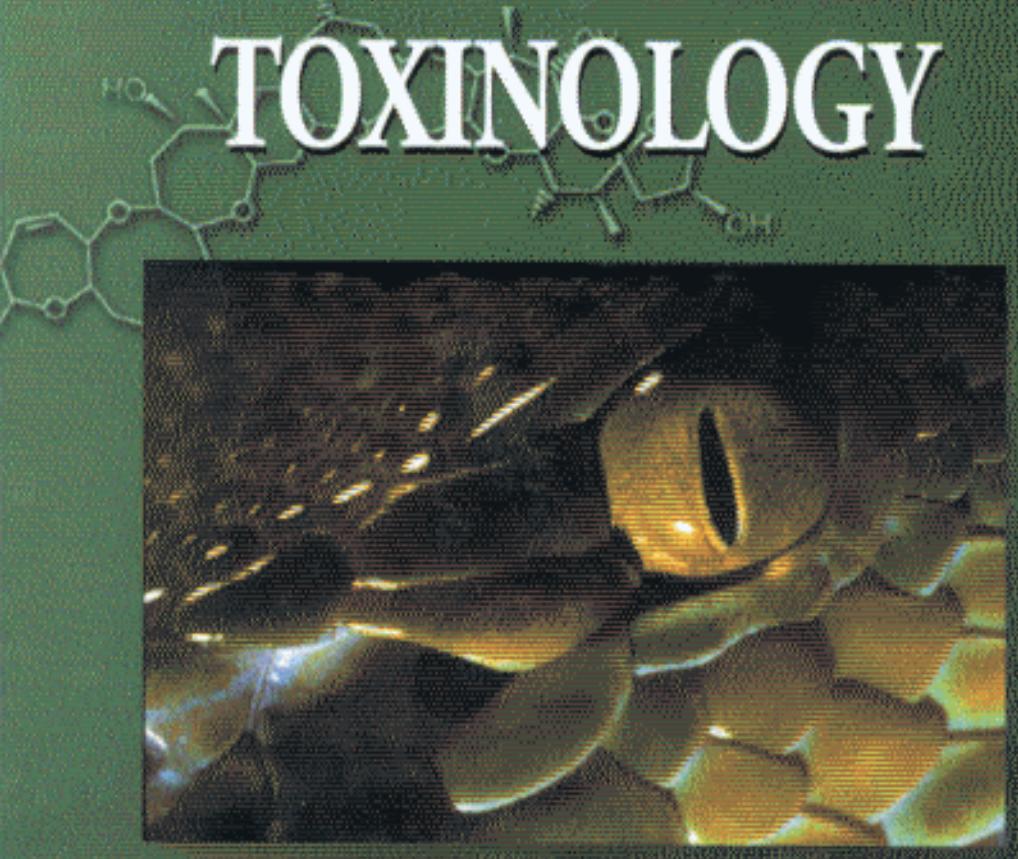


 WILEY

PERSPECTIVES IN MOLECULAR TOXICOLOGY



Edited by
André Ménez

CONTENTS

List of Contributors xix

Preface xxix

I TOXINS FROM MICROORGANISMS 1

1 Bacterial Toxins with Metalloprotease Activity 3

O. Rossetto and C. Montecucco

1.1 Introduction 3

1.2 Tetanus and botulinum neurotoxins 4

 1.2.1 Introduction 4

 1.2.2 Genetics and structure 4

 1.2.3 Neuronal intoxication 7

1.3 The anthrax lethal factor 10

 1.3.1 Introduction 10

 1.3.2 The protective antigen PA and toxin internalisation
 and translocation 10

 1.3.3 The metalloproteolytic activity of the lethal
 factor LF 12

1.4 Fragilysins from *Bacteroides fragilis* 13

 1.4.1 Introduction 13

 1.4.2 Genetics and structure of fragilysin (BFT) 13

 1.4.3 The metalloproteolytic activity of fragilysin 15

Acknowledgements 17

References 17

2 The Cholesterol-dependent Cytolysins: Current Perspectives on Membrane Assembly and Insertion 23

E. Hotze and R.K. Tweten

2.1 Introduction 23

2.2 CDC structure 24

2.3 Membrane recognition 25

 2.3.1 Domain 4 25

 2.3.2 Cellular recognition 26

- 2.4 Formation of oligomeric complexes and insertion of the transmembrane domains 28
 2.4.1 Identification of the transmembrane domain of PFO 28
 2.4.2 Oligomer assembly and insertion 29
- 2.5 The role of the CDCs in bacterial pathogenesis 31
 2.5.1 Listeriolysin O 32
 2.5.2 Pneumolysin 33
 2.5.3 Perfringolysin O 33
 2.5.4 Streptolysin O 33
- 2.6 Summary and perspectives 34
- References 35
- 3 Toxin-producing Dinoflagellates 39
M.J. Holmes and R. Lewis
- 3.1 Review of toxin-producing dinoflagellates 45
 3.1.1 Dinoflagellates with motile cell predominantly planktonic 45
 3.1.2 Dinoflagellates with motile cell predominantly benthic 50
- References 55
- 4 Involvement of Na^+ in the Actions of Ciguatoxins and Brevetoxins that Stimulate Neurotransmitter Release and Affect Synaptic Transmission 67
J. Molgó and E. Benoit
- 4.1 Introduction 67
- 4.2 Origins and chemical structures of ciguatoxins and brevetoxins 68
- 4.3 Voltage-dependent Na^+ channels as targets for ciguatoxins and brevetoxins 72
- 4.4 Alterations of neuronal Na^+ current and membrane potential by ciguatoxins and brevetoxins 73
- 4.5 Ciguatoxin- and brevetoxin-mediated swelling of the nodes of Ranvier of myelinated axons 75
