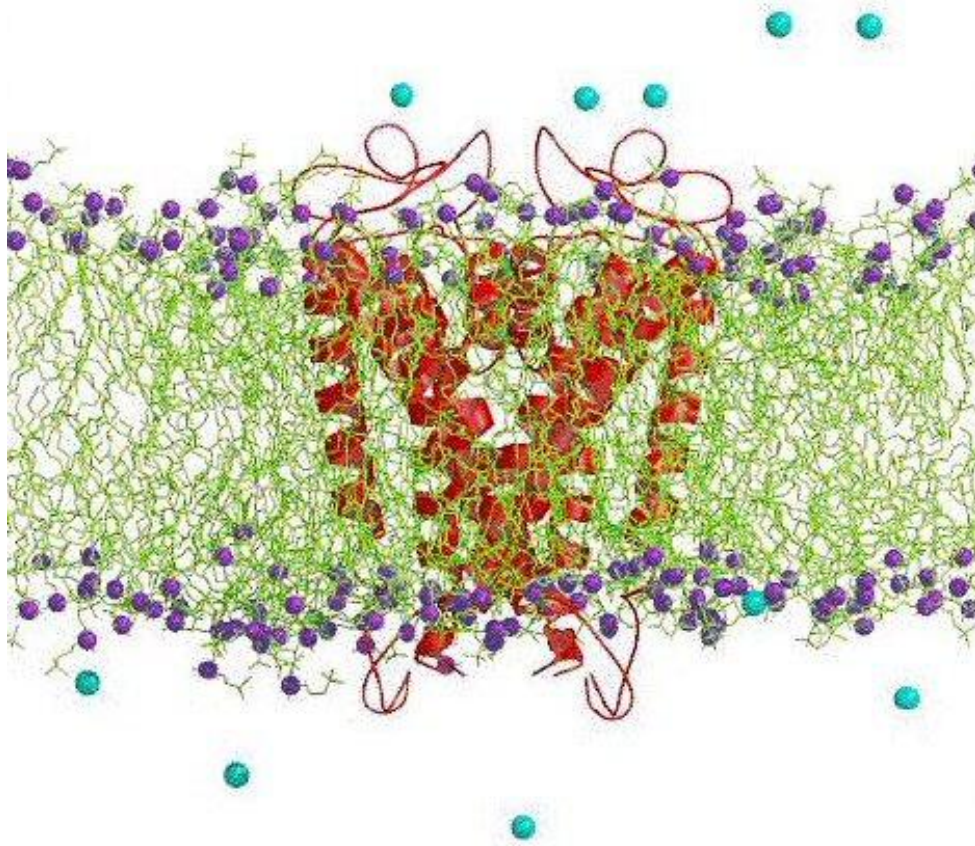


STRUCTURE-FUNCTION STUDIES OF INSECTICIDAL ATRACOTOXINS



PhD Thesis

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ABSTRACT

Part I

The κ -atracotoxins (κ -ACTXs, previously the Janus-faced atracotoxins) are a family of five insect-selective excitatory peptide neurotoxins containing 36-37 residues with four disulfide bonds. Toxins from this family were isolated from the venom of the Blue Mountains funnel-web spider (*Hadronyche versuta*) and Toowooba funnel-web spider (*Hadronyche infensa*). The NMR solution structure and primary sequence of the prototypic member κ -ATCT-Hv1c provided few clues as to the likely molecular target. In order to characterise the site of action and phylogenetic specificity of these toxins, whole-cell patch-clamp electrophysiology was employed using isolated DUM neurons from the American cockroach (*Periplaneta americana*). κ -ACTX-Hv1c had no effect on the gating or kinetics of I_{Na} or I_{Ca} at concentrations up to 1 μ M. However, at the same concentration, κ -ATCT-Hv1c reduced K_v channel currents by $56 \pm 7\%$ ($n = 5$). Subsequent experiments in insect DUM neurons indicated that inhibition of the macroscopic I_K was due to a block of calcium-activated K_v (K_{Ca}) channels, with an IC_{50} of 2.3 nM and 2.9 nM for peak and late $I_{K(Ca)}$ respectively ($n = 5$), and not 'A-type' or delayed-rectifier K_v channels. Insect selectivity was confirmed by a lack of activity on rat dorsal root ganglion (DRG) neuron global I_K as well as $I_{K(Ca)}$ at doses up to 1 μ M. κ -ACTX-Hv1c is a selective insect K_{Ca} (BK_{Ca}) channel pore-blocker, not a gating modifier, as inhibition of insect $I_{K(Ca)}$ occurred in the absence of any voltage-dependent actions on channel activation. Specificity for the insect BK_{Ca} channel was validated by κ -ACTX-Hv1c induced inhibition of $I_{K(Ca)}$ from the cloned insect K_{Ca} channel α -subunit (*pSlo*) expressed in HEK293 cells (IC_{50} of 240 nM). The 80-fold reduction in IC_{50} , most likely indicates that κ -ACTX-Hv1c interacts with the auxiliary subunits that form part of the wild-type channel, in a manner similar to the BK_{Ca} blocker, charybdotoxin (ChTX), as previously reported. Phyletic selectivity of κ -ACTX-Hv1c was confirmed by the 9776-fold

