In vitro interactions between C-terminal residues of the β_{1a} subunit of DHPR and RyR1 from skeletal muscle

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Excitation-contraction (EC) coupling is the process which links the excitatory action potential to contraction of the muscle. Essential to this process in skeletal muscle is the interaction between two calcium channels: the L-type voltage gated dihydropyridine receptor (DHPR) located in the transverse tubule and the ryanodine receptor (RyR1) located in the sarcoplasmic reticulum (SR). This interaction between the two channels triggers SR Ca^{2+} release through the RyR1, which raises cytosolic [Ca^{2+}] from ~100 nM to 1-10 μM and enables contraction. The physical components of this channel-to-channel interaction are yet to be elucidated.

The last 35 C-terminal residues (V490-M524) and a hydrophobic heptad repeat motif (L478, V485 and V492) of the β_{1a} subunit of the DHPR have been shown to play an important role in EC coupling (Beurg *et al.* 1999; Sheridan *et al*., 2004). However, the importance of the heptad repeat in EC coupling is controversial as Dayal *et al.* (2010) has recently shown that expression of a heptad repeat mutant (L478A, V485A, V492A) only slightly hinders EC coupling in a β_{1a}-null zebrafish model. Our aim was to investigate the functional and physical interaction between RyR1 and two 35 residue peptides: β_{1a} C-tail peptide, corresponding to the last 35 C-terminal residues of the β_{1a} subunit, (V490-M524; which contains only one of the hydrophobic residues in the heptad repeat) and β_{1a} A474-A508 peptide, which contains the full heptad repeat.

New Zealand rabbits were euthanized by captive bolt. Fractions of SR membrane, containing native RyR1, were collected from rabbit back and leg muscle by gradient centrifugation. RyR1 was purified by solubilising the SR and further gradient centrifugation. The physical interaction between RyR1 and the biotinylated β_{1a} peptides was investigated using streptavidin-agarose affinity chromatography. The functional action of β_{1a} peptides on RyR1 were measured by reconstituting RyR1 into artificial lipid bilayers and assessing channel response to peptide addition, and with [^{3}H]ryanodine binding.

We show that native and purified RyR1 bound to both β_{1a} peptides. As a functional consequence of the physical interaction between RyR1 and β_{1a} C-tail peptide, RyR1 activity increased 2 to 3-fold in the presence of the β_{1a} C-tail peptide (100 pM to 500 nM). Consistently, the β_{1a} peptide significantly increased [^{3}H]ryanodine binding to native RyR1 at Ca^{2+} concentrations between 1 and 10 μM. Significantly, there was also a 2-fold increase in native RyR1 activity in the presence of 10 and 100 nM β_{1a} A474-A508 peptide. These results demonstrate that the C-terminus of the β_{1a} subunit can modulate RyR1 activity by directly binding to the RyR1 and that the heptad repeat is not crucial for the functional interaction between the DHPR and RyR1. Preliminary data however indicate that three hydrophobic residues located along one surface of an α-helix between L496 and W503 are essential for the action of the C-tail peptide on RyR1 channel activity. Overall, the results suggest that through its C-terminus, the β_{1a} subunit modulates RyR1 activity under Ca^{2+} concentrations that occur in skeletal muscle fibres during EC coupling, but that the heptad repeat is not essential for this regulation.

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