Biophysical investigations of the cyclised skeletal muscle dihydropyridine receptor II-III loop

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An association between the dihydropyridine receptor (DHPR) and the skeletal muscle ryanodine receptor (RyR1)is essential during skeletal muscle excitation-contraction coupling (ECC). A cytoplasmic loop connecting the 2nd and 3rd transmembrane domains (II-III loop) of the DHPR-α1s subunit has been shown to be essential for ECC (Tanabe *et al.,* 1990). A conserved RyR1-domain known as SPRY2 has been shown to bind to the DHPR II-III loop (Cui *et al.,* 2008; Leong & Maclennan, 1998). To date, *in vitro* studies on the DHPR II-III loop have been performed using the linear form of the loop (Dulhunty *et al.,* 1999). However this does not reflect the II-III loop's actual physiological state where its ends are anchored to the DHPR-α1s subunit transmembrane domains in the t-tubule membrane.

Our aim was therefore, to mimic the *in vivo* geometry of the loop using intein-mediated protein cyclisation and examine the effects. The II-III loop cyclisation was achieved through the self-catalysed ligation of the termini of the *Synechocystis sp.* PCC6803 DnaB split inteins. The results of cyclisation were determined using several techniques: i) functional-effects on native RyR1 channel activity using single channel lipid bilayer technique; ii) binding affinity to the their SPRY2 domain using fluorofluorescence spectrophotometry; and iii) secondary structural determination conducted using circular dichroism. The results of these experiments revealed that the cyclised loop produced a more pronounced effect on RyR1 channel activity, a 2-fold increase in the open probability, which was mostly due to an increase in the channel relative mean open time from 2.5 ± 0.03 ms to 8.5 ± 0.04 ms. In addition, SPRY2 domain binds to the cyclised loop 4-fold more tightly compared than to the linear loop. Circular dichroism spectroscopy suggested that the cyclised loop is considerably more α helically-structured than its intrinsically unstructured linear counterpart. This structural change could be responsible for the observed stronger effects of the cyclised loop.

In conclusion, tethering the ends of the DHPR II-III loop; to mimic its *in vivo* condition geometry resulted in: (i) a greater activator of RyR1; (ii) a tighter binding to the SPRY2 domain; and (iii) a more structured protein. This study suggests that the structure imposed on the loop by its tethering to the DHPR-transmembrane domains of could be essential in its ability to interact with RyR1 during *in vivo* ECC.

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