In Vivo Assessment of Calcium Influx in alpha-Dystrobrevin Knock-Out Mouse by Manganese-Enhanced MRI

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Introduction

Calcium (Ca^{2+}) influx through the L-type Ca^{2+} channel plays an important role in regulating cardiac function by directly regulating Ca^{2+} release from sarcolemma reticulum. Recently, manganese (Mn^{2+}), with its dual properties as a T_1 contrast agent and a Ca^{2+} surrogate that enters myocytes through Ca^{2+} channels, has been used for in vivo delineation of changes in Ca^{2+} influx under dramatically altered physiological states such as inotropic stimulation and inhibition of the Ca^{2+} channels [1]. In the current study, we aimed to explore whether in vivo manganese-enhanced MRI (MEMRI) is sensitive to more subtle changes in Ca^{2+} influx by characterizing the α-dystrobrevin knockout ($adbn^{-/-}$) mouse that shows altered Ca^{2+} homeostasis due to disruption in cellular signaling proteins.

Table 1. Signal intensity (SI) enhancement (normalized to external water and baseline SI) and rising rate time constant τ(min^{-1}) in $adbn^{-/-}$ and control mice pre- and post-$MnCl_2$ injection.

		Pre-$MnCl_2$	Post-$MnCl_2$	τ(min^{-1})
Control (n=3)	Normalized SI	1±0.01	1.37±0.02	13.3
	Heart Rate	549±39	506±3	
$adbn^{-/-}$ (n=3)	Normalized SI	1±0.02	1.37±0.03	19.4
	Heart Rate	523±3	508±32	

Methods

Subjects 3-4 months old male (28.3±3.3g) $adbn^{-/-}$ mice (n=3) were characterized in this study. Their healthy littermates (n=3) were used as the controls. The animals were anesthetized with 1% isoflurane. Heart rate was maintained at around 500 bpm with 0.8 – 1.8 % isoflurane. A 126 mM $MnCl_2$ solution was intraperitoneal injected at a rate of 0.2 mL/hr for 30 minutes.

MRI Study T_1-weighted images were acquired on a horizontal 9.4T Bruker scanner (Bruker Biospec, Germany) using fast low flip angle gradient-echo (FLASH) pulse sequence. A 35 mm inner diameter volume coil was used to transmit and receive at ^1H frequency. ECG and respiratory signal was monitored by a physiological monitoring system (SA Instruments, Billerica, MA). A 1 mm thick mid-ventricle short-axis slice was imaged with the following parameters: TE, 1.9 msec; TR, ECG-gated R-R interval (110-130 ms); flip angle, 30°; number of averages, 6; FOV 2.5x2.5 cm^2; matrix size, 128x64; resolution, 195x390 μm^2. Eight baseline images were acquired prior to Mn^{2+} injection. To delineate the kinetics of Mn^{2+}–induced contrast enhancement, T_1-weighted images were acquired continuously at 2 minute temporal resolution during the 30 minutes of Mn^{2+} infusion and the following 30 minutes washout period.

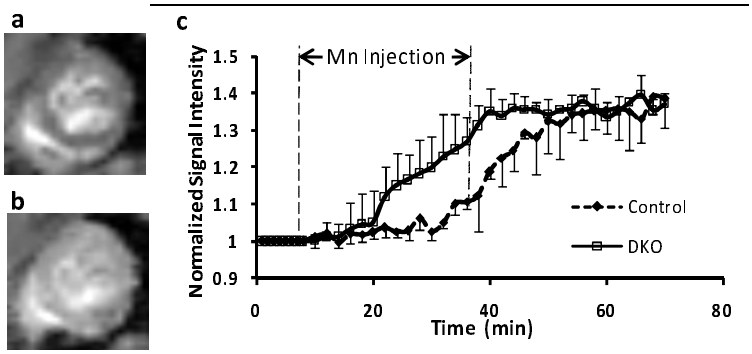

Figure 1. T_1 weighted images before (a) and after (b) Mn^{2+} injection; (c) Time courses of normalized signal intensity. Each point represents the average pixel value taken from the selected LV segment and is normalized to external water and baseline SI.