- 4.6 Ciguatoxins and brevetoxins increase intracellular Ca^{2+} levels in neuronal cells 77
- 4.7 Quantal transmitter release changes caused by ciguatoxins and brevetoxins 78
 4.7.1 Synchronous evoked quantal transmitter release 79
 4.7.2 Asynchronous quantal transmitter release 80
- 4.8 Swelling of motor nerve terminals *in situ* 82
- 4.9 Impairment of synaptic vesicle recycling by ciguatoxins and brevetoxins 83

4.10 Perspectives	86
Acknowledgements	87
References	87

II ANIMAL TOXINS AND NEW METHODOLOGIES 95

5 Role of Discovery Science in Toxicology: Examples in Venom Proteomics 97

J.W. Fox, J.D. Shannon, B. Stefansson, A.S. Kamiguti, R.D.G. Theakston, S.M.T. Serrano, A.C.M. Camargo and N. Sherman

5.1 Introduction	97
5.2 2-D gel electrophoresis/proteome analysis of snake venoms	98
5.2.1 <i>Dispholidus typus</i> (Boomslang) proteome	99
5.3 Proteomic comparison of the venoms from two different snake species producing similar pathologies of envenoming	100
5.4 Use of different pI ranges for venom 2-D PAGE proteome analysis	102
5.5 Proteome comparison of venoms from identical snake species	102
References	105

6 Proteomics of Venom Peptides 107

R. Stöcklin and P. Favreau

6.1 Proteomics	107
6.2 Mass spectrometry	108
6.2.1 Ion sources	109
6.2.2 Mass analysers	109
6.2.3 Mass spectrometers	110
6.3 Animal venom proteomics	111
6.3.1 Proteomic strategies	112
6.3.2 Direct analysis of crude venoms	113
6.3.3 Identification and characterisation of isolated toxins	114
6.3.4 On-line LC-ES-MS of crude <i>Apis mellifera</i> (honey bee) venom	114
6.3.5 MALDI-TOF-MS analysis of crude venoms	115
6.3.6 Proteomics of <i>Conus</i> venom	117
6.3.7 Discovery of novel sarafotoxins in <i>Atractaspis</i> snake venoms	119
6.4 Conclusion	119
Acknowledgements	121
References	121

7 High-resolution NMR of Venom Toxins in Nanomolar Amounts 125*M. Delepiere*

7.1 Introduction 125

7.2 Structural characterisation by NMR using small amounts
of material 126

7.2.1 The signal-to-noise ratio 127

7.2.2 Small-volume probes 127

7.3 Nano.nmr probe applications 133

7.4 Conclusions 135

Acknowledgements 136

References 136

**III ANIMAL TOXINS: FROM FUNDAMENTAL STUDIES
TO DRUGS? 141****8 Cone Snails and Conotoxins Evolving Sophisticated
Neuropharmacology 143***B.M. Olivera, J.S. Imperial and G Bulaj*8.1 *Conus* venoms: the molecules 143

