal perfusion with 1 μM SEA0400 during the Mn²⁺ wash-in period (CNTL+SEA, n=6); and 4) ischemia/reperfusion+SEA0400: 20-min no flow ischemia, followed by Mn²⁺ wash-in and washout protocol with 1 μM SEA0400 during the Mn²⁺ 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 T₁ mapping during Mn²⁺ perfusion and washout. 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 cm². Prior to Mn²⁺ perfusion, two baseline T₁ maps were acquired. To delineate the kinetics of Mn²⁺ induced contrast enhancement, T₁ 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 Mn²⁺ content by flame atomic absorption spectrophotometry.

Results
Changes in relaxation rate (R₁) during the time course of Mn²⁺ perfusion and washout are shown in Fig. 1. All four groups showed progressive increase in R₁ during the washin period. Consistent with our previous observation, NCX inhibition led to increased Mn²⁺ uptake in the control hearts. T₁ at the end of Mn²⁺ 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 Mn²⁺ retention with NCX inhibition.

Compared to the controls, IR hearts showed decreased Mn²⁺ uptake with NCX inhibition. T₁ at the end of Mn²⁺ 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, Mn²⁺ 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 significantly reduced in IR hearts.

Comparison between the two IR group suggests that IR hearts also showed a trend of decreased T₁ reduction with NCX inhibition, which is consistent with the reversal of NCX. T₁ at the end of Mn²⁺ wash-in was 0.66±0.09 s and 0.73±0.11 s in IR and IR+SEA hearts, respectively. However, Mn²⁺ 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 R₁ in IR hearts was almost identical to that of the controls.

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 Mn²⁺ uptake via the L-type Ca²⁺ channels, which is compensated by Mn²⁺ uptake via the NCX due to its reversal, leading to the same R₁ dynamics as the controls (Table 1).

Table 1. Comparison of Mn²⁺ uptake and efflux in control and IR.

<table>
<thead>
<tr>
<th></th>
<th>CNTL</th>
<th>IR</th>
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<tbody>
<tr>
<td>Mn²⁺ via Lca</td>
<td>Influx</td>
<td>Influx ↓</td>
</tr>
<tr>
<td>Mn²⁺ via NCX</td>
<td>Efflux→</td>
<td>Influx↓</td>
</tr>
<tr>
<td>Total Mn²⁺</td>
<td>Normal</td>
<td>High</td>
</tr>
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Reference
2. Chen Y. et al., ISMRM 2010