In vitro modulation of the cardiac ryanodine receptor (calcium release channel) activity by human homer 1b

P. Pouliquin, S.M. Pace and A.F. Dulhunty, Division of Molecular Bioscience, John Curtin School of Medical Research, Australian National University, Canberra ACT 0200, Australia.

Calcium signalling controls a wide variety of physiological processes and depends on the activity of protein signalling complexes which are 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. The Homer protein family allows both clustering and functional modulation of a plethora of different calcium signalling complexes. Homer 1 has recently been implicated in the interaction between RyR2 and a surface membrane L-type Ca^{2+} channel (dihydropyridine receptor, or $Ca_v1.2$) in the mice urinary bladder (Huang *et al.*, 2007). *In vitro* studies indicate that Homer 1b/c activates the skeletal muscle RyR1 (Pouliquin *et al.*, 2006 and Feng *et al.*, 2007) while it inhibits RyR2, the main RyR isoform in the heart and in neurons (Westhoff *et al.*, 2003). In the present study, we revisit the *in vitro* regulation of RyR2 by Homer 1.

New Zealand male white rabbits were euthanized by a captive bolt and back and leg muscle used to prepare skeletal sarcoplasmic vesicles. Cardiac sarcoplasmic vesicles were isolated from Merino sheep euthanized by overdose of 20 ml valabarb euthanasia solution (300 mg/ml) injected into the jugular vein. All sarcoplasmic vesicles were stored in liquid nitrogen. Human Homer 1b (H1b) fusion protein (with C-myc and 6His tags) was expressed in *E. Coli* and affinity purified on Ni-agarose columns (Pouliquin *et al.*, 2006). Sequence encoding human Homer 1b 120 N-term residues (Short Homer) was amplified by PCR and subcloned into the plasmid pHUE, Short Homer fusion protein was expressed and purified in the same way as H1b. Purified fusion proteins were dialysed overnight at 4°C against >1000 volumes of PBS, aliquots were stored at -80°C. Protein purity and identity were assessed using SDS-PAGE and Western blotting after electro-transfer onto nitrocellulose. Primary and secondary antibodies were monoclonal anti-C-myc and anti-mouse IgG POD conjugated (Sigma), immuno-decorated proteins were revealed by chemiluminescence (Roche). Activity of RyR present in sarcoplasmic vesicles was assessed from $[^3H]$ ryanodine binding (which increases when channel open probability increases) and from RyR channels incorporated into artificial lipid bilayers.

We show here that the *in vitro* activity of RyR2 is modulated in a complex manner by human Homer1b. Both ryanodine binding and single channel recordings indicated that at resting and activating cytosolic calcium concentrations, Homer1b, and a shorter version of the protein lacking the coil-coil domain, activated RyR2 in a dose dependent manner, in contrast to previously reported actions of Homer 1 (Westhoff *et al.*, 2003). Maximum activity was reached with ~500 nM Homer1, and activity fell with higher Homer concentration. Homer1b increased RyR2 activity at all cytosolic Ca^{2+} concentrations without altering the Ca^{2+} concentration dependence of RyR2 activity (RyR2 was active in the same Ca^{2+} concentration range and reached its maximal activity at the same Ca^{2+} concentration either in the absence or in the presence of Homer1 b). When RyR2 was maximally activated by 100 μM cytosolic calcium, RyR2 modulation by Homer1 b was complex: RyR2 activity was inhibited by ~10 nM Homer1 b and >500 nM Homer1 b, while intermediate concentrations activated the channel. These results show conclusively that Homer would activate Ca^{2+} release in the heart, as in skeletal muscle, in the presence of physiological cytoplasmic Ca^{2+} concentrations. We suggest that the differences between the results obtained in the present study and previously reported data (Westhoff *et al.*, 2003) may be due to different intrinsic properties of RyR2 from different species. Rodent Homer1 c was shown to inhibit rodent RyR2 activity in various conditions (Westhoff *et al.*, 2003), while we show that human Homer1 b modulates sheep RyR2. These two Homer1 proteins are highly homologous and to date no functional difference between them has been reported, while clear differences in the regulation of RyR2 from different species by other crucial proteins has been reported (Jeyakumar *et al.*, 2001). The complex modulation of RyR2 by Homer1 b is physiologically relevant in the heart and in neurons, where high levels of the two proteins are expressed.

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