Review

Snake Toxins from Mamba Venoms: Unique Tools for the Physiologist

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Dedicated to the memory of Professor Franc Gubenšek

Abstract

Snake venoms are complex mixtures of small molecules, peptides and proteins. Most of the biologically active toxins are peptides or enzymes. The peptides belong to several structural classes, and they have many different biological actions. The best characterised are the so-called three-finger toxins that have three peptide loops stabilised by four disulphide bridges. Despite their common 3D shape, these peptides can interfere selectively with different biological targets, including nicotinic and muscarinic acetylcholine receptors, acetylcholinesterase, ion channels, and cell membranes. Other small peptides can block K^+ or Ca^{2+} channels and are based on Kunitz serine proteinase inhibitors. This article summarises the proteins and peptides isolated from venoms of mamba snakes (*Dendroaspis* genus) that have been useful as experimental tools for physiologists and pharmacologists.

Keywords: Mamba toxins; neurotoxin; peptide; mamba venom; receptor; ion channel

1. Introduction

This review will focus on pharmacologically active peptides/toxin from mamba (*Dendroaspis)* snake venoms that have been used to help physiologists explore physiology. It is now well known that snake venoms have potent physiologically and pharmacologically active molecules that are proteins or peptides. The use of gel filtration and ion-exchange chromatographic techniques to isolate and purify venom components has uncovered molecules with highly selective pharmacology and aided our understanding of how peptide structure contributes to biological activity. Research on venom peptides has further progressed by the use of molecular biology techniques and proteomics.[1,2]

2. Nicotinic Receptor Toxins

The first snake toxins to be extensively studied were those with potent and selective blocking actions at nicotinic acetylcholine receptors. These "α-neurotoxins" were identified and subsequently isolated from venoms of snakes of the Elapidae (cobras, kraits, mambas, etc) and Hydrophidae (sea snakes) families. They have 60–74 amino acid residues in a single chain cross-linked by four or five disulphide bonds and contain a central core stabilised by four S-S bridges and three protruding peptide loops (like three fingers of a hand).[3] There are around 300 different examples of snake peptides with the three-finger motif, and examples are now known that have several quite different pharmacological actions from the α-neurotoxins. This makes the group extremely interesting in terms of determining structure-activity relationships and for mapping the molecular evolution of venom based peptides.[4] The α-neurotoxins can be subdivided into "short" (60–62 residues; four disulphides), and "long" (66–74 residues; five disulphides), including the kappa-neurotoxins (66 residues; five disulphides) and "weak" neurotoxins and other homologues (63–66 residues; five disulphides) (see[5] for review). The short and long α-neurotoxins paralyse skeletal muscle by blocking the ability of the neurotransmitter acetylcholine to bind to and activate the nicotinic receptors at the neuromuscular junction. Most early work con-

centrated on the receptors at the neuromuscular junction or on electroplaques of electric fish. These "muscle-type" receptors are pentameric proteins with two α subunits and one each of β, γ and ε (or δ), and such receptors are blocked by the high affinity binding of both short and long α-neurotoxins; for example, the short chain neurotoxin erabutoxin b has a very high affinity (Kd: 4×10^{-11} M) for acetylcholine receptors. Venom of *Bungarus multicinctus* and other kraits was shown to contain κ-bungarotoxins [6] that have little affinity for muscle-type nicotinic receptors but which bind to some neuronal isoforms (particularly the α3β2 subtype). Long chain α-neurotoxins were found to bind not just to muscle-type nicotinic receptors but also to homo-oligomeric nicotinic receptors (comprising five α subunits) that are found on neurones. The structural basis of this interaction with the α7 nicotinic receptor has been studied.[7] The so-called "weak" toxins can also bind with relatively high affinity to such neuronal nicotinic receptors.[8] More recently it has been found that α-bungarotoxin binds to and blocks a subset of $GABA_A$ receptors (Kd = 50 nM) that contain the $GABA_A$ β3 subunit.[9] This observation may limit the value of this toxin and possibly all short and long neurotoxins and will require a re-evaluation of the interpretation of all data where both receptors are present. α-Neurotoxins have been isolated and identified from venoms of the black mamba, *Dendroaspis polylepis*, and the Western green mamba, *Dendroaspis viridis*.

