From DHPR to RyR and back again: What lies along the way?

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In skeletal muscle, excitation-contraction coupling depends on a bi-directional interaction between the dihydropyridine receptor (DHPR), a voltage-gated calcium channel in the plasma membrane, and the type 1 ryanodine receptor (RyR1), a homo-tetrameric calcium release channel in the sarcoplasmic reticulum (SR). As a consequence of this bi-directional interaction: (i) the DHPR, in response to depolarization of the plasma membrane, elicits Ca^{2+} release *via* RyR1 without an intervening second messenger, (ii) RyR1 increases the amplitude of Ca^{2+} currents *via* the DHPR, and (iii) DHPRs within the plasma membrane are organized into groups of four (tetrads) such that each DHPR is apposed to a subunit of RyR1. A number of approaches have been used to probe the protein-protein interactions that link the DHPR and RyR1, including expression of cDNAs in muscle cells null for DHPR subunits or for RyR1, biochemical analyses of binding, and application of peptides to isolated RyR1. However, these have not yet produced a consistent picture. We have been examining several alternative approaches for establishing the spatial interrelationships between DHPRs and RyR1. To determine the orientation of DHPRs within tetrads, the fluorescent proteins ECFP or EYFP were fused to sites of α_{1S} or β_{1a}. Between N- and C-terminals, fluorescence resonance energy transfer (FRET) occurred between α_{1S} subunits adjacent within tetrads, but not between adjacent β_{1a} subunits, consistent with the idea that the N- and C-terminals are oriented towards, and away from, the center of tetrads for α_{1S} and β_{1a}, respectively. As a second approach, we have been determining which sites of the DHPR may be in close proximity to RyR1. This is accomplished by attachment of an ECFP-EYFP tandem ("CY", 23 residue linker) or a biotin acceptor domain (BAD: 70 or 97 residues) to DHPR sites. For CY-β_{1a} and α_{1S}-CY, FRET efficiency increased after expression in dyspedic myotubes (no RyR1) compared to dysgenic myotubes, suggesting that RyR1 may closely appose the β_{1a} N-terminal and α_{1S} C-terminal. In the case of the BAD fusions, expressing myotubes were fixed and permeabilized and exposed to fluorescently labeled NeutrAvidin (~60 kDa). NeutrAvidin had access to BAD at the N- and C-terminals of β_{1a} and to the α_{1S} N-terminal and II-III loop ("peptide A" region). NeutrAvidin did *not* have access to α_{1S}-BAD in dysgenic myotubes, but did have access to α_{1S}-BAD in dyspedic myotubes. Thus, two independent approaches suggest that the C-terminal of α_{1S} may be closely apposed to RyR1.