Data Processing For each T_1-weighted image, signal intensity (SI) in selected segments of left ventricle was normalized to an external water phantom placed on the side of the mouse. Baseline SI was calculated as the mean of eight control images. The time course of SI was further normalized to the baseline SI. The rate constant (τ) describing the rise time for signal enhancement was obtained from first-order exponential fitting to the normalized SI [2].

Measurement of L-type Calcium Current Conventional whole cell patch clamp techniques were used to measure the current through the L-type Ca^{2+} channel in isolated ventricular myocytes. Data acquisition was performed using pClamp 9 software and an Axopatch 200A patch clamp amplifier (Molecular Devices). Patch pipettes had a resistance of 2-4 M. Series resistance compensation (50-80%) was used to minimize voltage control errors. I_{Ca} recordings began 3 min after patch rupture. Electrophysiological experiments were performed under continuous flow conditions at 36.5±0.5°C.

Statistical Analysis All results were expressed as mean ± SD. Unpaired student's *t*-test was used for intergroup comparison. A 2-tailed value of P<0.05 was considered significant.

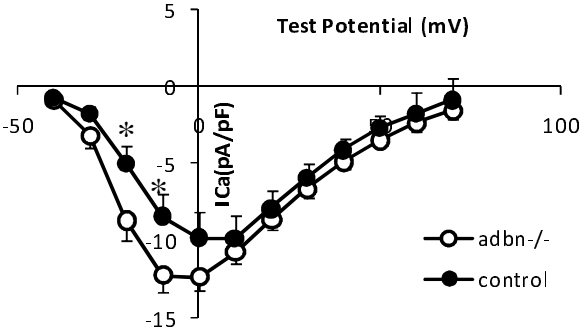

Figure 2. L-type calcium current at test potential from -40mV to +70mV. * P<0.05 compared with $adbn^{-/-}$ group.

Results

The heart rate was similar in both groups before and after Mn^{2+} injection (Table 1). Mn^{2+} led to significant SI enhancement in both $adbn^{-/-}$ and the control mice. Figure 1 shows representative T_1-weighted short-axis images before (Fig.1a) and after 30 minutes of Mn^{2+} injection (Fig.1b). Time courses of SI response to Mn^{2+} injection are shown in Fig.1c. The SI enhancement was observed immediately after Mn^{2+} injection in $adbn^{-/-}$ mice and about 15 minutes later in control group. More specifically, SI increased by 36±2% and 33±5% for $adbn^{-/-}$ and control groups respectively. The $adbn^{-/-}$ mouse showed significantly faster enhancement kinetics than the controls. The rate constant was 19.4 min^{-1} for $adbn^{-/-}$ mouse and 13.3 min^{-1} for the controls (Table 1). Consistent with the earlier and faster response to Mn^{2+} injection, $adbn^{-/-}$ mice exhibited significantly higher L-type calcium influx from whole cell patch clamp characterization (Fig.2).

Conclusion

In current study, $MnCl_2$ injection in both groups showed no adverse impact on cardiac function. Increased calcium influx through the L-type Ca channels, measured by whole cell patch clamp study, was sensitively reflected by earlier and faster Mn-induced signal enhancement for $adbn^{-/-}$ mouse *in vivo*. This study was the first one to show the relationship between the L-type Ca^{2+} influx measured *in vitro* and the Mn^{2+} uptake induced SI enhancement *in vivo*. Our results suggest that MEMRI may serve as a sensitive probe to detect subtle changes in calcium influx associated with altered Ca regulation.

Reference

1. Hu TC et al. *Magn Reson Med* 2001;46:884-90.
2. Yang Y et al. *NMR Biomed* 2008.