Mg Inhibits Spontaneous SR Ca Release and Modulates Calmodulin Binding to RyR2

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INTRODUCTION

The sarcoplasmic reticulum (SR) ryanodine receptor channel (RyR2) is responsible for the Calcium (Ca) release that activates cardiac contraction, influences ionic currents and when abnormal can lead to cardiac arrhythmias and heart failure. Lipid bilayer and SR vesicle studies showed that magnesium (Mg) strongly inhibits Ca release from the SR. However, the effect of Mg on RyR2 function within cardiac myocytes is not well studied. Neither is the effect of Mg on RyR2 binding by regulatory accessory proteins such as calmodulin (CaM), which binds to and inhibits RyR2 channel gating. In this project we assessed the effects of Mg on spontaneous SR Ca release at graded concentrations of 0.1 mM Mg (low), 1.0 mM Mg (physiological), and 3 mM Mg (high). We also studied the effects of Mg on binding affinity of wild-type (wt) CaM and two CPVT CaM mutants (N54I and N98S) in the native myocyte environment.

MATERIAL & METHODS

Ca2+ Sparks Measurements

Freshly isolated mouse ventricular cardiomyocytes were permeabilized by short exposure (30 seconds) to saponin (50 μg/ml) and superfused by internal solution with free 50 mM [Ca2+]p and 10 μM Fluo-4. Ca sparks were measured using a confocal microscope (BioRad, Radiance 2100, 40X objective) with line scan mode. SR Ca2+ content was evaluated by the Ca2+ transient on rapid caffeine application (10 mM).

CaM Affinity to RyR2

Mouse ventricular cardiomyocytes were permeabilized by 50 μg/ml saponin for 3 minutes and then superfused by internal solution with free 10 mM [Ca2+]p to wash away endogenous FKBP and CaM. The cells were then incubated for at least 1 hour with 100 nM FKBP labeled with Alexa Fluor 488 and varying concentrations of CaM labeled with Alexa Fluor 568. Fluorescent images were taken with a confocal microscope (BioRad, Radiance 2100, 40X).

RESULTS

Mg Decreases Ca Sparks Frequency

Ca sparks were imaged in line scan mode using a confocal microscope. Ca sparks were measured at 50 nM free Ca. Three Mg concentrations were tested: 0.1 mM (low), 1 mM (physiological), and 3 mM (pathologically high).

Mg Increases SR Ca Load and Ca Sparks Amplitude

Increasing [Mg] in the presence of endogenous wild type (wt) CaM correlated with an increase in SR Ca load and a concurrent increase in the amplitude of Ca sparks.

Measurement of CaM Affinity for RyR2 with FRET

A.

B.

The affinity of wtCaM to RyR2 peaked at 1 mM Mg and decreased for both 0.1 mM Mg and 3.0 mM Mg. This CaM-RyR2 binding affinity was also consistent for two CPVT CaM mutants (N54I and N98S) at 0.1 mM, 1.0 mM, and 3.0 mM Mg.

CONCLUSIONS

Our findings suggest that Mg reduces spontaneous SR Ca release and increases SR Ca load. Under physiological Mg concentration (0.1-1 mM), higher Mg inhibits RyR2 opening and increases CaM-RyR2 binding affinity. On the other hand, in pathologic Mg concentration (3 mM), Mg inhibits RyR2 opening despite reduced CaM-RyR2 binding affinity. Thus [Mg] influences the ability of CaM to quiet diastolic SR Ca release in a biphasic manner.

REFERENCES


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