8.1.1 Overview 143

8.1.2 Post-translational modification of conopeptides 144

8.1.3 Biosynthesis of conopeptides 144

 8.1.4 Functional domains of conopeptide precursors: the
generation of molecular diversity 146

8.1.5 Perspectives 147

8.2 Conopeptides: functional aspects 147

8.2.1 Overview 147

8.2.2 Biology of cone snails: a synopsis 148

8.2.3 Molecular mechanisms 148

8.2.4 Toxin cabals 149

8.2.5 Interspecific divergence: an overview 151

8.3 Conopeptides in medicine and neuroscience 152

8.3.1 Introduction 152

8.3.2 Medical applications 152

 8.3.3 *Conus* venoms: a toolbox for the neuroscientist 153

8.4 Future directions 153

8.4.1 Introduction 153

8.4.2 Structure and folding 154

8.4.3 Delivery design and post-translational modification 155

 8.4.4 *In vitro* extension of peptide evolution: drug
development 156

Acknowledgements 156

References 156

9 Toxin Structure and Function: What Does Structural Genomics Have To Offer? 159*R.S. Norton*

- 9.1 Many functions but few folds 159
 - 9.2 Structural genomics 161
 - 9.3 Membrane proteins: a major challenge 164
 - 9.4 Does protein structure explain function? 167
 - 9.5 Future prospects 168
- Acknowledgements 169
References 169

10 The Binding Sites of Animal Toxins Involve Two Components: A Clue for Selectivity, Evolution and Design of Proteins? 175*A. Ménez, D. Servent and S. Gasparini*

- 10.1 Introduction 175
 - 10.2 On the molecular targets 176
 - 10.2.1 The voltage-gated potassium channels 176
 - 10.2.2 The nicotinic acetylcholine receptors (AChRs) 177
 - 10.3 On the binding sites displayed by animal toxins 178
 - 10.4 What can we learn from comparisons of binding sites displayed by animal toxins? 178
 - 10.4.1 An understanding of the structural basis for the biological diversity of animal toxins: one mould for multiple missions 178
 - 10.4.2 A molecular rationale for the pleiotropic properties of animal toxins 179
 - 10.5 How do binding site properties help to understand the evolution of animal toxins? 188
 - 10.6 Design of novel toxins 189
 - 10.6.1 Modulating the original specificity profiles of natural toxins 189
 - 10.6.2 Design of novel functions on toxin folds 190
 - 10.7 Conclusions 191
- Acknowledgements 191
References 191

11 Scorpion Genes and Peptides Specific for Potassium Channels: Structure, Function and Evolution 201*L.D. Possani, E. Merino, M. Corona and B. Becerril*

- 11.1 Introduction 201
- 11.2 Isolation and primary sequences 202

- 11.3 Three-dimensional characterisation 204
11.4 Physiological effects 205
11.5 Structure of genes encoding K⁺-peptides 207
11.6 Phylogenetic tree and evolution 208
11.7 Perspectives and concluding remarks 209
Acknowledgements 211
References 211
- 12 Scorpion Toxins Differentiating among Neuronal Sodium Channel Subtypes: Nature's Guide for Design of Selective Drugs 215**
D. Gordon, N. Gilles, D. Bertrand, J. Molgó, G.M. Nicholson, M.P. Sauviat, E. Benoit, I. Shichor, I. Lotan, M. Gurevitz, R.G. Kallen and S.H. Heinemann
- 12.1 Introduction 215
 12.1.1 Mammalian excitable tissues display different sodium channel subtypes 216
 12.1.2 Scorpion toxins affecting sodium channels 216
- 12.2 Scorpion α -toxins and receptor site-3 on sodium channel subtypes 218
 12.2.1 Localisation of receptor site-3 on the sodium channel 219
- 12.3 Selective interaction of α -toxins with distinct mammalian sodium channel subtypes 219
 12.3.1 Differences in toxicity of α -toxins in CNS and periphery 219
 12.3.2 α -Toxins differentiate between neuronal sodium channel subtypes in CNS 221
 12.3.3 Difference in sub-cellular localisation of Lqh-II and Lqh-III sodium channel targets 223
 12.3.4 Effects of scorpion α -toxins on peripheral sodium channels 224
 12.3.5 Sodium channels of similar tissues differ in various vertebrates 225
- 12.4 Do the various α -toxins interact identically with receptor site-3? 225
- 12.5 Scorpion β -toxins that bind to receptor site-4 227
 12.5.1 Excitatory and depressant toxins exclusively bind to insect sodium channels 227
 12.5.2 Localisation of receptor site-4 on the sodium channel 229
- 12.6 Sodium channel domain-2 confers selective binding of excitatory toxins 230

