Molecular mechanism of type 1 ryanodine receptor-linked muscle diseases: toward diagnosis and therapy

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Type 1 ryanodine receptor (RyR1) is a Ca\(^{2+}\) release channel in the sarcoplasmic reticulum of skeletal muscle and is mutated in several diseases, including malignant hyperthermia (MH) and central core disease (CCD). Most MH and CCD mutations cause accelerated Ca\(^{2+}\) release, resulting in abnormal Ca\(^{2+}\) homeostasis in skeletal muscle. However, how specific mutations affect the channel to produce different phenotypes is not fully understood. We have recently developed a method to quantitatively evaluate the CICR activity of RyR1 channels by \[^{3}H\]ryanodine binding and ER luminal Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{\text{ER}}\)) measurements (Murayama et al., 2015). Using this method, we analyzed RyR1 channels carrying different MH and CCD mutations in the amino-terminal and central regions by expressing them in HEK293 cells (Murayama et al., 2015; Murayama et al., 2016). The mutations divergently affected two parameters for CICR, i.e., the gain that determines the attainable maximum activity and the sensitivity to activating Ca\(^{2+}\) in a site-specific manner. The CICR activity of the mutants correlated well with the severity of diseases. Based on these observations, we have developed a novel screening method for drugs inhibiting the RyR1 channel. [Ca\(^{2+}\)]\(_{\text{ER}}\) of HEK293 cells expressing wild-type (WT) or mutant RyR1s were monitored with R-CEPIA1er, a genetically-encoded Ca\(^{2+}\) indicator (Suzuki et al., 2014). We screened 1,535 compounds in a library of well-characterized drugs and identified four compounds that specifically increased [Ca\(^{2+}\)]\(_{\text{ER}}\) of the cells expressing mutant RyR1. Dose dependency and subtype specificity of these compounds found a candidate for novel RyR1 inhibitor. This novel screening method will be useful for exploring RyR1 inhibitors.

