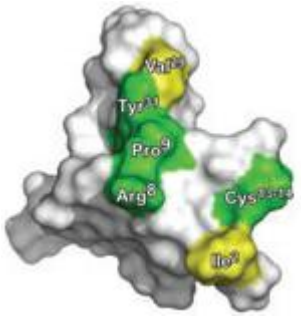
Interactions of insecticidal spider peptide neurotoxins with insect voltage- and neurotransmitter-gated ion channels



(Molecular representation of κ-HXTX-Hv1c including key binding residues, adapted from Gunning et al, 2008)

PhD Thesis

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UTS 2012

CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Monique J. Windley
2012

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Abstract

Two families of peptide neurotoxins that target insect large-conductance calcium-activated potassium channels (BK_{Ca}) have been isolated from the venom of two unrelated spiders. The κ-TRTX-Ec2 toxins are a family of three homologous peptides isolated from the African tarantula, *Eucratoscelus longiceps* and κ-HXTX-Hv1c is the prototypic member of a family of insect-selective neurotoxins isolated from the venom of the Blue Mountains funnel-web spider, *Hadronyche versuta*. This thesis describes the characterisation of these insecticidal toxins using voltage-clamp and current-clamp analysis of cockroach dorsal unpaired neurons utilising the whole-cell patch-clamp technique. The ability of these toxins to modulate the gating and kinetics of both voltage- and neurotransmitter-gated ion channels were assessed. Insect bioassays were also utilised to validate the insecticidal activities of various toxins that target K_V channel subtypes in house crickets.

The κ-TRTX-Ec2 family of toxins were found to be high affinity blockers of the insect BK_{Ca} channel while failing to modify voltage-gated sodium (Na_V) and calcium (Ca_V) channels. κ-TRTX-Ec2a, -Ec2b and -Ec2c block cockroach BK_{Ca} channels with IC_{50} values of 3.7, 25.3 and 24.6 nM, respectively. Additionally, κ-TRTX-Ec2a was found to inhibit delayed-rectifier K_V channel currents ($I_{K(DR)}$), but only at significantly higher concentrations. κ-TRTX-Ec2 toxins induced voltage-independent channel block and are thus proposed to interact with the turret and/or loop region of the external vestibule of the insect BK_{Ca} channel.

κ-HXTX-Hv1c has also been characterised to block the insect BK_{Ca} channel, while failing to modulate insect Na_V and Ca_V channels. The unique insect-selective action of κ-HXTX-Hv1c involves a rare vicinal disulphide ring (Cys13-Cys14) that has been determined to act as part of the bioactive surface (pharmacophore) interacting with the molecular recognition site on the insect BK_{Ca} channel. However, despite the high affinity and selectivity for the BK_{Ca} channel it was discovered that the BK_{Ca} channel is unlikely to be the lethal target of κ-HXTX-Hv1c. Acute toxicity tests of classical non-phylum selective BK_{Ca} blockers such as paxilline, charybdotoxin and iberiotoxin did not induce acute toxicity in insects. Furthermore, while κ-HXTX-Hv1c was found to prolong action potential repolarisation, increase spontaneous firing frequency and reduce spike afterhyperpolarisation, these results were markedly reduced in the presence of the BK_{Ca} channel blocker iberiotoxin.

Subsequent testing of cockroach K_V channel currents revealed that κ-HXTX-Hv1c failed to modify sodium-activated or delayed-rectifier K_V channel currents, but 1 μM κ-HXTX-Hv1c did produce a 29% block of 'A-type' fast-transient K_V channel currents ($I_{K(A)}$). This suggests that κ-HXTX-Hv1c additionally targets insect K_V1- or K_V4-like channel subtypes. The lethal insecticidal action of 4-AP in crickets further supports an action of κ-HXTX-Hv1c to block $I_{K(A)}$. The results of co-application experiments revealed that κ-HXTX-Hv1c blocks the same channel as the non-phylum selective vertebrate K_V4 channel toxin, κ-sparatoxin-Hv1b. However, it was found that κ-sparatoxin-Hv1b, either alone or in combination with iberiotoxin, was not insecticidal and thus the K_V4 and BK_{Ca} channels are unlikely to be the lethal targets of κ-HXTX-Hv1c. To determine if the lethal target was a neurotransmitter-gated ion channel, the effects of κ-HXTX-Hv1c were investigated on chloride-gated $GABA_A$ (GABA-Cl) and glutamate (Glu-Cl) channel currents and nAChR channel currents. It was revealed that 1 μM κ-HXTX-Hv1c failed to modify $GABA_A$ channel currents while causing only a moderate 21% increase in Glu-Cl channel currents. Alternately, it was found that κ-HXTX-Hv1c caused a concentration-dependent (EC_{50} 183 nM) slowing of nicotinic acetylcholine receptor (nAChR) channel current decay and reversed channel desensitisation. In addition, κ-HXTX-Hv1c moderately increased nAChR sensitivity to nicotine. These findings are consistent with a positive allosteric modulation of insect nAChRs to slow receptor desensitisation. The nAChR is a validated insecticidal target for various agrochemical insecticides, including the allosteric modulator spinosyn A. Therefore it is believed that the lethal target of κ-HXTX-Hv1c is the insect nAChR, whose modulation would lead to an increase in neurotransmission consistent with the excitotoxic phenotype of the toxin. This action is possibly augmented by additional actions on BK_{Ca} and K_V4 like channels to increase neuronal excitability.