- 12.7 Concluding remarks and perspectives 230
12.7.1 Structural differences at the toxin-receptor interacting surfaces of various sodium channels 230
12.7.2 Selective recognition 231
References 232
- 13 Diversification of Toxic Sites on a Conserved Protein Scaffold – a Scorpion Recipe for Survival 239**
M. Gurevitz, N. Zilberman, O. Froy, M. Turkov, R. Wilunsky, I. Karbat, J. Anglister, B. Shaanan, M. Pelhate, M.E. Adams, N. Gilles and D. Gordon
- 13.1 Diverse pharmacology of scorpion toxins affecting sodium channels 239
13.2 Structure-activity relationship 240
13.2.1 Cloning and functional expression 240
13.2.2 Determination of three-dimensional structures 240
13.2.3 Elucidation of bioactive surfaces 242
13.3 Diversification of toxins 243
13.3.1 Convergent and divergent toxin evolution 243
13.3.2 Scorpion toxin gene families and genomic organisation 244
13.3.3 A putative mechanism for diversification of bioactive surfaces in scorpion 'long' toxins 245
13.4 Perspectives in toxin design 246
13.4.1 Mobilisation of bioactive sites on a conserved scaffold 246
13.4.2 Future prospects in design of target-selective toxins 247
Acknowledgements 248
References 248
- 14 Methodological Approaches to the Study of Ion Channels Using Peptide Toxins: Proposed Comprehensive Guidelines 255**
M. De Waard, J.-M. Sabatier and H. Rochat
- 14.1 Ion channels are important targets for pharmacological and therapeutic interventions 256
14.2 Toxins as molecular traces for the purification of cloned ion channels 257
14.3 On the search for new natural ligands acting on ion channels 258
14.3.1 Biological sources for novel compounds acting on ion channels 258
14.3.2 Toxin screening tests 259
14.3.3 Production of toxins 261

- 14.4 Strategies in the design of therapeutically active molecules 263
14.5 Structure–function relationship approach for the design of novel therapeutic agents 263
 14.5.1 Toxin and ion channel structure 263
 14.5.2 Design of novel compounds 264
14.6 Conclusion 266
References 267
- 15 Toxins as Probes for Structure and Specificity of Synaptic Target Proteins 271**
P. Taylor, B. Molles, S. Malany and H. Osaka
- 15.1 Introduction 271
15.2 Site selectivity of the toxins 273
15.3 Towards a structure of the subunit binding face 276
15.4 Delineation of individual residue contributions to the binding energy 276
15.5 Concluding remarks 278
References 278
- 16 Allosteric and Steric interactions of Polyamines and Polyamine-containing Toxins with Nicotinic Acetylcholine Receptors 281**
T.J. Brier, I.R. Mellor and P.N.R. Usherwood
- 16.1 Introduction 281
16.2 Electrophysiological studies 284
 16.2.1 Potentiation 284
 16.2.2 Inhibition 284
 16.2.3 Ligand-binding studies 290
 16.2.4 Photoaffinity labelling experiments 290
16.3 Discussion 292
References 293
- 17 Anabaseine as a Molecular Model for Design of $\alpha 7$ Nicotinic Receptor Agonist Drugs 297**
W.R. Kem
- 17.1 Introduction 297
17.2 Neuronal nicotinic receptors as drug targets 299
17.3 Anabaseine, a natural toxin 300
17.4 Benzylidine and cinnamylidene derivatives of anabaseine 303
17.5 *In vitro* studies of DMXBA interaction with nicotinic receptors 303
17.6 DMXBA interaction with other receptors 305
17.7 Effects of DMXBA on cognition and sensory gating 306
17.8 Effects of DMXBA upon other neurotransmitter systems 306
17.9 Neuroprotective actions 307

