Aberrant splicing of ryanodine receptor reduces Ca^{2+} release *via* an inter-domain interaction in myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a multisystem disorder with autosomal dominant inheritance. Expansion of CTG repeats in the 3' untranslated region of a putative protein kinase gene occurs in DM1. Downstream, it was reported that several mRNAs were aberrantly spliced in muscles from DM1 patients, but the cause of muscle weakness is unknown. We investigated splicing of two major proteins of the sarcoplasmic reticulum, the ryanodine receptor 1 (RyR1) and sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase. The fetal variants, ASI(-) of RyR1 which lacks residue 3481-3485, and SERCA1b which differs at the C-terminal were significantly increased in skeletal muscles from DM1 patients and the transgenic mouse model of DM1 (HSA^{LR}).

To examine the functional difference between the ASI(+) and the ASI(-) RyR1 isoforms, we characterized [^{3}H]ryanodine binding to microsomal vesicles of HEK293T cells transfected with ASI(+) and ASI(-) RyR constructs. [^{3}H]ryanodine binding is a standard technique for assessing the open probability of RyR channels, because ryanodine binds solely to open channels and the binding is proportional to open probability. Channel open probability was also measured form RyRs incorporated into using artificial lipid bilayers. Finally Ca^{2+} release was examined using Ca^{2+} imaging techniques in dypedic myotubes (lacking RyR1) transfected with ASI(+) and ASI(-) RyR1 cDNA.

The affinity of [^{3}H]ryanodine binding to ASI(+) was higher than that to ASI(-). Channel open probability was significantly decreased and mean open time was significantly shorter in ASI(-) than in ASI(+). Consistent with the lower activity of ASI(-) channels, the RyR1-knockout myotubes expressing ASI(-) exhibited a decreased incidence of Ca^{2+} oscillations during caffeine exposure compared with that observed form myotubes expressing ASI(+) RyR (Kimura *et al.*, 2005). To determine how this aberrant splicing affects the activity of RyR channels, we tested whether the splicing region is involved in inter-domain interaction using synthetic peptides (Yamamoto *et al.*, 2000). Both peptides corresponding to the Thr(3471)-Gly(3500) around the ASI region in the presence (ASI(+)) and the absence (ASI(-)) of exon ASI activated native RyRs. However, peptide ASI(-) activated the channels more than peptide ASI(+).

The results suggest that ASI(-) peptide interrupts an inhibitory interdomain interaction in the native RyR more strongly than the ASI(+) peptide. We therefore suggest that ASI(-) region may interact more tightly with other domains and produce stronger inhibition of ASI(-) RyR, resulting in reduced activity of the ASI(-) RyR.

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