Two key regions of the human cardiac ryanodine receptor calcium release channel modulate its gating properties in a dual manner

C. Viero1; J. Euden2; SA. Mason2; MK. Seidel2; NL. Thomas2; S. Zissimopoulos2; AJ. Williams2

1Saarland University Hospital, Experimental and Clinical Pharmacology and Toxicology, Hamburg, Germany; 2Wales Heart Research Institute, Cardiff, United Kingdom

Ryanodine receptors type 2 (RyR2) regulate excitation-contraction coupling. Their dysfunction leads to pathological states, such as arrhythmia, heart failure and sudden death. Our homology model detected comparable structural determinants in the pores of the K+ channel KcsA and RyR2. A Glycine hinge motif in the inner transmembrane helix of many K+ channels is also found in RyR2. We tested the hypothesis that the Glycine at this position (4864) was critical for RyR2 function, as it is in K+ channels. A single substitution by Alanine in the human sequence did not alter Ca2+ release in transfected HEK293 cells, [3H]ryanodine binding or ion translocation and gating properties of purified channels, indicating that Glycine is not necessary. However Valine and Proline substitutions led to significant changes. Though well expressed in HEK293 cells, Valine mutant channels were not active as Ca2+ release entities in cells or as isolated channels reconstituted in planar lipid bilayers, and did not bind ryanodine (P<0.001 vs wild-type). The Proline mutation preserved the responses to caffeine and single channel activity, albeit with dramatic modifications, but [3H]ryanodine binding was abolished (P<0.01 vs wild-type). Our data suggest that a lack of flexibility in the pore impairs proper gating. To go further, we examined the NH2-terminal parts of RyR2 and showed by co-immunoprecipitation, chemical crosslinking and gel filtration that these domains can oligomerize within the tetrameric channel due to the presence of disulphide bonds. We used constructs (GST-BT4L) corresponding to the first 906 amino acids of the human RyR2 protein to test their interaction with endogenous NH2-terminal domains in HEK293 microsomes expressing RyR2. At diastolic Ca2+ concentrations (≤ 250 nM), GST-BT4L significantly induced twofold increase in [3H]ryanodine binding (P<0.05 vs control). Moreover GST-BT4L raised the open probability of single channels (P<0.05 vs GST alone), in low Ca2+ conditions and with a physiological ion flow. Our results provide evidence that GST-BT4L can disrupt the NH2 terminus self-association, which moderately opens RyR2 channels. Hence, we propose that while a general flexibility of the pore inner helix is required to allow the channel to open, the tetramerization of the NH2 terminus domains facilitates RyR2 closure and prevents Ca2+ leak at diastolic Ca2+ concentrations. Since these two regions are hot spots of RyR2 mutations in Catecholaminergic Polymorphic Ventricular Tachycardia 1, these insights may have implications in the understanding of the mechanisms underlying this Ca2+ leak-related channelopathy.