- 17.10 Pharmacokinetics and biotransformation of DMXBA 307
17.11 Effects of chronic nicotinic agonist administration 308
17.12 Nicotine dependence 308
17.13 Other compounds acting on $\alpha 7$ receptors 309
17.14 Possible toxic consequences of excessive $\alpha 7$ receptor stimulation 309
17.15 Concluding comments 310
Acknowledgements 310
References 311
- 18 Understanding the Structure–Function Relationship of Snake Venom Cardiotoxins 315**
T.K.S. Kumar, S. Srisailam, R.R. Vethanayagam and C. Yu
18.1 Introduction 315
18.2 The chemistry of cardiotoxins 315
18.3 Structure of cardiotoxins 316
18.4 Mode of action of cardiotoxins 321
18.5 Is there a receptor for cardiotoxins? 323
Acknowledgements 324
References 324
- 19 Structure and Function of Disintegrins and C-lectins: Viper Venom Proteins Modulating Cell Adhesion 327**
S. Niewiarowski, C. Marcinkiewicz, I. Wierzbicka-Patynowski, M.A. McLane and J.J. Calvete
19.1 Introduction 327
19.2 Viper venom anti-adhesive molecules 328
19.3 Monomeric disintegrins 328
19.4 Dimeric disintegrins 330
19.5 C-lectin-like proteins 333
19.6 Implication in biomedical research 335
References 337
- 20 Prothrombin Activators from Snake Venoms 341**
R.M. Kini, J.S. Joseph and V.S. Rao
20.1 Introduction 341
20.2 Group A prothrombin activators 342
20.3 Group B prothrombin activators 342
20.4 Group C prothrombin activators 344
20.5 Group D prothrombin activators 345
20.6 Implications of structural studies of prothrombin activators 348
20.7 Physiological role of prothrombin activators 349
Acknowledgements 351
References 351

21	C-type Lectins from Snake Venoms: New Tools for Research in Thrombosis and Haemostasis	357
<i>A. Wisner, M. Leduc and C. Bon</i>		
21.1	Introduction	357
21.2	Snake venom CTL affecting platelet functions	362
21.2.1	Interaction of snake venom CTLs with platelet GPIb	363
21.2.2	Snake venom CTLs interacting with vWF	365
21.2.3	Snake venom CTL interacting with the collagen receptor GPVI	366
21.3	Snake venom CTLs affecting blood clotting	366
21.3.1	Snake venom CTLs with anti-coagulant activity	366
21.3.2	Snake venom CTLs with pro-coagulant activity	367
21.4	Conclusion	368
References		369
22	Toxins Leading to Medicines	377
<i>A.L. Harvey</i>		
22.1	Introduction	377
22.2	Neuromuscular blocking agents	377
22.3	Analgesic effects of ω -conotoxins and their analogues	378
22.4	Conantokins as potential anti-epileptic drugs	379
22.5	From arrow-poison to analgesic	380
22.6	Leads from scorpion toxins	380
22.7	Anti-platelet agents from snake venoms	381
22.8	Hypoglycaemics from Gila monster venom	381
References		382
IV	EVOLUTION OF ANIMAL TOXINS	385
23	Accelerated and Regional Evolution of Snake Venom Gland Isozymes	387
23.1	Association of accelerated evolution with functional diversities in snake venom isozymes	387
23.1.1	Structures of PLA ₂ isozymes from the venoms of <i>Trimeresurus</i> snakes	387
23.1.2	Diverse physiological functions of PLA ₂ isozymes from <i>T. flavoviridis</i> venom	389
23.1.3	Structures of the cDNA and genes encoding <i>Trimeresurus</i> venom-gland PLA ₂ isozymes	390
23.1.4	Accelerated evolution of venom-gland PLA ₂ isozymes of <i>Trimeresurus</i> snakes	391
23.1.5	Mechanism of accelerated evolution in snake venom-gland PLA ₂ isozyme genes	392

