Mapping the insectophore of κ-atracotoxins: insect-selective BK_{Ca} channel blockers that reveal a novel insecticide target

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Arthropod pests vector numerous pathogens of human and veterinary health importance, and they destroy ~10% of the world's food supply (Oerke & Dehne, 2004). Despite the introduction of transgenic crops and other biological control methods, chemical insecticides remain the dominant approach for combating these arthropods. Unfortunately, the vast majority of these insecticides act on one of just five nervous system targets and reliance on just a handful of targets has promoted the evolution of insecticide resistance. Along with human and environmental health concerns, the problem of insecticide resistance has intensified the search for new insect-control methods.

The Janus-faced atracotoxins (J-ACTXs) are a family of insect-selective excitatory neurotoxins from Australian funnel-web spiders that are lethal to both agronomically and clinically relevant insects (Wang *et al.*, 2000; Maggio & King, 2002b) but display no activity in vertebrates (Wang *et al.*, 2000). These toxins possess a rare vicinal disulfide bond between two adjacent cysteine residues that is critical for activity (Wang *et al.*, 2000). An alanine scan of the representative family member J-ACTX-Hv1c has delineated the key functional residues of the toxin (Maggio & King, 2002 a&b). This epitope is restricted to five key residues (Arg^8, Pro^9, Cys^{13}-Cys^{14} and Tyr^{31}) that form a bipartite surface patch on a single face of the toxin structure. Despite this wealth of structural data, their molecular target has proved elusive.

We previously speculated (Maggio & King, 2002b) that the J-ACTXs might target voltage-gated potassium (K_V) channels since the pharmacophore residues Arg^8 and Tyr^{31} overlay well with the conserved LysTyr/Phe dyad of vertebrate K_V channel blockers (Dauplais *et al.*, 1997). In the present study, we tested this hypothesis by whole-cell patch-clamp analysis of cockroach dorsal unpaired median (DUM) neurons. Based on this finding we have renamed this toxin family κ-ACTX-1 based on the previously established nomenclature for atracotoxins.

κ-ACTX-Hv1c selectively blocked cockroach calcium-activated K^+ (K_{Ca}) channels with an IC_{50} of 2 nM, but not other insect voltage-gated K_V, Na_V or Ca_V channels. κ-ACTX-Hv1c also blocked heterologously expressed cockroach BK_{Ca} (*pSlo*) channels without a significant shift in the voltage-dependence of activation. Moreover, the block was voltage-dependent, indicating that κ-ACTX-Hv1c is likely to be a pore blocker rather than a gating modifier. The molecular basis of the insect selectivity of κ-ACTX-Hv1c was established by its failure to significantly inhibit mouse *mSlo* currents (IC_{50} ~10 μM) and its lack of activity on rat dorsal root ganglion neuron $I_{K(Ca)}$. We used a panel of point mutants to identify the molecular epitope (insectophore) on the toxin that mediates its interaction with K_{Ca} channels, and we show that this insectophore is strikingly different to that of vertebrate K_V channel toxins.

κ-ACTX-Hv1c is the first insect-specific K^+ channel blocker identified from spider venom and its lethal block of insect BK_{Ca} channels validates these ion channels as a potential insecticide target. Moreover, the channel-binding epitope of the toxin mapped in the current study provides a template for the rational design of novel chemical insecticides that act specifically on insect BK_{Ca} channels.

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