increase in IC_{50} against *mSlo* channels. Interestingly κ -ACTX-Hv1c, like ChTX, failed to potently block the *dSlo* channel with the $IC_{50} > 10 \mu\text{M}$.

Additional experiments on DUM neuron $I_{K(\text{Ca})}$ using alanine mutants confirmed the pharmacophore of bioactive residues κ -ACTX-Hv1c comprises Arg⁸, Pro⁹, Val²⁹ and Tyr³¹, previously identified by acute toxicity tests in house flies (*Musca domestica*). Interestingly, the functionally critical Arg⁸ and Tyr³¹ residues align extremely well with the Lys-Phe/Tyr diad conserved amongst structurally dissimilar K_v channel toxins, providing a possible basis for targeting of the toxin to K^+ channels. Using a panel of 8 mutants (R8E, R8Q, R8K, R8H, Y31W, Y31F, Y31L and Y31V) the mechanism of interaction was investigated further. The Arg⁸ residue appears to interact with the channel via hydrogen bonding from the δ -guanido group to carbonyl groups on the extracellular surface of the channel, as evidenced by the high potency of the R8H mutant. The imidazole group of His is an adequate substitute for the δ -guanido group of arginine. In contrast the R8E, R8Q and R8K had reduced potency indicating that the positive charge of the amino group of Arg does not directly interact with the target nor is the alkyl group of Arg critical for binding to the target. The critically important Tyr³¹ interacts with the channel via non-specific hydrophobic interactions as substitution for an aromatic ring (Y31F & Y31W) maintains the potency of the toxin. In contrast substitution to small less hydrophobic side chains (Y31V, Y31L and Y31A) reduced potency. It appears therefore that Tyr³¹ in conjunction with Ile² and Val²⁹, that lie at either side of the primary pharmacophore, appear to act as 'gasket' residues to exclude bulk solvent from disrupting the Arg⁸-channel interaction.

This study has identified κ -atracotoxins as potential lead compounds in the development of new biopesticides and validates insect BK_{Ca} channels as potential insecticide targets.

Part II

The second part of this thesis was to determine the target site for the 'hybrid' toxin FW178 from the venom of the Blue Mountains funnel-web spider (*H. versuta*). FW178 is a unique toxin that shares little homology to other known atracotoxins. In order to identify the site of action of this toxin, whole-cell patch-clamp electrophysiology was employed using isolated DUM neurons from the American cockroach (*Periplaneta americana*). FW178 failed to inhibit insect I_{Na} , $I_{K(DR)}$ or $I_{K(A)}$ at doses up to 1 μ M. However, further studies demonstrated that η -ACTX-Hv1a blocks voltage-gated calcium (Ca_V) channel currents in DUM neurons as well as $K_{(Ca)}$ channel currents carried by $pSlo$ channels with IC_{50} values of 409 nM and 671 nM, respectively. FW178 therefore blocks cockroach Ca_V currents with approximately the same potency as ω -ACTX-Hv1a, a known insect M-LVA and HVA Ca_V channel blocker, while it blocks cockroach $pSlo$ channels with about a 4-fold lower potency than κ -ACTX-Hv1c.

Interestingly FW178 has an LD_{50} of 38 ± 3 pmol/g when injected into *M. domestica* as compared to the LD_{50} values for ω -ACTX-Hv1a (86.5 ± 1.3 pmol/g) and κ -ACTX-Hv1c (91 ± 5 pmol/g). This makes FW178 at least two-fold more potent than any other atracotoxin isolated from Australian funnel-web spiders. Despite this, FW178 only blocks cockroach Ca_V channels with a similar potency to ω -ACTX-Hv1a, and blocks cockroach $BK_{(Ca)}$ with 4-fold less potency than κ -ACTX-Hv1c. Therefore the striking potency of FW178 may result from a synergistic action to block insect Ca_V and $BK_{(Ca)}$ channels. Not surprising the pharmacophore of FW178 (refer section 4.3) contains elements of the pharmacophore of both ω -ACTX-Hv1a and κ -ACTX-Hv1c. Thus FW178 directly block insect $K_{(Ca)}$ channels, but the toxin also enhances this action by indirectly reducing current through these channels by block of the transient inward flow of calcium through Ca_V channels. Therefore FW178 represents the first known dual-target, self-synergizing toxin and is an excellent lead compound for the development of a novel insecticide.

