Quantitative Assessment of L-type Calcium Channel Activity by Manganese-Enhanced MRI

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Calcium (Ca^{2+}) channel mediated Ca^{2+} cycling is central to the excitation-contraction coupling in heart. Manganese-enhanced MRI (MEMRI) allows the assessment of Ca^{2+} uptake via the L-type Ca^{2+} channels in living tissues (1). Previously, we have developed a rapid T_1 mapping method to follow the dynamics of Mn^{2+}-induced R_1 changes in hearts at high temporal resolution (2). In the current study, a mathematical model was developed for quantitative assessment of the L-type Ca^{2+} channel activity by least-squares fitting of the model to experimental MEMRI data acquired from perfused hearts.

Materials and 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. There were four experimental groups: 1) hearts perfused with 1.5 mM Ca^{2+} under normal workload (n=5); 2) hearts perfused with 1.5mM Ca^{2+} and 500 nM isoproterenol (ISO) to induce β-adrenergic stimulation (n=6); 3) and 4) hearts perfused with 2.5 and 3.0 mM Ca^{2+} to increase the workload without altering the the L-type Ca^{2+} channel activity (n=4). 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 30 min washout period.

MRI Study MRI images were acquired on a 9.7T vertical-bore scanner (Bruker Biospec, Germany) 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-Locker sequence was used for rapid T_1 mapping during Mn^{2+} perfusion and washout period. During image acquisition, the heart was paced at 480 BPM during β-adrenergic stimulation and at 360 BPM for the other three groups. The pacing signal was also used to trigger the image acquisition. Imaging parameters were: TE, 2 ms; TR, trigger interval (125 ms for ISO stimulation and 166 ms for the rest); flip angle, 10°; FOV, 2.5x2.5 cm^2; matrix size, 128x64. These parameters allowed continuous acquisition of T_1 maps at 3 min (2 min for ISO stimulated hearts) temporal resolution during Mn^{2+} infusion and washout.

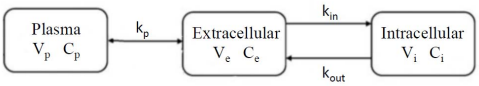

Figure 1. The three-compartment model.

Compartment Model A compartment model was developed that included the plasma, extracellular, and intracellular spaces (Fig. 1). Changes in extracellular (C_e) and intracellular (C_i) Mn^{2+} concentration were described by the following kinetic equations:

$$\frac{dC_e(t)}{dt} = k_p[C_p(t) - C_e(t)] - k_{in}C_e(t) + k_{out}C_i(t)\frac{V_i}{V_e} \quad [1]$$

$$\frac{dC_i(t)}{dt} = k_{in}C_e(t)\frac{V_e}{V_i} - k_{out}C_i(t) \quad [2]$$

where C_p is the Mn^{2+} concentration in plasma, and V_p, V_e, and V_i are the corresponding volume fraction of the three spaces. The relaxation rate (R_1) in each imaging pixel is linearly related to the average Mn^{2+} concentration in that pixel, C_{tot}, as $R_1(t)=R_0+r_1C_{tot}(t)$, where C_{tot} is the volume-weighted sum of Mn^{2+} concentration in each compartment, i.e., $C_{tot}(t)=C_e(t)V_e+C_i(t)V_i+C_p(t)V_p$.

For the perfused heart study, C_p was 30 μM during Mn^{2+} perfusion. R_0 and r_1 were measured experimentally using MRI and flame atomic absorption spectrophotometry. The value of k_p was fixed to a large value (100 min^{-1}) because the exchange between plasma and extracellular spaces was too fast to be resolved from the data. The rate of Mn^{2+} uptake (k_{in}) and efflux (k_{out}) was determined by minimizing the sum-of-squares difference between measured and model-predicted R_1 changes.

Results

Experimentally-determined r_1 was 4.28 $mM^{-1}\cdot s^{-1}$. MRI-measured and model-fitted R_1 curves are shown in Fig. 2. The estimated rate constants are presented in Table 1. ISO stimulation induced a significant increase in Mn^{2+} uptake. As a result, k_{in} for the ISO group was >3-fold higher than the other three groups. However, the quality of the fitting is less satisfactory as compared to the other three groups, suggesting that there might be other mechanisms not involved in the current model.

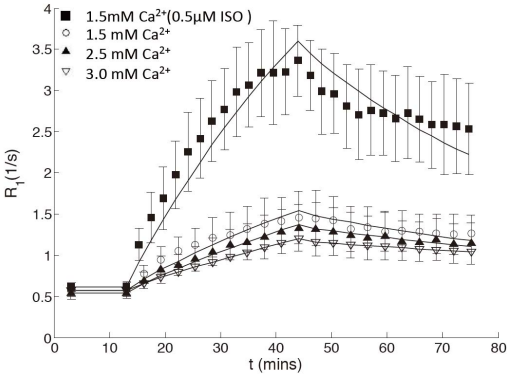

Figure 2. The experiment data and fitted curves of the four study groups.

Mn^{2+} uptake showed a trend of dependence on the Ca^{2+} concentration in the perfusate, with the MRI-measured R_1 increase being slightly slower at high Ca^{2+} concentration. Parameter estimation also showed a progressive decrease in k_{in} as the Ca^{2+} concentration increases. This trend may suggest substrate competition between Ca^{2+} and Mn^{2+} for the entrance through the L-type Ca^{2+} channels.

Conclusion

In the current study, a compartment model for quantitative evaluation of Mn^{2+} uptake and efflux in cardiac myocytes by fitting the model to the MEMRI data. Our results suggest a trend of dependence of Mn^{2+} uptake on the Ca^{2+} concentration.

Table 1. Model-fitted k_{in} and k_{out}.

$[Ca^{2+}]$ (mM)	1.5 (0.5 μM ISO)	1.5	2.5	3
k_{in} (min^{-1})	8.689	2.499	2.025	1.446
k_{out} (min^{-1})	0.021	0.015	0.012	0.008

References

1. Arne Skjold et al. Journal of Magnetic Resonance Imaging 2006; 24:1047–1055
2. Wen Li et al. Magnetic Resonance Imaging in Medicine 2010; 64:1296-1303