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Review

Structure–function properties of venom components from Australian elapids

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Abstract

A comprehensive review of venom components isolated thus far from Australian elapids. Illustrated is that a tremendous structural homology exists among the components but this homology is not representative of the functional diversity. Further, the review illuminates the overlooked species and areas of research.

1. Introduction

Australian elapids are well known to be the most toxic in the world, with all of the top ten and nineteen of the top 25 elapids with known LD_{50}s residing exclusively on this continent (Broad et al., 1979). Thus far, three main types of venom components have been characterised from Australian elapids: prothrombin activating enzymes; lipases with a myriad of potent activities; and powerful peptidic neurotoxins. Many species have the prothrombin activating enzymes in their venoms, the vast majority contain phospholipase A_2s and all Australian elapid venoms are suspected to contain peptidic neurotoxins.

In addition to the profound neurological effects such as disorientation, flaccid paralysis and respiratory failure, characteristic of bites by many species of Australian elapids is hemorrhaging and incoagulable blood. As a result, these elapids can be divided into two main classes: species with procoagulant venom (Table 1) and species with non-procoagulant venoms (Table 2) (Tan and

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Table 1. Species with known procoagulant venom

Common name	Scientific name
Stephen's banded snake	*Hoplocephalus stephensi*
Krefft's tiger snake	*Notechis ater ater*
Tasmanian/King Island tiger snake	*Notechis ater humphreysi*
Peninsula tiger snake	*Notechis ater niger*
Western tiger snake	*Notechis ater occidentalis*
Chappell island tiger snake	*Notechis ater serventyi*
Inland taipan	*Oxyuranus microlepidotus*
Coastal taipan	*Oxyuranus scutellatus*
Red-bellied black snake	*Pseudechis porphyriacus*
Dugite	*Pseudonaja affinis*
Speckled brown snake	*Pseudonaja guttata*
Gwardar	*Pseudonaja nuchalis*
Eastern brown snake	*Pseudonaja textilis*
Rough-scaled snake	*Tropidechis carinatus*

Ponnudurai, 1990). Procoagulant species can produce effects with similarities to viper envenomation. These variances in pharmacology has direct impact upon the clinical pathology as well as antivenom use (Table 3).

A central feature of the clinical pathology produced by envenomations by members of the procoagulant group is DIC (disseminated intravascular coagulation) produced by powerful coagulants present in the venoms. This feature of the clinical pathology is characterised by the disappearance of fibrinogen from the blood through the procoagulant process prothrombin activation which results in a net effect of incoagulable blood. In contrast, non-procoagulant species produce effects that are more along the lines of classical elapid envenomation and neurological effects from post- and pre-synaptic blockage may be severe. Some of these species, *Pseudechis australis* (Mulga snake) in particular, can also produce severe disruption of hemostasis but anticoagulation is only produced without fibrinolysis. Further, certain members of this group can produce relatively pronounced myonecrosis.

Table 2. Species with known non-procoagulant venom

Common name	Scientific name
Common death adder	*Acanthophis antarticus*
Lowland copperhead	*Austrelaps superbus*
Mulga	*Pseudechis australis*
Collett's snake	*Pseudechis colletti*
Spotted black snake	*Pseudechis guttatus*

Table 3. Australian antivenoms and species covered

Antivenom	Species covered
Australian and New Guinea Polyvalent	Can be used for bites from any Australian or New Guinean elapid but monovalent venoms are more effective
Brown snake monovalent	All *Pseudonaja* (brown snakes) species
Black snake monovalent	*Pseudechis australis* (Mulga) and likely *P. butleri* (Butler's snake/Spotted Mulga)
Death adder monovalent	All *Acanthophis* (Death adder) species
Sea snake monovalent	All members of the sea snake families Hydrophiidae and Laticaudidae
Taipan monovalent	All *Oxyuranus* (Taipan) species
Tiger snake monovalent	All species of *Austrelaps, Hoplocephalus* (banded/broad-headed/pale-headed snakes), *Notechis* (Tiger snakes), *Tropidechis carinatus* (Rough-scaled snakes) as well as all *Pseudechis* (Black snake) species except *P. australis* and perhaps *P. butleri.*

