A novel scorpion toxin activates cardiac RyR2 calcium release channels at fM concentrations

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Ryanodine receptor (RyR) calcium release channels in the sarcoplasmic reticulum of skeletal and cardiac muscle fibres supply Ca^{2+} to allow striated muscle contraction and ex-utero survival. The membrane permeable scorpion toxins, Imperatoxin A and Maurocalcine, are high affinity modulators of RyR channels and are used as tools in defining molecular mechanisms in the gating of these ion channels (Dulhunty *et al.*, 2003). The toxins contain a 3-disulfide inhibitor cystine knot (ICK) motif found in venom peptides from spiders, scorpions and cone snails. The ICK motif was predicted to have evolved from an ancestral two-disulfide-directed β-hairpin (DDH) fold. The first native peptide toxin containing a DDH fold, U1-LITX-Lw1a (Lw1a), was recently isolated from *Liocheles waigiensis* and shown to have potent insecticidal activity in a broad range of insect species (Smith *et al.*, 2011). We now show that Lw1a interacts with RyR1 channels with very high affinity. This was a surprising finding as the toxin did not have the necessary charge density for Imperatoxin A and Maurocalcine's membrane permeability and RyR activation (Mabrouk *et al.*, 2007; Lee *et al.*, 2004).

We examined skeletal RyR1 channels isolated from rabbit back and leg muscle and cardiac RyR2 channels from sheep heart, inserted into lipid bilayers for current recording using standard techniques (Wei *et al.*, 2009). Channel activity was recorded in the presence of symmetrical 250 mM Cs^{+} as the current carrier, with 1 μM Ca^{2+} on the cytoplasmic side (Ca^{2+} buffered with BAPTA and checked with a Ca^{2+} electrode) and 1 mM Ca^{2+} on the side of the channel facing the lumen of the sarcoplasmic reticulum. The wild type (WT) Lw1A toxin or the W36A mutant toxin was added to either the cytoplasmic or luminal solutions bathing the channels.

An increase in the open probability of skeletal RyR1 channels was routinely seen with 10 pM to 100 pM WT Lw1a, and occasionally seen with 1 pM of the toxin in the cytoplasmic solution. Cardiac RyR2 channels were more sensitive to the WT toxin. The channels were significantly activated by only 10 fM of the toxin in the cytoplasmic solution. The W36A mutant peptide did not increase the activity of RyR1 or RyR2 channels. Indeed, there was a strong trend towards a drop in activity at 100 pM and 1 nM of the mutant toxin. Both Lw1a and W36A at concentrations of ~1 nM induced strong submaximal conductance activity in RyR1 and RyR2 channels with long opening to 33% and 50% of the maximal opening. The subconductance activity was reminiscent of that seen in the presence of Imperatoxin A and Maurocalcine, although this activity was less pronounced than with the ICK toxins. Finally, the WT toxin added to the luminal solution had no consistent effect on channel activity indicating that, unlike Imperatoxin A or maurocalcine, Lw1a did not easily cross the lipid bilayer. The results show a very high affinity interaction between the DDH fold toxin and the cardiac RyR2 channel despite predictions that the toxin did not possess the required charge for membrane permeation and RyR activation. It is notable that Imperatoxin A binds to multiple sites on the RyR protein, with open probability enhanced by binding to a high affinity site and substate activity induced by binding to a lower affinity site (Dulhunty *et al.*, 2003). If Lw1a binds to the same sites, the results indicate that its binding to the site that increases open probability is enhanced and requires W36, while binding to a second charge-sensitive substate-induction site that also enhances membrane permeability is less effective in Lw1a and is insensitive to the W36A mutation.

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