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TABLE OF CONTENTS

•	DISCLAIMER	I
•	ABSTRACT	II
•	ACKNOWLEDGEMENTS	V
•	LIST OF FIGURES AND TABLES	XIII
•	LIST OF ABBREVIATIONS	XIX
•	PUBLICATIONS ARISING FROM THIS THESIS	XXII
	CHAPTER 1: INTRODUCTION	1
•	1.1: BIOPESTICIDES	2
○	1.1.1 Global Pest Problem	2
○	1.1.2 Pesticide Usage	3
○	1.1.3 Transgenic Plants	5
○	1.1.4 Baculoviruses	6
○	1.1.5 Recombinant Baculoviruses	7
•	1.2: AUSTRALIAN FUNNEL WEB SPIDERS	9
•	1.3: AUSTRALIAN FUNNEL WEB SPIDER TOXINS	13
○	1.3.1 Spider Venoms	13
○	1.3.2 Vertebrate-Selective Atracotoxins	13
○	1.3.3 Invertebrate-Selective Atracotoxins	17

CHAPTER 2: ION CHANNELS AND ION CHANNEL TOXINS 18

- **2.1: Na_v CHANNELS** 18
 - 2.1.1 Insect Na_v channels 25
 - 2.1.2 Na_v channel toxins and toxin binding sites 26

- **2.2: K_v CHANNELS** 32
 - 2.2.1 Insect K_v channels 40
 - 2.2.2 K_v channel toxins 41

- **2.3 Ca_v channels** 44
 - Insect Ca_v channels 47
 - Insect-selective Ca_v channel toxins 48

CHAPTER 3: DORSAL UNPAIRED MEDIAN (DUM) NEURONS 50

- **3.1: CHARACTERISTICS OF DORSAL UNPAIRED
MEDIAN NEURONS FROM AMERICAN COCKROACHES
*PERIPLANETA AMERICANA*** 51
 - 3.1.1 Basic electrophysiological characteristics 53
 - 3.1.2 Sodium currents 55
 - 3.1.3 Potassium currents 58
 - 3.1.4 Calcium currents 64

CHAPTER 4: ATRACOTOXINS 69

- **4.1: JANUS-FACED ATRACOTOXINS (J-ACTX's)** 69
 - 4.1.1 Toxicity of J-ACTX Family 70
 - 4.1.2 Structure of J-ACTX-Hv1c and

	pharmacophore Mapping	72
○	4.1.3 What is the target of J-ACTX-Hv1c?	75
•	4.2: ω-ATRACOTOXINS	79
○	4.2.1 Structure and function of the ω -ACTX-Hv1 Family	79
○	4.2.2 Insectophore mapping of ω -ACTX-Hv1a	82
○	4.2.3 Structure and function of the ω -ACTX-Hv2 Family	83
○	4.2.4 Homology with ω -Agatoxin-IVA and Possible Mode of Action	86
•	4.3: THE 'HYBRID' TOXIN FW178	88
○	4.3.1 Toxicity of the 'hybrid' toxin Fw178	89
○	4.3.2 Structure of the 'hybrid' toxin Fw178 and pharmacophore mapping	89
○	4.3.3 What is the target of the 'hybrid' toxin Fw178?	91
•	4.4: THESIS AIMS AND OBJECTIVES	94
•		
	CHAPTER 5: MATERIALS AND METHODS	98
•	5.1: INSECT ELECTROPHYSIOLOGICAL EXPERIMENTS	98
○	5.1.1 Research animals	98
○	5.1.2 Dissection of DUM neurons	99
○	5.1.3 Enzyme treatment of DUM neurons	99
○	5.1.4 Trituration and tissue culture of DUM neurons	100
○	5.1.5 Preparation of tissue culture plates	101
○	5.1.6 Electrophysiological whole-cell patch-clamp set-up	101

○	5.1.7 Microelectrodes and bath electrodes	102
○	5.1.8 Electrophysiological solutions	103
○	5.1.9 External and internal solutions for recording Na ⁺ , K ⁺ and Ca ²⁺ currents from DUM neurons	103
○	5.1.10 Voltage-clamp protocols	106
•	5.2: IDENTIFICATION OF DUM NEURONS	107
•	5.3: MAMMALIAN ELECTROPHYSIOLOGICAL EXPERIMENTS	108
○	5.3.1 Preparation of tissue culture plates	108
○	5.3.2 Dissection and isolation of dorsal root ganglion (DRG) neurons	109
○	5.3.3 Enzyme treatment of DRG neurons	109
○	5.3.4 Trituration and plating of DRG neurons	110
○	5.3.5 External and internal solutions for recording K ⁺ currents from DRG neurons	111
○	5.3.6 Voltage-clamp protocols	111
•	5.4: SLO-CHANNEL ELECTROPHYSIOLOGICAL EXPERIMENTS	112
○	5.4.1 <i>S/o</i> channel expression in HEK293 cells	112
○	5.4.2 External and internal solutions for recording K _(Ca) currents from transfected HEK293 cells	113
○	5.4.3 Voltage-clamp protocols	113
•	5.5: VERTEBRATE TOXICITY TESTING	113
•	5.6: DATA ANALYSIS	115