2. Prothrombin activating enzymes

Many, but not all, species of Australian elapids contain factor Xa-like prothrombin activating enzymes responsible for most of the disruption of blood chemistry and hemorrhage clinically seen but in only a handful of species has the specific procoagulant component been isolated. As thoroughly reviewed by Rosing and Tans (1992), prothrombin activators found in snake venoms can be divided into four groups: group I convert prothrombin to meizothrombin with activity insensitive to the presence of the non-enzymatic prothrombinase complex cofactors ($CaCL_2$, factor V, and phospholipid); group II and III activators are able to cleave both peptide bonds in prothrombin essential for the conversion of prothrombin to thrombin, the difference between the II and III being that the converting activity of II is strongly stimulated by phospholipids and factor Va in the presence of calcium while III is only stimulated by $CaCl_2$ and phospholipid; group IV activators are proteases that cleave prothrombin into non-active precursor forms of thrombin rather than converting prothrombin into the enzymatically active products. Blood disrupting venoms from Australian elapids fall into either group II or group III (Table 4).

The clinical effects are diagnosed through blood studies showing severe coagulopathy. Coagulation times are typically grossly abnormal in addition to sharp decreases in fibrinogen levels accompanied by the rapid appearance of extreme quantities of fibrin and fibrin degradation products, consisting of both crosslinked and non-crosslinked species. The laboratory results are consistent with venom induced defibrination due to the action of a procoagulant.

2.1. Group II species

The prothrombin activators from members of the tiger snake complex (Genera *Austrelaps* (Copperheads), *Notechis* (Tiger snakes), and *Tropidechis* (Rough-scaled

Table 4. Isolated prothrombin activators

Source	Group	MW	Subunits	Subunit's	References
Notechis ater niger	II	58	2	37, 23	(Williams and White, 1989)
Notechis scutatus	II	54	2	32, 23	(Tans et al., 1985)
Oxyuranus scutellatus scutellatus	III	200	2	110, 80	(Speijer et al., 1986)
Pseudonaja affinis affinis	III	80	unknown	unknown	(Williams et al., 1994)
Pseudonaja textilis	II	53	unknown	unknown	(Stocker et al., 1994)
Pseudonaja textilis	III	200	2	105, 65	(Masci et al., 1988)
Tropidechis carinatus	II	41.5	unknown	unknown	(Marsh et al., 1997)

snakes)) and species with related venom (*Hoplocephalus* (Banded, Broad-headed, and Pale-headed snakes) species and *Pseudechis porphyriacus* (Red-bellied black snake)) are factor Xa-like in structure and function. Representative of prothrombin activators from species in this group is the prothrombin activator from *Notechis scutatus* (Mainland tiger snake). This venom component is an approximately 54 kDa disulfide-linked dimer, consisting of an approximately 32 kDa heavy chain and an approximately 23 kDa light chain, with eight phospholipid interacting gamma-carboxyglutamic acid residues (Tans et al., 1985). This prothrombin activator cleaves bovine prothrombin at Arg 274–Thr 275 and Arg 323–Ile 324, not being particular for either bonds as demonstrated by prethrombin-2 and meizothrombin both occurring as prothrombin activation intermediates. The presence of Ca^{2+}, factor Va, and phospholipid increase the enzymatic activity, with factor Va resulting in a 7-fold increase in substrate conversion as the venom activator forms a strongly bonded 1:1 complex with factor Va. The presence of human platelets stimulates the prothrombin activation by this activator, as well as that from *Oxyuranus scutellatus scutellatus* (Coastal taipan), in direct contrast to the prothrombin activator ecarin from the African viper *Echis carinatus* (Saw-scaled viper) which is insensitive to platelet presence (Speijer et al., 1987).

The procoagulant from *Notechis ater niger* (Peninsula tiger snake) is an approximately 58 kDa protein made up of two subunits that show up on gel electrophoresis at approximately 37 kDa and 23 kDa respectively (Williams and White, 1989). This toxin converts normal prothrombin, requiring factor V, and has an ecarin-like characteristics such as not needing Ca^{2+} and phospholipid for activity in addition to an ability to clot heparinized plasma. The bioactivity, however, is sped up in the presence of the cofactors. The procoagulant property of the *Pseudechis porphyriacus* venom (Chester and Crawford, 1982) and the 64.5 kDa (41.5 kDa under reducing conditions) coagulant factor from *Tropidechis carinatus* (Rough-scaled snake) (Mebs, 1982; Marsh et al., 1997) display similarities to that from *N. scutatus* and in the requirement for calcium, factor V and phospholipid to activate normal prothrombin.

