F157A and Y160A substitutions in the helix 6 Region of GSTM2-2 C terminus reduces the inhibitory action of helix 6 on RyR2 channels

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The ryanodine receptor (RyR) Ca^{2+} release channel, located in the membrane of the internal sarcoplasmic reticulum Ca^{2+} store, is central to Ca^{2+} signalling and contraction in skeletal and cardiac muscle (Abdellatif *et al.,* 2007). Some glutathione transferases (GSTs) and structurally related proteins are potent modulators of ryanodine receptor (RyR) Ca^{2+} release channels. Recently it was discovered that muscle specific GSTM2-2 inhibits cardiac muscle ryanodine receptors (RyR2), but not skeletal muscle ryanodine receptors (RyR1) (Dulhunty *et al.*, 2001). The selective inhibition of RyR2 by GSTM2-2 has significant clinical potential in the treatment of chronic heart failure. Data obtained thus far suggests that the main inhibitory effect of GSTM2-2 on RyR2 is associated with α-helix 6. Since the flexibility and exposure of α-helix 6 may be important for membrane entry and for the modulation of RyR2 by GSTM2-2, specific residues in the C terminal domain, within α-helix 6 were mutated. Effects of these modifications on SR Ca^{2+} release and single channel experiments with lipid bilayers were evaluated.

Recombinant GST M2-2 C terminus and its mutants, F157A and Y160A were expressed and purified using the vector, pHUE. Cardiac SR vesicles were prepared from sheep heart following euthanasia by barbiturate overdose. Levels of extravesicular Ca^{2+} were monitered with antipyrylazo III, a Ca^{2+} indicator, at 710 nm using a Cary 3 spectrophotometer. Ryanodine receptor channel activity was measured using single channel lipid bilayer technique. The degree of helicity of the secondary structure of the wild type and the mutants were determined using Circular Dichroism spectroscopy.

Effects of the GSTM2-2 C terminus and its mutants, F157A and Y160A on cardiac SR Ca^{2+} release revealed that, compared to the control, all three molecules reduced the caffeine-induced Ca^{2+} release significantly ($p < 0.001$). But the inhibition produced by the mutants was significantly less ($p < 0.05$) than that produced by the wild type protein. Open probability at +40 mV was significantly reduced ($p < 0.05$) when the mutant F157A was added to the *cis* bath solution when single channel lipid bilayer experiments were conducted with cardiac SR vesicles. This mutant didn't have any voltage dependent effect on RyR2, since it showed a similar reduction in the open probability of 17.5% at -40 mV. The mutant, Y160A, when added to the *cis* bath solution produced a voltage dependent inhibition of the RyR2 with a significant open probability decrease (30%, $p < 0.05$) at +40 mV only. Similar to the results obtained for the Ca*2+* release assay, the relative open probability at +40 mV produced a significantly ($p < 0.05$) less inhibition with the mutants, F157A and Y160A, compared to the wild type GSTM2-2 C terminus. Inhibition produced by the wild type was not voltage dependent. CD spectrum revealed that the secondary structure of all three molecules has a high content of alpha helical content. In conclusion, F157A and Y160A, mutants of the GSTM2 C terminal domain have an inhibitory effect on cardiac ryanodine receptor activity but this differs from the effect of the wild type protein in magnitude (F157A and Y160A) and voltage dependence (Y160A). These differences are consistent with our hypothesis that helix 6 is important in inhibition of RyR2 and suggest that destabilizing the interaction between helix 6 and C terminal domain reduces the inhibitory action of helix 6. Since destabilization of helix 6 interactions should facilitate membrane entry, the results suggest that membrane entry is not essential for RyR2 inhibition.

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