Manganese Uptake in Heart Is Dependent of L-Type Calcium Channel Activity but Not Extracellular Calcium Concentration

Y. Chen1,2, W. Li1,2, W. Li1,2, and X. Yu1,2
1Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, United States, 2Case Center for Imaging Research, Case Western Reserve University, Cleveland, OH, United States

Introduction
Calcium channel mediated Ca\(^{2+}\) cycling is central to the excitation-contraction coupling (ECC) in heart. Abnormal Ca\(^{2+}\) cycling is associated with contractile dysfunction and arrhythmogenesis. However, current investigation of ECC has largely relied on the characterizing of Ca\(^{2+}\) handling in isolated cells using fluorescence dyes. Manganese is a potent MRI contrast agent that enters the cell through the L-type calcium channels. Manganese-enhanced MRI (MEMRI) thus provides the potential for in vivo evaluation of Ca\(^{2+}\) uptake in myocardium. The objective of this study was to quantify manganese (Mn\(^{2+}\)) uptake in hearts under altered physiological and biochemical conditions. We aimed to investigate that whether altered Ca\(^{2+}\) concentration can also change the dynamics of Mn\(^{2+}\) accumulation in myocardium.

Methods
Heart Perfusion Protocol Male Sprague Dawley rats were anesthetized. The heart was excised, cannulated, and perfused with Krebs-Henseleit buffer equilibrated with 95% O\(_2\) - 5% CO\(_2\) at 37\(^{\circ}\)C. A water-filled latex balloon was inserted into the left ventricle, connected to a pressure transducer to record the left ventricular pressure and heart rate. The rate-pressure product (RPP) was calculated as an index of workload. There were three experimental groups: 1) hearts perfused with 1.5 mM Ca\(^{2+}\) under normal workload (n=5); 2) hearts perfused with 500 nM isoproterenol (ISO) to induce \(\beta\)-adrenergic stimulation (n=6); and 3) hearts perfused with 2.5 mM Ca\(^{2+}\) to increase the workload (n=4). The heart was paced at 360 BPM at baseline and 480 BPM during \(\beta\)-adrenergic stimulation. Once the heart rate and pressure were stabilized, the perfusate was switched to modified Krebs-Henseleit buffer containing 30 \(\mu\)M MnCl\(_2\) for 30 min, followed by a 30 min washout period.

MRI study MRI images were acquired on a 9.4T Bruker vertical scanner (Bruker Biospin Co. Billerica, MA) using 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-Lock sequence was used for rapid T\(_1\) mapping during Mn\(^{2+}\) perfusion and washout period (1). Signals from the pacing instrument were used to trigger the image acquisition. Imaging parameters were: TE, 2 ms; TR, trigger interval (166 ms for baseline, 125 ms for ISO stimulation); flip angle, 10\(^{\circ}\); FOV, 2.5x2.5 cm\(^2\); matrix size, 128x64. 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 (2 min for ISO stimulated hearts) temporal resolution during the 30 min Mn\(^{2+}\) infusion and the following 30 min washout period.

Results
Changes in relaxation rate (R\(_1\)) during the time course of perfusion are shown in Fig. 1. R\(_1\) increased significantly in ISO stimulated hearts. At elevated Ca\(^{2+}\) concentration, changes in R\(_1\) were essentially the same as the baseline with 1.5 mM Ca\(^{2+}\) concentration (Fig. 1).

At a Ca\(^{2+}\) concentration of 1.5 mM, T\(_1\) relaxation time was effectively reduced from 1.76±0.31 s at baseline to 0.65±0.10 s after 30 min Mn\(^{2+}\) perfusion. ISO induced a significant increase in ventricular workload. Average RPP during image acquisition increased from (38.5±10.7) \(\times\)10\(^3\) mmHg/min at baseline to (90.9±27.3) \(\times\)10\(^3\) mmHg/min during ISO stimulation. With increased L-type Ca\(^{2+}\) channel activity induced by ISO stimulation, Mn\(^{2+}\) uptake was also increased, leading to further T\(_1\) reduction to 0.28±0.03 s at the end of Mn\(^{2+}\) infusion (Fig. 2) (P<0.05 compare to no ISO stimulation).

Changes in T\(_1\) during the perfusion with 2.5 mM Ca\(^{2+}\) were similar to that of 1.5 mM Ca\(^{2+}\) perfusion, from 1.73±0.44 s at baseline to 0.55±0.12 s after 30 min Mn\(^{2+}\) perfusion (p=N.S.). Average RPP was also similar at (37.2±1.8) \(\times\)10\(^3\) mmHg/min.

At the end of 30 min washout period, T\(_1\) was 0.33±0.03 s for ISO perfused hearts, and 0.78±0.10 s and 0.73±0.17 s for 1.5 mM and 2.5 mM Ca\(^{2+}\) groups respectively. The slightly increased in T\(_1\) reflected the elimination of Mn\(^{2+}\) from the circulatory buffer and coronary vasculature.

Conclusion
Mn\(^{2+}\) uptake in isolated perfused heart is dependent on altered L-type Ca\(^{2+}\) channel activity but not on Ca\(^{2+}\) concentration in the perfusate. The minimal increase in T\(_1\) relaxation time during washout suggests prolonged Mn\(^{2+}\) retention in myocytes.

Reference