- **5.7: SUPPLY OF CHEMICALS** 116

CHAPTER 6: RESULTS 117

- **6.1: DETERMINATION OF THE TARGET SITE FOR J-ACTX-HV1C** 117
 - 6.1.1 Effect of J-ACTX-Hv1c on insect Na_V channels 118
 - 6.1.2 Effect of J-ACTX-Hv1c on insect Ca_V channels 120
 - 6.1.3 Effect of J-ACTX-Hv1c on macroscopic insect K_V channels 122
 - 6.1.4 Effect of κ -ACTX-Hv1c on insect $K_{(DR)}$ channels 125
 - 6.1.5 Effect of κ -ACTX-Hv1c on insect $K_{(A)}$ channels 127
 - 6.1.6 Isolation of insect $K_{(Ca)}$ channels using charybdotoxin (ChTX) 130
 - 6.1.7 Effect of κ -ACTX-Hv1c on insect $K_{(Ca)}$ channels 133
 - 6.1.8 Effect of κ -ACTX-Hv1c on mammalian $K_{(Ca)}$ channels 139
 - 6.1.9 Effect of κ -ACTX-Hv1c on *pSlo* currents expressed in HEK293 cells 141
 - 6.1.10 Effect of κ -ACTX-Hv1c on *dSlo* currents expressed in HEK293 cells 145
 - 6.1.11 Effect of κ -ACTX-Hv1c on *mSlo* currents expressed in HEK293 cells 148

- **6.2: EFFECT OF ALANINE-SCANNING MUTANTS OF κ -ACTX-HV1C ON DUM NEURON K_{Ca} CHANNELS** 150
 - 6.2.1 Preface 150
 - 6.2.2 R8A mutant 150
 - 6.2.3 Y31A mutant 152
 - 6.2.4 P9A mutant 154

○	6.2.5 V29A mutant	156
•	6.3: DETERMING THE CHEMICAL FEATURES OF THE TOXIN PHARMACOPHORE: FUNCTIONAL ROLE OF ARG⁸	158
○	6.3.1 Preface	158
○	6.3.2 R8E mutant	159
○	6.3.3 R8Q mutant	159
○	6.3.4 R8K mutant	160
○	6.3.5 R8H mutant	163
•	6.4: DETERMING THE CHEMICAL FEATURES OF THE TOXIN PHARMACOPHORE: FUNCTIONAL ROLE OF Tyr³¹	165
○	6.4.1 Preface	165
○	6.4.2 Y31V mutant	165
○	6.4.3 Y31L mutant	167
○	6.4.4 Y31F mutant	169
○	6.4.5 Y31W mutant	171
	CHAPTER 7: RESULTS (Part II)	176
•	7.1: DETERMINATION OF THE TARGET SITE FOR THE 'HYBRID' TOXIN FW178	176
○	7.1.1 Preface	176
○	7.1.2 Vertebrate Toxicity of the 'hybrid' toxin FW178	177
○	7.1.3 Effect of the 'hybrid' toxin FW178 on insect Na _v channels	179
○	7.1.4 Effect of the 'hybrid' toxin FW178 on insect Ca _v channels	181

○	7.1.5 Effect of the 'hybrid' toxin FW178 on insect $K_{(A)}$ and $K_{(DR)}$ channels	183
○	7.1.6 Effect of the 'hybrid' toxin FW178 on <i>pSlo</i> currents expressed in HEK293 cells	186
CHAPTER 8: DISSCUSSION		190
○	8.1. What is the molecular target of κ -ACTX-Hv1c?	190
○	8.2 κ -ACTX-Hv1c targets insect BK_{Ca} channels	193
○	8.3 Interaction of the pharmacophore with the channel target	197
○	8.3.1 The role of the critical arginine	198
○	8.3.2 The role of the critical tyrosine	200
○	8.4 Model of κ -ACTX-Hv1c binding	201
○	8.5 BK_{Ca} channels, a potential insecticide target?	203
○	8.6 Design of a novel chemical insecticide	205
CHAPTER 9: THE 'HYBRID' TOXIN FW178		207
CHAPTER 10: REFERENCES		211

