The variably spliced ASI region of RyR1 contains a basic α-helix domain that modifies EC coupling

A.F. Dulhunty,[1] T. Kimura,[2] J.D. Lueck,[3] P.J. Harvey,[1] S.M. Pace,[1] N. Ikemoto,[4] M.G. Casarotto[1] and R.T. Dirksen,[3] [1]JCSMR, ANU, PO Box 334, ACT, 2601, Australia, [2]Hyogo College of Medicine, 1-1 Mukogawa-cho Nishinomiya, HYOGO 663-8501, Japan, [3]University of Rochester Medical Center, PO Box 711, 601 Elmwood Avenue, Rochester, NY, 14642 USA and [4]Boston Biomedical Research Institute, Watertown, MA, USA.

The function of the alternatively spliced ASI residues (Ala^{3481}-Gln^{3485}) in the skeletal muscle ryanodine receptor (RyR1) Ca^{2+} release channel has been examined. The residues are present in the adult (ASI(+)RyR1) isoform but absent in the juvenile (ASI(-)RyR1) splice variant. ASI(−)RyR1 is over-expressed in myotonic dystrophy type 1 (DM1) and is less active than ASI(+) (Kimura *et al.*, 2005). The ASI region contributes to an inhibitory inter-domain interaction which is stronger in ASI(−)RyR1 since its interruption by ASI domain peptides causes greater activation in AS1(−)RyR1 (Kimura *et al.*, 2007). We predicted that interruption of this ASI interaction may contribute to excitation-contraction (EC) coupling and if it did, EC coupling would be stronger in AS1(−)RyR1. The ASI domain peptides include the ASI residues and a sequence of 5 contiguous positively charged residues which bind to the β1a subunit of the skeletal dihydropyridine receptor (DHPR) and whose deletion depresses EC coupling (Cheng *et al.*, 2005). We predicted that this sequence may contribute to ASI peptide activity. Interruption of an inhibitory inter-domain interaction by domain peptide DP4 also activates RyR1 (Yamamoto *et al.*, 2000). Based on their positions in the RyR1 sequence, we predicted that the ASI and DP4 regions must support different inter-domain interactions.

EC coupling was examined in RyR-null myotubes injected with cDNA for ASI(−)RyR1 or ASI(+)RyR1. Intracellular Ca^{2+} was measured in intact myotubes loaded with fura-FF AM. Voltage-gated L-channel activity and SR Ca^{2+} release were measured simultaneously using whole cell patch clamp. The structure of the ASI peptides was examined using nuclear magnetic resonance (NMR). Ca^{2+} release from isolated skeletal SR vesicles was measured using spectrophotometry. RyR activity was assessed from [^{3}H] ryanodine binding (which increases when channel open probability increases) or from RyR channels incorporated into artificial lipid bilayers.

Ca^{2+} release during EC coupling was greater in myotubes expressing ASI(−)RyR1 than in those expressing ASI(+)RyR1. The L-type Ca^{2+} current was similar in both ASI(−)RyR1 and ASI(+)RyR1 expressing myotubes (indicating similar DHPR expression, function and alignment with RyRs). As with caffeine (Kimura *et al.*, 2005), maximal 4-chloro-m-cresol induced Ca^{2+} release was less for ASI(−)RyR1. Total SR Ca^{2+} load and resting cytoplasmic Ca^{2+} concentrations were the same in both cases. These results were consistent with ASI(−) region supporting a stronger inter-domain interaction with greater inhibition, which then allowed greater activation when the interaction was interrupted during EC coupling.

The NMR-derived structures of ASI(−) and ASI(+) peptides both have random coil N-terminal regions bracketing (Ala^{3481}-Gln^{3485}) and an α-helical C-terminal part spanning the 5 contiguous basic residues. The activity of the ASI peptides on RyR1 activity was critically dependent on the basic residues and their inclusion in an α-helical structure. The structure and action of the ASI peptides mimicked that of the basic α-helical ***A*** region of the DHPR α1s II-III loop. In addition, the ASI peptides competed with peptide ***A*** for RyR1 activation. It remains to be determined whether this similarity between the DHPR α1s II-III loop and the ASI region is co-incidental or whether it has a functional significance in the intact cell. RyR activation by the ASI peptides and DP4 exhibited different Ca^{2+}-dependence and the effects of the two domain peptides were additive, suggesting that they acted at separate sites on RyR1 and interrupted distinctly different inter-domain interactions.

The results show that the ASI residues have a strong influence on the efficacy of EC coupling which is stronger when they are deleted. Our findings further indicate that disruption of an inter-domain interaction involving the ASI region may play a role in EC coupling. Overall, the data suggest that enhanced Ca^{2+} release during EC coupling may contribute both to developmental changes in Ca^{2+} release and to the myopathy in DM1.

Kimura T, Nakamori M, Lueck JD, Pouliquin P, Aoike F, Fujimura H, Dirksen RT, Takahashi MP, Dulhunty AF & Sakoda S. (2005) *Human Molecular Genetics*, **14:** 2189-200.

Kimura T, Pace SM, Wei L, Beard NA, Dirksen RT & Dulhunty AF. (2007) *Biochemical Journal*, **401:** 317-24.

Yamamoto T, El-Hayek R & Ikemoto N. (2000) *Journal of Biological Chemsitry*, **275:** 11618-25.

Cheng W, Altafaj X, Ronjat M & Coronado R. (2005) *Proceedings of the National Academy of Science USA*, **102:** 19225-30.