A HEK293 bioreporter cell line demonstrates temporally differentiated effects on impedance and ryanodine receptor modulation by venom fractions from the Australian scorpion *Liocheles waigiensis*

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Scorpion venoms contain a diverse variety of neuropeptides that potently and specifically act across a broad array of ion channels to modulate physiological function, and are responsible for significant mortality, especially in tropical countries. A small number of scorpion toxin (SCTX) peptides, termed calcins, have been identified to possess the ability to rapidly cross the cell membrane and bind with high affinity to ryanodine (RyR) channels to induce long-lived channel subconductance states, and thereby modulate cytosolic Ca^{2+}. Additionally, these calcins are also able to carry impermeable molecules across the membrane, making them potentially valuable small molecule tools for intracellular drug delivery. The most potent calcin-like peptide discovered is ϕ-LITX-Lw1a, derived from the Australian scorpion *Liocheles waigiensis*. This is the only SCTX peptide characterized from *L. waigiensis* to date, and as such the venom from this scorpion represents a considerable source of potential ion channel modulators, with likely action on RyR channels. RyR mutations underlie several disease states such as polymorphic ventricular tachycardia and malignant hypertension, and as such identification of SCTX peptides with action on these channels has considerable therapeutic potential. In the present study, size-exclusion Fast Protein Liquid Chromatography (FPLC) was used to fractionate 12 protein peaks of *L. waigiensis* into 14 individual peak components. We then broadly screened these components for bioactivity on cell membrane properties and cytosolic Ca^{2+} stores using the development of a novel biosensor, consisting of HEK293 cells co-expressing the type 1 ryanodine receptor (RyR1) and the genetically encoded Ca^{2+} reporter GCaMP5G (rHEK293). xCELLigence (ACEA Biosciences) was used to screen for venom-induced alterations in rHEK293 cellular conductivity, finding broad venom activity with distinct temporal responses attributable to particular venom components. This broad activity was supported by subsequent Ca^{2+} imaging experiments, where whole venom and a large number of components significantly increased cytosolic Ca^{2+} comparably to that produced by 4 mM caffeine, a RyR agonist. We were largely able to correlate both venom activity and potency across these two platforms, supporting impedance-based measurements as a high-throughput screening method to identify SCTX bioactivity. We revealed that *L. waigiensis* venom contains a large number of components that are able to modulate cytosolic Ca^{2+}, with potential to help improve understanding of ion channel function and facilitate the development of novel therapeutics.

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