Structural determination of interactions between the the ryanodine receptor and the recombinant DHPR II-III loop and synthetic "C" peptides

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Skeletal muscle excitation-contraction coupling (S-ECC) is independent of extracellular Ca^{2+} influx, but requires a skeletal sequences in the II-III loop of the dihydropyridine receptor (DHPR) particularly in "*C*" region (residues 724-760). It was recently shown that substitution of cardiac for skeletal residues at positions 737, 741 or 742 in the loop abolishes S-ECC in myocytes[1]. To explore the hypothesis that S-ECC depends on a physical interaction between the skeletal ryanodine receptor (RyR1) and the II-III loop, we examined the effect of these substitutions on functional interactions between native RyR1 channels in lipid bilayers and the recombinant skeletal DHPR II-III loop (*SDCL*), or skeletal *C* region peptide (C_S).

Mutants $SDCL_{A\text{-}P}$, $SDCL_{F\text{-}T}$ and $SDCL_{P\text{-}T}$ contained individual substitutions A737P, F741T and P742T and $SDCL_{AFP\ PTT}$ contained the triple substitution. Wild type *SDCL* activated RyR1 with high affinity (≥10 nM). In contrast $SDCL_{AFP\text{-}PTT}$ inhibited channels (≥100 nM). $SDCL_{A\text{-}P}$ mimicked the inhibitory action of the triple mutant, while activation by $SDCL_{F\text{-}T}$ or $SDCL_{P\text{-}T}$ was indistinguishable from wild-type. Curiously, the cardiac II-III loop (*CDCL*) activated RyR1 so that the mutations did not functionally convert *SDCL* to *CDCL*. The triple A737P, F741T and P742T mutation in peptide C_S (a) converted its function from skeletal-like to cardiac-like and (b) changed its structure from skeletal-like random coil to cardiac-like nascent helix (assessed from NMR analysis). The data indicate that *SDCL* interacts with RyR1 through both it's *C* region and N-terminal *A* region. The results show that A373 is critical for the structure of the *C* region and for functional consequences of interactions between RyR1 and the II-III loop and support the hypothesis that S-ECC depends on a physical interaction between RyR1 and the DHPR.

(1) Kugler, G., Weiss, R.G., Flucher, B.E., Grabner, M. 2004. Structural requirements of the dihydropyridine receptor alpha1S II-III loop for skeletal-type excitation-contraction coupling. J. Biol. Chem. 279: 4721-8.