3. Muscarinic Toxins

Competition binding studies of mamba venoms with synaptosomal membranes led to the discovery of three-finger type peptides that bound to a number of central muscarinic receptors (see[10] for a review). The mamba venoms were initially characterised for their ability to displace selective muscarinic ligands ^{3}H-quinuclidinyl benzilate (QNB) and ^{3}H-N-methylscopolamine (NMS) from membranes prepared from the CNS or from the membranes of cells that have been engineered to express a particular subtype of muscarinic receptor. The first peptides to be characterised from the venom of the eastern green mamba, *Dendroaspis angusticeps* were named muscarinic toxin 1 (MTx1) and 2 (MTx2), and subsequently other toxins from mamba venoms (*Dendroaspis polylepis* and *Dendroaspis viridis*) were discovered with different selectivities. Currently, 10 different MTx have been isolated.[11] More recently, homologous peptides have been isolated from cobra venoms,[12] and a larger peptide BM14 (82 amino acid residues including 10 cysteines) was found in *Bungarus multicinctus* venom that bound to m2 muscarinic receptors.[13] γ-Bungarotoxin also binds to m2 receptors.[14] MTx1 is relatively selective for the m1 subtype; MTx3 is highly selective for the m4 subtype; and MTx7 (or m1-toxin) is extremely selective and virtually irreversibly blocks the m1 subtype.[15] MTx1 and 2 from green mamba venoms appear to be very unusual for toxins in that they cause an allosteric activation of their receptor target rather than a block.[16] When injected into the hippocampus of mice, these peptides can enhance learning and memory processes, whereas MTx3 (which blocks m4 muscarinic receptors) is inhibitory.[11] An unresolved issue with these toxins that impacts upon any interpretation of their pharmacology is that they have been reported to interact with G-protein coupled receptors both in the CNS and the periphery.[15,16] More recently a new three-finger fold toxins from the venom of the green mamba (*Dendroaspis angusticeps*) have been characterised with high affinity for α1a and α2 adrenergic receptor subtypes.[17]

4. Anticholinesterases

The anticholinesterase activity of green mamba venom was predicted from muscle fasciculations caused by injection of venom fractions into mice.[18] Peptides were subsequently isolated that had potent anticholinesterase activity and were called "fasciculins". Interestingly, when the structure of fasciculin 1 was solved[19], it was similar to the growing family of three-finger toxins. Fasciculins produce a long-lasting inhibition of acetylcholinesterase activity, thereby prolonging the action of the neurotransmitter acetylcholine.[18] When combined with other toxins that are present in mamba venoms, the fasciculins can cause muscle paralysis by inducing a depolarization-type neuromuscular blockade due to prolonged activation of acetylcholine receptors at the neuromuscular junction and the subsequent inactivation of sodium channels that are involved in the generation of muscle action potential. Fasciculins bind tightly to a peripheral anionic site on acetylcholinesterases from most species, but not from birds. They also have very little affinity for butyrylcholinesterases.

5. Anticoagulant Toxins

Some peptides isolated from mamba venoms that have a three-finger fold structure have been found to inhibit platelet aggregation (mambin).[20] Mambin (also known as dendroaspin) contains the sequence RGD that is common to proteins that bind to the glycoprotein receptor IIb-IIIa on platelets. Glycoprotein IIb-IIIa normally binds to fibrinogen to promote platelet aggregation. Mambin inhibits ADP-induced platelet aggregation (IC_{50} = 172 ± 22 nM) and inhibits the binding of platelet fibrinogen receptor GP IIb-IIIa to fibrinogen (IC_{50} = 3.1 ± 0.8 nM). Homologous sequences were noted in thrombostatin and peptide S5C1, which are also from mamba venoms and assumed to be platelet inhibitors.

6. Ca^{2+} Channel Blockers

A 60 amino acid residue peptide was isolated and named calciseptine following the screening of components of black mamba venom for activity on preparations of isolated smooth muscle.[21] Calciseptine is in the three-finger class of toxins. The peptide binds to L-type Ca^{2+} channels, probably via the recognition site for 1, 4-dihydropyridines. [21] Calciseptine blocks Ca^{2+} currents through L-type Ca^{2+} channels in cardiac and smooth muscle cells, and was found to act as an agonist on L-type channels in skeletal muscles.[21–23] Calciseptine does not interact with N-type or T-type Ca^{2+} channels.[21] A 60-residue Ca^{2+} channel blocking peptide (calcicludine) was also isolated from the venom of the green mamba but is structurally homologous to Kunitz-type protease inhibitors, as are the dendrotoxins (next section). The peptide potently blocks L-type Ca^{2+} channels in cerebellar granular cells ($K_{0.5}$ = 0.2 nM) and cardiac myocytes (EC_{50} = 15 nM). [24]

