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

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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°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 β-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 β-adrenergic stimulation. Once the heart rate and pressure were stabilized, the perfusate was switched to modified Krebs-Henseleit buffer containing 30 μM $MnCl_2$ 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°; 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) $x10^3$ mmHg/min at baseline to (90.9±27.3) $x10^3$ 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) $x10^3$ 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.

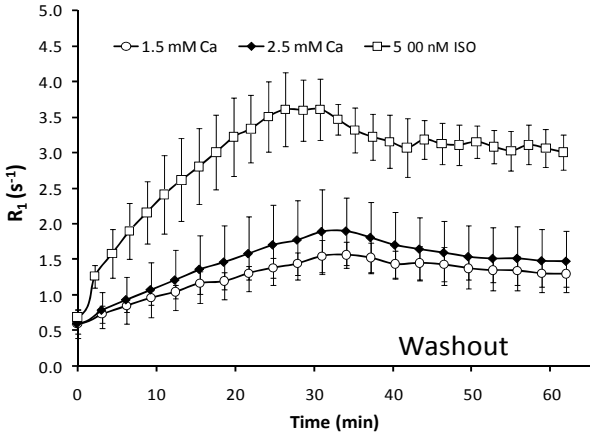

Figure 1. Dynamic changes in relaxation rate (R_1).

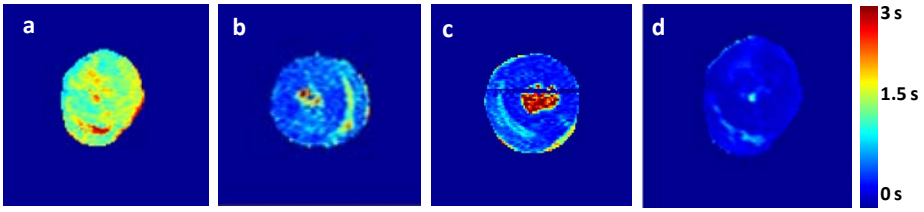

Figure 2. T_1 maps. a. baseline. b-d. 30 min after Mn^{2+} perfusion with 1.5 mM Ca^{2+} (b), 2.5 mM Ca^{2+} (c), and 500 nM ISO (d).

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

1. Li W et al. ISMRM Proc. 2009, No.442.