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Therapeutic Potential of GSTM2-2 C-Terminal Domain and Its Mutants, F157A and Y160A on the Treatment of Cardiac Arrhythmias: Effect on Ca2+ Transients in Neonatal Ventricular Cardiomyocytes

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Abstract : The ryanodine receptor (RyR) is an intracellular ion channel that releases Ca2+ from the sarcoplasmic reticulum and is essential for the excitation-contraction coupling and contraction in striated muscle. Human muscle specific glutathione transferase M2-2 (GSTM2-2) is a highly specific inhibitor of cardiac ryanodine receptor (RyR2) activity. Single channel-lipid bilayer studies and Ca2+ release assays performed using the C-terminal half of the GSTM2-2 and its mutants F157A and Y160A confirmed the ability of the C terminal domain of GSTM2-2 to specifically inhibit the cardiac ryanodine receptor activity. Objective of the present study is to determine the effect of C terminal domain of GSTM2-2 (GSTM2-2C) and the mutants, F157A and Y160A on the Ca2+ transients of neonatal ventricular cardiomyocytes. Primary cardiomyocytes were cultured from neonatal rats. They were treated with GSTM2-2C and the two mutants F157A and Y160A at 15μM and incubated for 2 hours. Then the cells were led with Fluo-4AM, fluorescent Ca2+ indicator, and the field stimulated (1 Hz, 3V and 2ms) cells were excited using the 488 nm argon laser. Contractility of the cells were measured and the Ca2+ transients in the stained cells were imaged using Leica SP5 confocal microscope. Peak amplitude of the Ca2+ transient, rise time and decay time from the peak were measured for each transient. In contrast to GSTM2C which significantly reduced the % shortening (42.8%) in the field stimulated cells, F157A and Y160A failed to reduce the % shortening.Analysis revealed that the average amplitude of the Ca2+ transient was significantly reduced ($P<0.001$) in cells treated with the wild type GSTM2-2C compared to that of untreated cells. Cells treated with the mutants F157A and Y160A didn't change the Ca2+ transient significantly compared to the control. A significant increase in the rise time ($P< 0.001$) and a significant reduction in the decay time ($P< 0.001$) were observed in cardiomyocytes treated with GSTM2-2C compared to the control but not with F157A and Y160A. These results are consistent with the observation that GSTM2-2C reduced the Ca2+ release from the cardiac SR significantly whereas the mutants, F157A and Y160A didn't show any effect compared to the control. GSTM2-2C has an isoform-specific effect on the cardiac ryanodine receptor activity and also it inhibits RyR2 channel activity only during diastole. Selective inhibition of RyR2 by GSTM2-2C has significant clinical potential in the treatment of cardiac arrhythmias and heart failure. Since GSTM2-2C-terminal construct has no GST enzyme activity, its introduction to the cardiomyocyte would not exert any unwanted side effects that may alter its enzymatic action. The present study further confirms that GSTM2-2C is capable of decreasing the Ca2+ release from the cardiac SR during diastole. These results raise the future possibility of using GSTM2-2C as a template for therapeutics that can depress RyR2 function when the channel is hyperactive in cardiac arrhythmias and heart failure.

Keywords : arrhythmia, cardiac muscle, cardiac ryanodine receptor, GSTM2-2
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