23.1.6	Evolution of <i>T. flavoviridis</i> serum inhibitors against its venom PLA ₂ s	394
23.2	Regional evolution of venom-gland PLA ₂ isozymes of <i>T. flavoviridis</i> snakes	395
23.2.1	Distribution of <i>T. flavoviridis</i> snakes	395
23.2.2	Regional differences in evolution of venom PLA ₂ isozymes	396
23.3	Problems in the future	397
	References	398
24	Functional Diversification of Animal Toxins by Adaptive Evolution	401
	<i>D. Kordis, I. Križaj and F. Gubenšek</i>	
24.1	Introduction	401
24.2	Biological roles of venoms	401
24.3	Toxins belong to different classes of proteins	402
24.4	Toxins have many molecular targets	404
24.4.1	Ion channels	404
24.4.2	Voltage-activated ion channels	404
24.4.3	Ligand-gated ion channels	406
24.4.4	Ca ²⁺ -binding proteins	406
24.4.5	Pentraxins	406
24.4.6	Lectins	407
24.4.7	Other targets	407
24.4.8	Targeting of toxins to receptor subtypes	407
24.4.9	Synergistic action of multiple toxins	407
24.5	Toxins are members of large multigene families	408
24.6	Adaptive evolution of animal toxins	409
24.6.1	Evolution of new functions by gene duplication	409
24.6.2	Introns in toxin multigene families are highly conserved	411
24.6.3	Very high evolutionary rate of toxins	411
24.6.4	Adaptive evolution diversifies different regions of toxins	412
24.6.5	What is the driving force for the adaptive evolution of toxins?	412
24.6.6	Mechanisms for evolving hypervariability of animal toxins	412
24.7	Extreme structural plasticity of toxins	413
	Acknowledgements	415
	References	415

V FROM VENOMS TO TREATMENT 421**25 The Venomous Function 423***M. Goyffon*

- 25.1 Venomous apparatus 423
 - 25.1.1 Venomous glands 424
 - 25.1.2 Injecting apparatus 425
- 25.2 Venoms: modes of action and composition 425
- 25.3 Venoms and molecular evolution 427
 - 25.3.1 vPLA₂s 427
 - 25.3.2 Toxins of Buthid scorpion venoms 428
- 25.4 Venoms and vital functions 429
- 25.5 Venoms and envenomations 429
- 25.6 Conclusion 431
- Acknowledgements 432
- References 432

26 Are Inhibitors of Metalloproteinases, Phospholipases A₂ and Myotoxins Members of the Innate System? 435*J. Perales and G.B. Domont*

- 26.1 Introduction 435
- 26.2 Snake venom metalloproteinases (SVMPs) 436
- 26.3 Snake venom metalloproteinase inhibitors (SVMPIs) 437
 - 26.3.1 Classification 437
 - 26.3.2 High molecular mass class 437
 - 26.3.3 Low molecular mass class 440
 - 26.3.4 Genic family 440
 - 26.3.5 Mechanisms of inhibition 441
- 26.4 Phospholipases A₂ (PLA₂s) and their receptors 442
- 26.5 PLA₂ inhibitors (PLIs): antineurotoxic and antimyotic 443
 - 26.5.1 Classification 443
 - 26.5.2 Specificity 444
 - 26.5.3 Mechanism 447
 - 26.5.4 Inhibitors of snake venom PLA₂s toxic effects 448
- 26.6 Perspectives 449
- 26.7 Finally, an answer to the title 451
- Acknowledgements 452
- References 452

- 27 The Treatment of Snake Bites: Analysis of Requirements and Assessment of Therapeutic Efficacy in Tropical Africa 457**
- J.-P. Chippaux*
- 27.1 Introduction 457
- 27.2 Analysis of requirements 459
- 27.2.1 Actual level of health: incidence, morbidity and lethality 459
- 27.2.2 Desirable levels of health: a difficult parameter to assess 464
- 27.3 Analysis of supply 464
- 27.3.1 Producers 464
- 27.3.2 Prescribers 465
- 27.4 Analysis of consumption 466
- 27.4.1 The decision to undergo treatment 466
- 27.4.2 Access to care 467
- 27.4.3 Staff training 467
- 27.5 Solutions 468
- 27.5.1 Improved identification of requirement 468
- 27.5.2 Improving supply 468
- 27.6 Conclusions 471
- References 471