Dynamic Manganese-enhanced MRI Reveals Dominant Modulation of Myocardial L-Type Calcium Channel Flux by Neuronal, not Endothelial Nitric Oxide Synthase in Mice

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Introduction

Modulation of L-Type Calcium Channel (LTCC) flux plays an important role in calcium cycling and contractility of cardiomyocytes. Based upon localization within the cardiomyocyte, prevailing opinion is that neuronal nitric oxide synthase (nNOS) modulates sarcoplasmic reticular calcium release, while endothelial nNOS (eNOS) modulates LTCC flux1,3. Counter to this hypothesis, a recent in vitro study suggests that nNOS modulates LTCC flux4. Manganese (Mn2+), a T1 shortening contrast agent for MRI, enters and accumulates in cardiomyocytes through the LTCC in proportion to calcium flux. As a result, Mn-enhanced MRI may be used to probe in vivo LTCC flux. Our prior study showed significantly attenuated contractile reserve but normal perfusion reserve in response to dobutamine (Dob) in nNOS−/− mice5. We tested the hypothesis that, consistent with prevailing opinion, LTCC flux is elevated in baseline and depressed in nNOS−/− mice with Dob.

Methods

All imaging was performed on a 4.7T MRI scanner (Varian, Palo Alto, CA) using a custom built cylindrical Litz coil (Doty Scientific, Columbia, SC) and an MR compatible physiological monitoring and gating system for mice (SA Instruments, Inc., Stony Brook, NY). Wild type (WT), nNOS−/−, and eNOS−/− mice (n = 8 per group) aged 16 ± 1 weeks were studied with Mn-enhanced MRI at baseline (BSL). One week after acquisition of BSL data, measurements were repeated with continuous infusion of 5µg/kg·min Dob into the intraperitoneal (IP) cavity. Body temperature was maintained at 37° C and anesthesia was administered using 1.25% isoflurane in O2.

The uptake of Mn2+ and resultant increase in signal intensity was probed in two mid-ventricular short axis slices using an ECG-gated saturation recovery pulse sequence with constant repetition time (TR) of 200ms. A variable delay was inserted after an ECG trigger to ensure a constant TR between a non-selective saturation pulse and an ECG gated 1mm thick 90° imaging pulse. Imaging parameters included 25.6x25.6mm FOV, matrix size of 128x96, 90º imaging pulse. Imaging parameters included 25.6x25.6mm FOV, matrix size of 128x96, 3 averages. Images were acquired every 2 to 90º imaging pulse. Imaging parameters included 25.6x25.6mm FOV, matrix size of 128x96, 3 averages. Images were acquired every 2 to 3 minutes for 20 minutes prior to and 45 minutes following a 30 minute IP infusion of MnCl2 at a rate of 0.42 mg/kg·min. Signal-to-noise ratio (SNR) was measured from the entire myocardium for each slice and plotted against time (Fig. 1). The portion of the SNR vs. time curve corresponding to MnCl2 infusion was isolated (solid gray line) and the slope of the linear fit to that data was used as an index of LTCC flux (LTCCI). Additionally, systolic blood pressure (BP) was measured using tail cuff system.

Results

Basal LTCCI (mean ± SEM) was significantly higher in nNOS−/− (0.94 ± 0.1 A.U./min) (Fig. 2) than in both eNOS−/− (0.68 ± 0.05 A.U./min, p < 0.05 vs. nNOS−/−) and WT mice (0.52 ± 0.04 A.U./min, p < 0.01 vs. nNOS−/−). In contrast, LTCCI did not differ significantly between groups in the presence of Dob (0.83 ± 0.04 A.U./min nNOS−/−, 0.85 ± 0.12 A.U./min eNOS−/−, 1.03 ± 0.06 A.U./min WT). Heart rate was similar in all groups at BSL (490 ± 29 nNOS−/−, 470 ± 16 eNOS−/−, 481 ± 18 WT, P=NS) and increased in the presence of Dob in all groups (522 ± 19 nNOS−/−, 503 ± 19 eNOS−/−, 560 ± 18 WT, P=NS). Higher blood pressure response was found in eNOS−/− mice compared to nNOS−/− (94 ± 3 nNOS−/−, 111 ± 4 eNOS−/−, 106 ± 4 WT, P=0.01 eNOS−/− vs. nNOS−/−).

Conclusions

Our results suggest that, counter to the prevailing model of nitric oxide signaling, nNOS and not eNOS plays a more important role in modulating basal LTCC flux. The modest increase in LTCCI in eNOS−/− mice may be attributable to higher blood pressure rather than modulation of LTCC flux by eNOS. Interestingly, despite heightened basal LTCC flux, nNOS−/− mice demonstrate similar in vivo basal contractile function5. Furthermore, the absence of an in vivo contractile reserve in nNOS−/− mice in response to Dob mirrors the absence of an LTCCI response to Dob seen in this study. LTCCI in eNOS−/− mice did not differ significantly from WT mice at both BSL and with Dob, suggesting that modulation of LTCC flux by eNOS may belong to a separate signalling pathway.

References

(2) Hare et al. J. Mol Cell Cardiol. 2003; 35(7):719-729.

Figure 1. (Top) Sample images from a WT mouse with and without Dob. The slope of the increase in SNR (underlined) during MnCl2 infusion shows increased LTCC flux during infusion of Dob.

Figure 2. (Top) Baseline LTCCI is significantly higher in nNOS−/− mice compared to WT and eNOS−/− mice. (Bottom) Increased LTCCI accompanying Dob seen in WT and eNOS−/− mice are not seen in nNOS−/− mice.