CLIC-2, FKBP12.0 and FKBP12.6 association with ryanodine receptor calcium release channels

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Ryanodine receptor (RyR) Ca^{2+} release channels are essential for the vital functions of skeletal and cardiac muscle contraction. The ion channel releases Ca^{2+} from the internal sarcoplasmic reticulum Ca^{2+} store in response to surface membrane depolarisation, setting off a cascade of events that result in muscular contraction. The ryanodine receptor is the largest known ion channel and its activity, and consequently the strength of muscle contraction, is modified by a large number of soluble factors and associated proteins. Among these are the FK506 binding proteins, the 12kDa FKBP12 and 12.6kDa FKBP12.6 as well as CLIC-2 (**c**h**l**oride **i**ntracellular **c**hannel type 2), a member of the GST structural family. FKBP proteins bind to RyRs with high affinity and stabilise channel activity by suppressing opening to sub-conductance levels. FKBP12 is associated with RyR1 *in vivo*, while FKBP12 and 12.6 are associated with RyR2. Muscle dysfunction, *i.e.*, cardiac arrhythmia and skeletal muscle weakness, can be attributed to hyperactivity of RyR channels due to dissociation of FKBP12 and FKBP12.6 (Bellinger *et al.*, 2009; Vinet *et al.*, 2012). FKBP dissociation is thought to result from excess nitrosylation or excess β-adrenergic stimulation under stress enhancing RyR phosphorylation. CLIC-2 proteins are expressed in striated muscle and the brain, with the soluble form of CLIC2 being a strong inhibitor of RyR channels (Board *et al.*, 2004). An H101Q variant of CLIC-2 has been found to activate RyR channels, with this activation being associated with heart failure, seizures and intellectual deficit (Takano *et al.*, 2012).

We observed, in single channel lipid bilayer experiments, an increased incidence of sub-state openings in RyR2 channels inhibited by WT CLIC-2 and in channels activated by H101Q CLIC-2. Sub-state activity reminiscent of the effect of FKBP dissociation. Thus our hypothesis was that both wild type (WT) and H101Q mutant CLIC-2 alter FKBP binding to RyR1 and RyR2, in addition to their opposite effects on overall channel open probability. We explored the ability of WT and H101Q CLIC-2 to dissociate FKBP12 and FKBP12.6 from RyRs. Microsomal vesicles containing RyR protein complexes were incubated with buffer, alone or containing wild type or mutant CLIC-2 or rapamycin, a drug that binds FKBP proteins to dissociate them from RyRs. Then FKBP associated with the RyRs was determined by co-immunoprecipitation. Both the WT and H101Q CLIC-2 (8 μM) indeed dissociated >50% FKBP12, and ˜50% FKBP12.6, from cardiac RyR2 channels, with the H101Q being more effective than the WT protein. Both WT and H101Q constructs were less effective in removing FKBP12 from skeletal RyR1 channels than from cardiac RyR2. Curiously, 20 μM rapamycin was ineffective in dissociating FKBP12 from RyR2, but removed >75% of FKBP12.6. In contrast 10 μM rapamycin stripped >95% of FKBP12 from RyR1. These observations indicate an unexpected and novel relationship between the CLIC-2 and FKBP binding to RyRs and reveal differences between CLIC-2 effects on FKBP binding in RyR1 and RyR2 that will be explored in the future. CLIC-2 is the first protein shown to be as effective as rapamycin or FK506 in dissociating FKBPs from RyRs and is more effective than rapamycin in removing FKBP12 from RyR2.

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