Concentration dependent modulation of the cardiac ryanodine receptor by Homer1

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Calcium signalling controls a wide variety of physiological processes and depends on the activity of protein signalling complexes clustered in specialised cellular sites. Ryanodine receptor (RyR) calcium release channels form the hub of the calcium signalling complex that is vital in muscle contraction. Homer proteins allow both clustering and functional modulation of a plethora of proteins from different calcium signalling complexes; interactions between the ryanodine receptors and Homer emerge as a new aspect of regulation of the crucial calcium signal in muscle. Homer1 modulates the sekeletal ryanodine receptor (RyR1) activity in a concentration dependent manner (Feng *et al.*, 2008), activating the receptor at ≤ 200nM, but strongly inhibiting its activity at concentrations > 200nM. Only one publication has specifically examined the effect of Homer1 on the cardiac ryanodine receptor (RyR2, the main ryanodine receptor isoform in the heart and in the brain), concluding that Homer1 inhibits channel activity, regardless of its concentration (Westhoff *et al.*, 2003). Here we re-examine the modulation of the RyR2 activity by Homer1 and test the hypothesis that modulation of RyR1 and RyR2 by Homer1 are fundamentally different.

Skeletal and cardiac muscle sarcoplasmic reticulum vesicles were isolated from New Zealand rabbits or sheep heart, respectively. Human recombinant Homer1b (a long isoform able to multinerise) and short Homer (an isoform unable to multimerise) were purified by affinity chromatography. We determined the activity of the RyRs by two different means: specific [^{3}H]-ryanodine binding, which is proportional to the activity of a population of RyRs; and single channel techniques. With the later we measure the ionic current flowing through a single channel molecule incorporated in an artificial lipid bilayer where the solutions bathing each sides of the channel can be manipulated and the intrinsic gating properties of individual channels measured.

Homer1b increased RyR1 and RyR2 activity at all cytosolic [Ca^{2+}] without altering the [Ca^{2+}]-dependence. At resting and activating cytosolic [Ca^{2+}], Homer1b activated RyR2 in a dose dependent manner. In the presence of 1μM cytosolic Ca^{2+}, 50nM Homer1b increased RyR2 open probability (from 0.009 ± 0.003 to 0.137 ± 0.053) and mean open time (from 0.57 ± 0.09ms to 1.85 ± 0.46ms) without a significant change in mean closed time. Maximum activity was reached with ~50-100nM Homer1b, and activity fell dramatically with Homer1b > 200nM. When RyR2 was maximally activated by 100μM cytosolic Ca^{2+}, RyR2 activation by Homer1b was reduced, while inhibition by ≥ 200nM Homer1b was sustained. Short Homer1 similarly modulated RyR2 activity in a biphasic manner, though with lower affinity than Homer1b.

We conclude that Homer1 modulates skeletal RyR1 and cardiac RyR2 in an intrinsically similar manner. RyR2 modulation by Homer1b is likely to be physiologically relevant in the heart and in neurons, where high levels of the two proteins are expressed.

Feng W, Tu J, Pouliquin P, Cabrales E, Shen X, Dulhunty A, Worley PF, Allen PD, Pessah IN. (2008) *Cell Calcium,* **43:** 307-14.

Westhoff JH, Hwang SY, Duncan RS, Ozawa F, Volpe P, Inokuchi K, Koulen P. (2003) *Cell Calcium,* **34:** 261-9.