LIST OF FIGURES AND TABLES

CHAPTER 1: INTRODUCTION

- Fig 1.1:** Partial taxonomy of Australian funnel-web spiders. 10
- Fig 1.2:** *Hadronyche versuta* is mainly distributed throughout the Blue Mountains region of New South Wales (NSW). 11
- Fig 1.3:** Adult male and female *Hadronyche versuta*. 12
- Fig 1.4:** Structural characteristics of the δ -ACTX family. 16

CHAPTER 2: ION CHANNELS AND ION CHANNEL TOXINS

- Fig 2.1:** Schematic representation of the molecular structure and membrane topology of Na_v channels. 24
- Fig 2.2:** Localization of known neurotoxin receptor sites on Na_v channels. 31
- Fig 2.3:** Membrane topologies and main features of Kv and Kir potassium channel subtypes. 34
- Fig 2.4:** Schematic representation of the molecular structure and membrane topology of the α - and β -subunits of BK_{Ca} channels. 39
- Fig 2.5:** HVA Ca_v channels typically comprise a single copy of each of the α_1 , α_2 - δ , β and γ subunits, whereas LVA Ca_v channels consist of only the pore-forming α_1 subunit. 46

CHAPTER 3: DORSAL UNPAIRED MEDIAN (DUM) NEURONS

Fig 3.1: DUM neurons of the terminal abdominal ganglia (TAG) of the Cockroach *Periplaneta americana*. 52

Fig 3.2: Intrinsic spontaneous electrical activity in DUM neuron somata *in vitro*. 54

Table 3.1: Sodium currents in cockroach DUM neurons. 57

Table 3.2: Potassium and chloride induced currents in cockroach DUM neurons. 62

Table 3.3: Calcium currents in cockroach DUM neurons. 68

CHAPTER 4: ATRACOTOXINS

Fig 4.1: Structural characteristics of the J-ACTX-1 family. 71

Fig 4.2: The bioactive surface of J-ACTX-Hv1c. 75

Fig 4.3: Inhibitory Cystine Knot motif (ICK). 76

Table 4.1: Sources and biological activity of cystine knot peptides. 77

Fig 4.4: Comparison of the primary structures of currently available members of the ω -ACTX-Hv1 family. 81

Fig 4.5: Molecular surface of ω -ACTX-Hv1a illustrating the proposed interaction between residues in the β -hairpin and insect Ca_v channels. 83

Fig 4.6: Comparison of the primary structures of currently available members of the ω -ACTX-Hv2 family.	85
Fig 4.7: Structural similarities between ω -ACTX-Hv2a and ω -agatoxin-IVA.	87
Fig 4.8: Comparison of the mature toxin sequences of ω -ACTX-Hv1a, κ -ACTX-Hv1c and FW178.	88
Fig 4.9: Ribbon representation of FW178 hybrid toxin.	90
Fig 4.10: Pharmacophore of FW178.	91
Fig 4.11: Overlay of the pharmacophore of ω -ACTX-Hv1a, κ -ACTX-Hv1c and FW178.	93

CHAPTER 5: MATERIALS AND METHODS

Table 5.1: Composition of external and internal solutions used for electrophysiological recordings of sodium currents from DUM neurons.	104
Table 5.1: Composition of external and internal solutions used for electrophysiological recordings of potassium currents from DUM neurons.	104
Table 5.3: Composition of external and internal solutions used for electrophysiological recordings of calcium currents from DUM neurons.	105
Fig 5.1: Light micrographs of DUM neurons stained with neutral red.	107

Table 5.4: Trypsin incubation times for newborn rats. 110

Table 5.5: Composition of external and internal solutions used for electrophysiological recordings of potassium currents from DRG neurons. 111

Table 5.6: Composition of external and internal solutions used for electrophysiological recordings of $I_{K(Ca)}$ currents from transfected HEK293 cells. 113

CHAPTER 6: RESULTS

Fig 6.1: Effects of J-ACTX-Hv1c on Na_V channels in cockroach DUM neurons. 119

Fig 6.2: Effects of J-ACTX-Hv1c on Ca_V channels in cockroach DUM neurons. 121

Fig 6.3: Effects of J-ACTX-Hv1c on K_V channels in cockroach DUM neurons. 124

Fig 6.4: Effects of κ -ACTX-Hv1c on $K_{(DR)}$ channels in cockroach DUM neurons. 127

Fig 6.5: Effects of κ -ACTX-Hv1c on $K_{(A)}$ channels in cockroach DUM neurons. 129

Fig 6.6: To record $I_{K(Ca)}$ in isolation from other K_V channel currents a current subtraction routine following perfusion with the $K_{(Ca)}$ channel blockers ChTX and $CdCl_2$ was used. 132

