

Multi-scale evaluation of spider toxins as potential anti-nociceptive agents: example of cyriotoxin-1a

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Our expertise is the identification and structural and functional characterization of original natural toxins targeting receptors and ion channels, and which may have applications in human health. In this context, and in collaboration with the *Institut du Thorax*, Smartox Biotechnology and Sanofi R & D, we are interested in the multi-scale evaluation (from the cell *in vitro* to the organism *in vivo*) of spider toxins as potential anti-nociceptive agents, as illustrated by the example of Cyriotoxin-1a (CyrTx-1a). Na_v1.7 channel subtype is highly expressed in dorsal root ganglia (DRG) of the sensory nervous system and plays a central role in the pain signaling process. We investigated a library prepared from original venoms of 117 different animals to identify new selective inhibitors of this target.

We used high-throughput screening of the venom library, using automated patch-clamp experiments on human voltage-gated sodium channel subtypes, and then *in vitro* and *in vivo* electrophysiological experiments to characterize the active peptides that have been purified, sequenced and chemically synthesized. Analgesic effects were evaluated in mice *in vivo*.

We identified and further characterized CyrTx-1a, a novel peptide isolated from *Cyriopagopus schioedtei* spider venom. This 33 amino acids toxin belongs to the inhibitor cystine knot structural family and inhibits hNa_v1.1-1.3 and 1.6-1.7 in the low nanomolar range, compared to the micromolar range for hNa_v1.4-1.5 and 1.8. CyrTx-1a was 920 times more efficient at inhibiting tetrodotoxin (TTX)-sensitive than TTX-resistant sodium currents recorded from adult mouse DRG neurons *in vitro*, and approximately 170 times less efficient than huwentoxin-IV at altering mouse skeletal neuromuscular excitability *in vivo*. CyrTx-1a exhibited an analgesic effect in mice by significantly increasing reaction time in the hot-plate assay.

Keywords: anti-nociceptive toxin, mouse dorsal root ganglia neuron, mouse tactile and hot pain sensitivity, pain, voltage-gated sodium channel subtype.