Abbreviations

4-AP	4-aminopyridine
α-BgTx	α-bungarotoxin
ACh	acetylcholine
AcNPV	*Autographa californica* nuclear polyhedrosis virus
AHP	afterhyperpolarisation
AP	action potential
ASICs	acid-sensing ion channels
ATP	adenosine tri-phosphate
BK_{Ca} channel	large-conductance Ca^{2+} and voltage-activated K^+ channel ($K_{Ca}1.1$, Maxi-K, BK, Slo1)
BSA	bovine albumin serum
Ca_V channel	voltage-activated Ca^{2+} channel
ChTx	charybdotoxin
DDH	disulfide-directed β-hairpin
DSE	dihedral strain energy
DUM	dorsal unpaired median
dSlo	*Drosophila* Slo-poke potassium channel
EC_{50}	median effective dose
EDTA	2,2',2",2"'-(Ethane-1,2-diyldinitrilo)tetraacetic acid
EGTA	ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*'*N*'-tetraacetic acid

ESI-QTOF	electrospray ionization quadrupoletime-of-flight mass spectrometry
FBS	fetal bovine serum
GABA-Cl channel	γ-aminobutyric acid-activated chloride channel
Glu-Cl channel	glutamate-activated chloride channel
GNA	*Galanthus nivalis* agglutinin
GSH	glutathione
HEPES	N-hydroxyethylpiperazine-N-ethanesulfonic acid
hSlo	human Slo-poke potassium channel
HVA	high-voltage-activated
HXTX	hexatoxin (from the venom of spiders belonging to the family Hexathelidae)
IbTx	iberiotoxin
IC_{50}	median inhibitory concentration
ICK	inhibitory cystine knot
IK_{Ca}	intermediate-conductance Ca^{2+}-activated K^+ channel ($K_{Ca}3.1$, $IK_{Ca}1$)
$I_{BK(Ca)}$	Ca^{2+}-activated K^+ channel current
I_{Ca}	voltage-activated Ca^{2+} channel current
I_K	voltage-activated K^+ channel current
$I_{K(A)}$	transient ‘A-type’ K^+ current
$I_{K(DR)}$	delayed-rectifier K^+ current
$I_{K(Na)}$	Na^{2+}-activated K^+ channel current
I_{Glu-Cl}	glutamate-activated chloride current

$I_{GABA\text{-}Cl}$	γ-aminobutyric acid-activated chloride current
I_{Na}	voltage-activated Na^+ channel current
I_{nAChR}	nicotinic-acetylcholine receptor current
α-KTx	potassium channel scorpion toxin
KD_{50}	median knockdown dose
K_V channel	voltage-activated K^+ channel
LD_{50}	median lethal dose
LIT	latroinsectotoxin (from the venom of spiders belonging to the genus *Latrodectus*)
LJP	liquid junction potential
MALDI-TOF	matrix-assisted laser desorption/ionization time-of-flight
MAMPs	membrane-acting antimicrobial peptides
M-LVA	mid- to low-voltage-activated
MOPS	3-morpholinopropane-1-sulfonic acid
MSCs	mechanosensitive ion channels
mSlo	*Mus musculus* Slo-poke potassium channel
nAChD	desensitising nicotinic-acetylcholine channel current
nAChN	non-desensitising nicotinic-acetylcholine channel current
nAChR	nicotinic-acetylcholine receptor
Na_V channel	voltage-activated Na^+ channel
NIS	normal insect saline
NMR	nuclear magnetic resonance

PAMs	positive allosteric modulators
PDB	protein data base
pSlo	*Periplaneta* Slo-poke potassium channel
rp-HPLC	reversed phase high performance liquid chromatography
Sec	selenocysteine
SK_{Ca} channel	small-conductance Ca^{2+}-activated K^+ channel ($K_{Ca}2.x$)
SPRTX	sparatoxin (from the venom of spiders belonging to the family Sparassidae)
TAG	terminal abdominal ganglia
TEA	tetraethylammonium
TFA	2,2,2-trifluoroacetic acid
TRP	transient receptor potential
TRTX	theraphotoxin (from the venom of spiders belonging to the family Theraphosidae)
TTX	tetrodotoxin
VDR	vicinal disulfide ring
V_h	holding potential
$V_{1/2}$	voltage at half-maximal activation
V_{rev}	reversal potential

Publications arising from this thesis

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