- Fig 6.7:** κ -ACTX-Hv1c blocks K_{Ca} channels in cockroach DUM neurons. 136
- Fig 6.8:** κ -ACTX-Hv1c and charybdotoxin share the same insecticidal target. 138
- Fig 6.9:** κ -ACTX-Hv1c failed to inhibit K_V channels in rat DRG neurons. 140
- Fig 6.10:** Inhibition of *pSlo* currents by TEA (10 mM) and ChTX (1 μ M) 143
- Fig 6.11:** Inhibition of *pSlo* currents by κ -ACTX-Hv1c. 144
- Fig 6.12:** κ -ACTX-Hv1c fails to significantly inhibit *dSlo* channels. 147
- Fig 6.13:** κ -ACTX-Hv1c significantly inhibits *mSlo* channels. 149
- Fig 6.14:** The mutant κ -ACTX-Hv1c constructs R8A & Y31A block K_{Ca} channels in cockroach DUM neurons. 153
- Fig 6.15:** The mutant κ -ACTX-Hv1c construct P9A blocks K_{Ca} channels in cockroach DUM neurons. 155
- Fig 6.16:** The mutant κ -ACTX-Hv1c construct V29A blocks K_{Ca} channels in cockroach DUM neurons. 157
- Fig 6.17:** The mutant κ -ACTX-Hv1c constructs R8E & R8Q block K_{Ca} channels in cockroach DUM neurons. 160
- Fig 6.18:** The mutant κ -ACTX-Hv1c construct R8K blocks K_{Ca} channels in cockroach DUM neurons. 162
- Fig 6.19:** The mutant κ -ACTX-Hv1c construct R8H blocks K_{Ca} channels

in cockroach DUM neurons. 164

Fig 6.20: The mutant κ -ACTX-Hv1c construct Y31V blocks K_{Ca} channels in cockroach DUM neurons. 166

Fig 6.21: The mutant κ -ACTX-Hv1c construct Y31L blocks K_{Ca} channels in cockroach DUM neurons. 168

Fig 6.22: The mutant κ -ACTX-Hv1c construct Y31F blocks K_{Ca} channels in cockroach DUM neurons. 170

Fig 6.23: The mutant κ -ACTX-Hv1c construct Y31W blocks K_{Ca} channels in cockroach DUM neurons. 172

Fig 6.24: Concentration-response curves for recombinant κ -ACTX-Hv1c mutants on cockroach DUM neuron $I_{K(Ca)}$. 174

Fig 6.25: Comparison of fold-reductions in DUM neuron $I_{K(Ca)}$ IC_{50} and housefly LD_{50} . 175

CHAPTER 7: RESULTS

Fig 7.1: Typical responses of the isolated chick biventer nerve-muscle preparation to 1 μ M FW178. 178

Fig 7.2: Effects of 'hybrid' toxin FW178 on Na_V channels in cockroach DUM neurons. 180

Fig 7.3: The 'hybrid' toxin FW178 blocks Ca_V channels in cockroach DUM neurons. 183

Fig 7.4 Effects of 'hybrid' toxin FW178 on macroscopic K_V channels comprising $I_{K(A)}$ and $I_{K(DR)}$ in cockroach DUM neurons. 186

Fig 7.5: Block of K_V channel currents by the 'hybrid' toxin FW178 in the absence of ChTX. 187

Fig 7.6: Inhibition of $pSlo$ currents by the 'hybrid' toxin FW178. 190

CHAPTER 8: DISSCUSION

Figure 8.1: Alignment of the pore region of vertebrate and invertebrate Slo channels. 194

Table 8.1: Phyletic-selectivity of κ -ACTX-Hv1c and ChTX for K_{Ca} channels. 196

LIST OF ABBREVIATIONS

ACh	Acetylcholine
ACTX	Atracotoxin
AP	Action Potential
ATP	Adenosine tri-phosphate
α -BGT	α -Bungarotoxin
α -LTx	α -Latrotoxin
α -ScTX	α -Scorpion toxin
4-AP	4-amino pyridine
BAPTA	1,2-bis-(2-aminophenoxy)ethane- <i>N,N,N,N</i> -tetra acetate
BK_{Ca}	Large-conductance Ca^{2+} -activated K^+ channels
BTX	Batrachotoxin
β -Sctx	β -Scorpion toxin

