Subcellular Distribution of Manganese and Its Impact on Mitochondrial Function in Rat Cardiac Myocytes

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Purpose

Calcium (Ca^{2+}) cycling is central to the excitation-contraction coupling in the heart. Abnormal Ca^{2+} cycling has been implicated in contractile dysfunction. Manganese (Mn^{2+})-enhanced MRI (MEMRI) provides the opportunity for in vivo evaluation of Ca^{2+} uptake in cardiac myocytes. However, the intracellular distribution of Mn^{2+} is not fully delineated. In addition, its impact on subcellular organelle is also not understood. In the current study, we analyzed Mn^{2+} distribution in subcellular organelles after 30 min of Mn^{2+} perfusion, and evaluated the impact of Mn^{2+} on mitochondrial function.

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 and 5% CO_2 at 37°C. 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 $MnCl_2$.

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. 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[1].

Subcellular Fractionation and Oxidation phyosphorylation Ventricular tissues were collected either at the end of 30 min Mn^{2+} perfusion (n=4) or at the end of washout (n=3). The tissues were minced and suspended in modified Chappel-Perry buffer at 4°C. Subsarcolemmal mitochondria (SSM), interfibrillar mitochondria (IFM), and nuclei fraction (NF) were separated by centrifugation as described previously[2]. Mitochondrial function was assessed by measuring the oxygen consumption rate (MVO_2) at state 3 and state 4. Respiratory control ratio (RCR) and P/O ratio were calculated accordingly[3].

Mn^{2+} Quantification Myocardium and separated subcellular organelles were burned in furnace at 600°C for 2 hours. The ashes were dissolved in 14% nitric acid over night. Mn^{2+} content was measured by ICP-OES (Agilent Technologies).

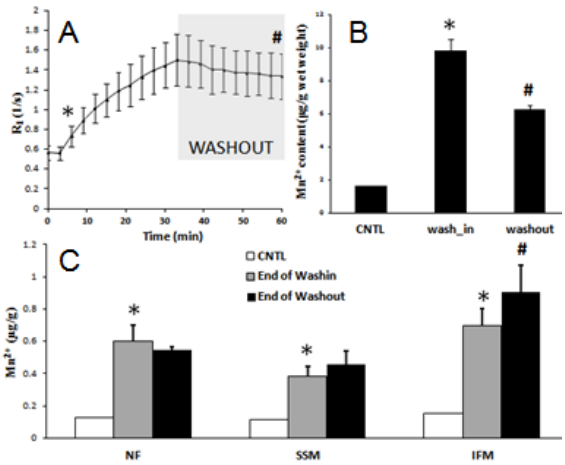

Figure 1. A. R_1 changes during Mn^{2+} perfusion and washout; B. Mn^{2+} content in the whoel myocardium; C. Mn^{2+} content in subcellular compartments. *p<0.05 compare to before Mn^{2+} perfusion, #p<0.05 compare to the end of Mn^{2+} perfusion.

Results

Changes in relaxation rate (R_1) during the time course of Mn^{2+} perfusion and washout are shown in Fig. 1A. R_1 increased progressively during Mn^{2+} perfusion. At the end of Mn^{2+} perfusion, Mn^{2+} content was significantly increased (Fig. 1B). Accordingly, Mn^{2+} content in nuclei fraction and two populations of mitochondria also increased significantly (Fig. 1C).

R_1 showed a rapid decrease in the initial 10 min of the washout period, followed by a slower rate of reduction. Consistent with changes in R_1, Mn^{2+} content at the end of washout also decreased significantly (Fig. 1B, $p<0.01$). Mn^{2+} content in NF remained unchanged. However, Mn^{2+} content in IFM increased significantly during the washout period (Fig. 1C, $p<0.05$). Mn^{2+} content in SSM also showed a trend of increase.

Mn^{2+} accumulation in mitochondria induced a significant increase in MVO_2 at both state 3 and state 4. However, RCR and P/O ratio were unaltered (Table 1)

Table 1. Oxidative properties of isolated mitochondria.

		CNTL	Mn^{2+} perfusion
SSM	State 3 MVO_2	228.45±66.21	464.70±47.66*
	State 4 MVO_2	11.39±3.13	48.75±6.01*
	RCR	21.68±11.77	9.67±2.17
	P/O ratio	2.51±0.06	2.43 ± 0.17
IFM	State 3 MVO_2	298.62±107.64	325.97±143.16
	State 4 MVO_2	18.27±0.66	57.99±43.86*
	RCR	16.25±5.30	8.97±0.90
	ADP/O	2.49±0.02	2.43±0.17

*p<0.05, unit for MVO_2 is nA O/min/mg

Discussion & Conclusion

Our data show that there was continuous Mn^{2+} uptake by the mitochondria during the washout period, which may contribute to the long Mn^{2+} retention in the myocytes. Further, the significantly increased MVO_2 induced by Mn^{2+} suggest that Mn^{2+} may play a similar role as Ca^{2+} in regulating mitochondrial respiration.

References

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