7. K^+ Channel Blockers

Venom from the Eastern green mamba *Dendroaspis angusticeps* was shown to increase the responses of skeletal muscle preparations to nerve-evoked twitches through increased acetylcholine release from motor nerve endings, an effect subsequently shown to be due to blocking of neuronal K^+ channels.[25–27] These components, dendrotoxins, are structurally homologous to Kunitz-type protease inhibitors, although they have no or very weak activity against serine proteases. α-Dendrotoxin from green mamba and dendrotoxin I from the black mamba block cloned voltage-dependent K^+ channels (Kv1.1, Kv1.2 and Kv1.6) in the low nanomolar range; dendrotoxin K from the black mamba selectively blocks Kv1.1 channels at picomolar concentrations.[27] Although not very toxic on peripheral injection, when injected centrally, dendrotoxins cause convulsions and death. More recently it has been shown that dendrotoxin K reduced tumour formation in nude mice, probably through a pathway governing G1-S transition.[28] With the use of dendrotoxins labelled with quantum dots, it is now possible to visualise living cells expressing functional Kv 1.1 channels.[29]

8. Natriuretic Peptides

A vasorelaxant peptide of 38 amino acid residues and with homology to atrial natriuretic peptide (ANP) was isolated from green mamba venom and named dendroaspis natriuretic peptide, DNP.[30] Homologous peptides have also been isolated from the venom of the inland taipan *Oxyuranus microlepidotus*.[31] DNP acts on ANP-A receptors to activate guanylate cyclase and increase levels of cyclic GMP, which cause vascular relaxation. DNP also causes natriuresis.[32] Due to the renal and vascular effects of DNP these peptides may have a role in treating and managing patients with congestive heart failure.[33] In addition to binding to ANP-A receptors, DNP has been recently shown to inhibit L-type calcium channel currents and delayed rectifier potassium currents I(K(V)) via cGMP-PKG-dependent signal pathway in guinea pig gastric myocytes[34,35]

9. Conclusions

Many peptides and proteins have been isolated from snake venoms because of their potent and selective biological activity. Venoms of mamba snakes have been exceptionally rich hunting grounds for toxinologists, yielding several apparently unique classes of bioactive snake toxins in addition to examples of more ubiquitous classes of toxins. Different biological activities can be provided through variations on a common structural theme. Examples are the different 3-finger toxins from mamba venoms that can bind selectively to nicotinic or muscarinic acetylcholine receptors, acetylcholinesterase, Ca^{2+} channels, etc. Because of such adaptations of core structures, new examples of venom components with different biological activities will not be pinpointed through application of genomic or proteomic techniques. Rather, new activities are likely to be discovered by testing venoms and their components on a broader range of biological targets and pathways.

10. References

1. J. W. Fox, S. M. Serrano, *Toxicon*. **2005**, *45,* 969–85
2. B. G. Fry, *Genome. Res*. **2005,** *15,* 403–20.
3. T. Endo, N. Tamiya. In: Harvey AL, editor. *Snake. Toxins*. New York: Pergamon, **1991**, p. 165–222.
4. B. G. Fry, N. G. Lumsden, W. Wuster, J. C. Wickramaratna, W. C. Hodgson, R. M. Kini, *J. Mol. Evol.* **2003,** *7,* 446–52.
5. V. I. Tsetlin, F. Hucho, *FEBS Lett*. **2004**, *557,* 9–13.
6. V. A. Chiappinelli. In: Harvey AL, editor. *Snake. Toxins*. New York: Pergamon, **1991**, p. 223–58.
7. C. Fruchart-Gaillard, B. Gilquin, S. Antil-Delbeke N. Le Novere, T. Tamiya, P. J. Corringer, J. P. Changeux, A. Menez, D. Servent, *Proc. Nat. Acad. Sci. USA*. **2002**, *99,* 3216–21.
8. Y. N, Utkin, V. V. Kubhtina, E. V. Kryukova, F. Chiodini, D. Bertrand, C. Methfessel, V. I. Tsetlin, *J. Biol. Chem*. **2001**, *276,* 15810–5.
9. C. M. McCann, J. Bracamontes, J. H. Steinbach, J. R. Sanes, *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103,* 5149–54.
10. D. Jerusalinsky, A. L. Harvey, *TIPS*. **1994**, *15,* 424–430.
11. D. Jerusalinsky, E, Kornisiuk, P. Alfaro, J. Quillfeldt, A. Ferreira, V. E. Rial, R. Duran, C. Cervenansky, *Toxicon*. **2000**, *38,* 747–61.
12. V. V. Kukhtina, C. Weise, T. A. Muranova, V. G. Starkov, P. Franke, F. Hucho, S. Wnendt, C. Gillen, V. I. Tsetlin, Y. N. Utkin, *Eur. J. Biochem*. **2000**, *267,* 6784–9.