CAMs	Cell adhesion molecules
CF-NIS	Ca ²⁺ -free normal insect saline
CICR	Ca ²⁺ -induced Ca ²⁺ -release
ChTX	Charybdotoxin
CMF-PBS	Ca ²⁺ -and Mg ²⁺ -free phosphate buffered saline
DHP	Dihydropyridine
DRG	Dorsal Root Ganglion
DUM	Dorsal Unpaired Median
ET ₅₀	Time to 50% paralysis/death
GABA	γ-aminobutyric acid
GSH	Glutathione
GST	Glutathione-S-transferase
HaTX-1	Hanatoxin-1
HEK293	Human embryonic kidney 293 cells
HpTX	Heteropodatoxin
HoTx	Hololenatoxin
IBX	Iberiotoxin
ICK	Inhibitory cystine knot
IFM	Hydrophobic triad of isoleucine, phenylalanine and methionine
Ig	Immunoglobulin
i.p	Intraperitoneal
<i>I</i> _{Ca(tLVA)}	Transient low voltage-activated calcium current
<i>I</i> _{Ca(mLVA)}	Maintained low voltage-activated calcium current
<i>I</i> _{Ca(HVA)}	High voltage-activated calcium current
<i>I</i> _{Ca(r)}	Calcium resting current
<i>I</i> _{Cl(Ca)}	Calcium-activated chloride current
<i>I</i> _{K(A)}	'A-like' potassium current
<i>I</i> _{K(tCa)}	Transient calcium-activated potassium current
<i>I</i> _{K(mCa)}	Maintained (or Late) calcium-activated potassium current
<i>I</i> _{K(DR)}	Delayed rectifier potassium current
<i>I</i> _{K(IR)}	Inward rectifier potassium current

$I_{K(r)}$	Potassium resting current
$I_{K(t)}$	Total (macroscopic) potassium current
$I_{K(Na)}$	Sodium-activated potassium current
I_{Na}	Sodium current
$I_{(mNa)}$	Maintained sodium current
$I_{(rNa)}$	Sodium resting (or background) current
LA	Local Anesthetics
MSG	Mushroom shaped accessory gland
NIS	Normal insect saline
NMR	Nuclear magnetic resonance
NPPB	5-nitro-2-(3-phenylpropylamino) benzoic acid
NSW	New South Wales
NVPs	Nucleopolyhedroviruses
PLTX	<i>Plectreurys</i> spider toxin
rp-HPLC	Reversed phase high performance liquid chromatography
SITS	4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid
SK _{Ca}	Small-conductance Ca ²⁺ -activated K ⁺ channels
<i>dSlo</i>	<i>Drosophila</i> Slo-poke potassium channel
<i>mSlo</i>	Murine Slo-poke potassium channel
<i>pSlo</i>	<i>Periplaneta</i> Slo-poke potassium channel
STX	Saxitoxin
TAG	Terminal abdominal ganglion
TEA-Cl	Tetra ethyl ammonium chloride
TTX	Tetrodotoxin
VGCC	Voltage-gated calcium channels
VGKC	Voltage-gated potassium channels
VGSC	Voltage-gated sodium channels
VUM	Ventral Unpaired Median

PUBLICATIONS ARISING FROM THIS THESIS

PATENTS

- [1] King GF, Sollod McFarland B, Nicholson GM, Gunning SJ (2005) INSECTICIDAL POLYPEPTIDES AND METHODS OF USE THEREOF. United States Provisional Application Serial No. 11/267,815 filed on November 4, 2005

PUBLICATIONS IN REFEREED JOURNALS AND MONOGRAPHS

IF = 2006 Science Citation Index journal impact factor; Cites = Times Cited

- [1] Gunning SJ, Maggio FJ, Windley MJ, Valenzuela SM, King GF, Nicholson GM (2008) The Janus-faced atracotoxins are specific blockers of invertebrate K_{Ca} channels. *FEBS Journal*, 275, 4045-4059. (IF = 3.033; Cites = 0)
- [2] Birinyi-Strachan LC, Gunning SJ, Lewis RJ, Nicholson GM (2005) Block of voltage-gated potassium channels by Pacific ciguatoxin-1 contributes to increased neuronal excitability in rat sensory neurons. *Toxicology and Applied Pharmacology*, 204, 175-186. (4.722; Cites = 7)
- [3] Gunning SJ, Chong Y, Khalife AA, Hains PG, Broady KW, Nicholson GM (2003) Isolation of δ -missulenatoxin-Mb1a, the major vertebrate-active spider d-toxin from the venom of *Missulena bradleyi* (Actinopodidae). *FEBS Letters*, 554, 211-218. (3.372; Cites = 7)

PAPERS IN PREPARATION

- [4] Gunning S, Sollod BL, Wen S, Quinton L, Chamot-Rooke J, Escoubas P, Nicholson GM, King GF (2008) Evolution of a dual-target, self-synergizing ion channel toxin. *Science*, in preparation.