13. C. Chung, B. N. Wu, C. C. Yang, L. S. Chang, *Biol. Chem.* **2002**, *383,* 1397–406.
14. L. S. Chang, C. Chung, B. N. Wu, C. C. Yang. *J. Protein. Chem.* **2002**, *21,* 223–9.
15. K. N. Bradley, *Pharmacol. Ther.* **2000**, *85,* 87–109.
16. A. L. Harvey, E. Kornisiuk, K. N. Bradley, C. Cervenansky, R. Duran, M. Adrover, G. Sanchez, D. Jerusalinsky, *Neurochem. Res.* **2002**, *27,* 1543–54.
17. C. Rouget, L. Quinton, A. Maïga, C. Gales, G. Masuyer, C. Malosse, J. Chamot-Rooke, R. Thai, G. Mourier, E. De Pauw, N. Gilles, D. Servent, *Br. J. Pharmacol*. **2010**, *161,* 1361–74.
18. C. Cervenansky, F. Dajas, A. L. Harvey, E Karlsson, In: Harvey AL, editor. *Snake. Toxins*. New York: Pergamon, **1991**, p. 303–21.
19. M. H. le Du, P. Marchot, P. E. Bougis, J. C. Fontecilla-Camps, *J. Biol. Chem*. **1992**, *267,* 22122–30.
20. R. S. McDowell, M. S. Dennis, A. Louie, M. Shuster, M. G. Mulkerrin, R. A. Lazarus, *Biochemistry.* **1992**, *31,* 4766–72.
21. J. R. de Weille, H. Schweitz, P. Maes, A. Tartar, M. Lazdunski, *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88,* 2437–40.
22. O. Yasuda, S. Morimoto, B. Jiang, H. Kuroda, T. Kimura, S. Sakakibara, K. Fukuo, S. Chen, M. Tamatani, T. Ogihara, *Artery*. **1994**, *21,* 287–302.
23. M. C. Garcia, Z. Hernandez-Gallegos, J. Escamilla, J. A. Sanchez, *J. Membr. Biol*. **2001**, *184,* 121–9.
24. H. Schweitz, P. Vigne, D. Moinier, C. Frelin, M. Lazdunski, *J. Biol. Chem.* **1992**, *267,* 13928–32.
25. A. L. Harvey, A. J. Anderson, In: A. L. Harvey, (Ed): *Snake Toxins*. New York: Pergamon. **1991**, p. 131–64.
26. A. L. Harvey, A. J. Anderson, P. M. Mbugua, E. Karlsson, *J. Toxicol. – Toxin Reviews*. **1984**, *3,* 91–137.
27. A. L. Harvey, B. Robertson, *Curr. Med. Chem*. **2004**, *11,* 3065–72.
28. S. H. Jang, P. D. Ryu, S. Y. Lee, *J. Vet. Sci*. **2011**, *12,* 35–40.
29. R. L. Orndorff, S. J. Rosenthal, *Nano. Lett.* **2009**, *9,* 2589–99.
30. H. Schweitz, P. Vigne, D. Moinier, C. Frelin, M. Lazdunski, *J. Biol. Chem*. **1992,** *267,* 13928–32
31. B. G. Fry, J. C. Wickramaratana, S. Lemme, A. Beuve, D. Garbers, W. C. Hodgson, P. Alewood, *Biochem. Biophys. Res. Comm*. **2005**, *327,* 1011–5
32. H. C. Chen, J. G. Lainchbury, J. C. Burnett Jnr, *J. Amer. Coll. Cardiol*. **2002**, *40,* 1186–91.
33. S. Vink, A. H. Jin, K. J. Poth, G. A. Head, P. F. Alewood. *Toxicon*. **2010** Dec 10. [Epub ahead of print] doi:10. 1016/j. toxicon. 2010. 12. 001
34. S. Y. Zhang, Z. X. Cai, P. Li, C. Y. Cai, C. L. Qu, H. S, Guo. *Regul Pept*. **2010**, *164,* 120–5.
35. X. Y. Gu, Z. X. Cai, H. Yuan, T. H. Wu, J. Li, H. S. Guo, *Regul. Pept.* **2009**, *155,* 115–20.

Povzetek

Kačji strupi so kompleksna zmes majhnih molekul, peptidov in proteinov. Večina biološko aktivnih toksinov je peptidov ali encimov. Peptidi sodijo v nekaj strukturnih skupin in izražajo več različnih bioloških učinkov. Najbolje opisani so tako imenovani triprstni toksini, ki imajo tri peptidne zanke stabilizirane s štirimi disulfidnimi vezmi. Kljub podobni 3D obliki, lahko ti peptidi selektivno interferirajo z različnimi biološkimi tarčami, na primer nikotinskimi in muskarinskimi acetilholinskimi receptorji, acetilholinesterazami, ionskimi kanalčki in celičnimi membranami. Drugi mali peptidi, podobni inhibitorjem serinskih proteinaz Kunitzovega tipa, lahko blokirajo K^+ ali Ca^{2+} kanalčke. Pričujoči članek povzema ugotovitve o proteinih in peptidih izoliranih iz strupov mamb (rod *Dendroaspis*), ki so se izkazali kot zelo uporabna eksperimentalna orodja za fiziologe in farmakologe.