INTERNATIONAL CONFERENCE PROCEEDINGS

- [1] Nicholson GM, Gunning SJ, Maggio FJ, Windley MJ, Valenzuela SM, King GF (2008) Identifying novel insecticide targets using insect-specific spider toxins. 3rd International Congress on Natural Peptides to Drugs, Zermatt, Switzerland 14-17 April, 2008.
- [2] Sollod BL, Gunning S, Wen S, Nicholson GM, King GF (2006) A dual-target, self-synergizing toxin from spider venom. 15th World Congress on Animal, Plant and Microbial Toxins, Glasgow, Scotland, 24-28 July, 2006
- [3] Gunning SJ, Maggio F, Valenzuela S, Broady KW, King GF, Nicholson GM (2006) Pharmacophore mapping of the κ -atracotoxins: selective insect potassium channel blockers that reveal a novel insecticide target. 15th World Congress on Animal, Plant and Microbial Toxins, Glasgow, Scotland, 24-28 July, 2006.
- [4] Gunning SJ, Maggio F, Valenzuela S, Broady KW, King GF, Nicholson GM (2005) Selective actions of κ -atracotoxins on insect K_{Ca} channels: electrophysiological validation of the insect target and pharmacophore. 7th Asia-Pacific Congress on Animal, Plant and Microbial Toxins, Cebu City, Philippines, 25-28 October, 2005.
- [5] Sollod BL, Gunning SJ, Wen S, Nicholson GM, King GF (2005) Evolution of a dual target, self-synergizing toxin: implications for insecticide and pharmaceutical discovery. Venoms to Drugs 3, Heron Island, 28 August - 2 September, 2005.

- [6] Gunning SJ, Maggio F, Valenzuela S, Broady KW, King GF, Nicholson GM (2005) κ -Atracotoxins: Insect potassium channels blockers that reveal a novel insecticide target Venoms to Drugs 3, Heron Island, 28 August - 2 September, 2005.
- [7] Gunning SJ, Maggio F, King GF, Nicholson GM (2004) κ -Atracotoxins: Insect potassium channels blockers that reveal a novel insecticide target. 8th Symposium of the Pan-American Section of the International Society of Toxinology, Angra dos Reis, Brazil, 19-23 September 2004.
- [8] Gunning SJ, Chong Y, Khalife AA, Hains PG, Broady KW, Nicholson GM (2003) Discovery of a novel sodium channel neurotoxin δ -missulenatoxin-Mb1a from the venom of the Eastern mouse spider *Missulena bradleyi*. 14th World Congress on Animal, Plant and Microbial Toxins, Adelaide, 14-19 September 2003.
- [9] Gunning SJ, Maggio F, King GF, Nicholson GM (2003) Do insecticidal J-atracotoxins target insect potassium channels? 14th World Congress on Animal, Plant and Microbial Toxins, Adelaide, 14-19 September 2003.
- [10] Gunning S, Khalife A, Padula M, Smith R, Broady KW and Nicholson GM (2002) Modulation of sodium channel gating and kinetics by δ -missulenatoxin-Mb1a from the Australian eastern mouse spider *Missulena bradleyi*. 6th Asia-Pacific Congress on Animal, Plant and Microbial Toxins, Cairns, 8-12 July 2002

LOCAL CONFERENCE PROCEEDINGS

- [1] Gunning SJ, Maggio FJ, Valenzuela SM, King GF, Nicholson GM (2007) Mapping the insectophore of κ -atracotoxins: insect-selective BKCa channel blockers that reveal a novel insecticide target. Proceedings of the Australian Physiological Society, 38, 37P. Newcastle, 2-5 December, 2007
RNSH
RNSH
- [4] Gunning SJ, Maggio F, Valenzuela SM, King GF, Nicholson GM (2004) κ -Atracotoxins: Insect potassium channels blockers that reveal a novel insecticide target. 21th RNSH/UTS Scientific Meeting, Sydney, November 2004
- [5] Gunning SJ, Maggio F, King GF, Nicholson GM (2003) Do insecticidal J-atracotoxins target insect potassium channels? 20th RNSH/UTS Scientific Meeting, Sydney, November 2003
- [6] Gunning SJ, Chong Y, Khalife AA, Hains PG, Broady KW, Nicholson GM (2003) Discovery of a novel sodium channel neurotoxin δ -Missulenatoxin-Mb1a from the venom of the Eastern Mouse spider *Missulena bradleyi*. 20th RNSH/UTS Scientific Meeting, Sydney, November 2003
- [7] Gunning SJ, Khalife AA, Padula M, Smith R, Broady KW, Nicholson GM (2002) Isolation and pharmacological characterisation of the neurotoxin δ -Missulenatoxin-Mb1a from the Eastern Mouse spider *Missulena bradleyi*. 19th RNSH/UTS Scientific Meeting, Sydney, November 2002