Textarin, the minor coagulative factor from *Pseudonaja textilis* (Eastern brown snake) venom, is a 53 kDa serine protease prothrombin activator that, in contrast to the main prothrombin activator found in this venom, is strongly stimulated by the presence of calcium and phospholipid (Stocker et al., 1994).

2.2. Group III species

Unlike the coagulative factors found in group II, the prothrombin activators of group III are multiple subunit enzymes of much larger size. The prothrombin activator from *Oxyuranus s. scutellatus* is made up of two subunits of 110 and 80 kDa in addition to two disulfide linked polypeptides, which contain the active site of the enzyme (Speijer et al., 1986). Like the proteolytic cleavage of factor VII, the activation of prothrombin is accelerated in the presence of calcium and phospholipid. Further, like the prothrombin activator from *N. scutatus*, the cleavage of the Arg274–Thr275 and Arg323–Ile324 bonds is random, with thrombin and meizothrombin both being products of the reaction and phospholipid interacting gamma-carboxyglutamic acid residues. Another similarity with *N. scutatus* is that prothrombin activation is stimulated by the presence of human platelets. This is in contrast to the venoms of *P. textilis* and *Oxyuranus microlepidotus* (Inland taipan) which do not require co-factors to activate both normal prothrombin or the decarboxylated form.

The specific procoagulant from the *Pseudonaja affinis affinis* (Dugite) is a 80 kDa serine protease with a sialic acid component which also contributes to the coagulant action. This factor has a different specific activity than the similar procoagulants from the other members from this genera: *P. inframacula* (Peninsula brown snake), *P. nuchalis* (Gwardar), and *P. textilis* but has a homologous primary structure (Williams et al., 1994). Like *O. s. scutellatus*, this specie is able to activate both the decarboxylated and normal form of prothrombin without the aid of the cofactors calcium, factor V, and phospholipids (Chester and Crawford, 1982). In contrast, the major coagulative factor from *P. textilis* is a 200 kDa prothrombin activator that is immunologically related to the prothrombin activator from *O. s. scutellatus*. The myriad of effects of this component include the ability to coagulate citrated plasma, factor V- and factor X-deficient plasmas, and warfarin plasma; to convert purified human prothrombin to thrombin; and to hydrolyze the peptide p-nitroanilide substrate S-2222; and as would be expected, calcium ions and phospholipids had little if any effect on the rates of coagulation of citrated plasma or S-2222 hydrolysis catalyzed by this enzyme (Masci et al., 1988).

3. Lipase enzymes

Australian snake venoms contain a myriad of lipases with the vast majority being phospholipases. The enzymatic action of phospholipases is cleavage of phospholipids and there are four main species of phospholipases (A_1, A_2, C, D) classified by the site at which they hydrolyze ester bonds of 3-*sn*-phosphoglycerides. Most venom phospholipases are phosphatidylcholine 2-acylhydrolase (PLA_2 EC 3.1.1.4), hydrolyzing the *sn*-2 ester of 3-*sn*-phosphoglycerides.

3.1. Lysophospholipase enzymes

Lysophospholipases hydrolyze the *sn*-1-acyl ester bond of lysophospholipids, an action that is often greatly accelerated in the presence of PLA_2s. Two lysophospholipases isolated from *Pseudechis australis* venom, LPLA-1 and -2, have a strong degree of homology. LPLA-1 is a homodimer with the monomer being 123 amino acids, linked by seven disulfide bonds, and is similar to PLA_2s not only in primary structure and N-terminal sequence, but also in the physiological requirement of Ca^{2+} for bioactivity (Takasaki and Tamiya, 1982). LPLA-1 was shown to hydrolyze lysophosphatidylcholine more rapidly than lysophosphatidylethanolamine but is unable to hydrolyze phosphatidylcholine and when PLA_2 is present LPLA-1 has a strong direct hemolytic activity.

3.2. Phospholipase A enzymes

A phospholipase A isolated from *P. australis* with direct hemolytic, as well as anticoagulant and myotoxic activity is MSPA. This 13 kDa basic PLA is composed of 119 amino acids cross-linked by seven disulfide bonds and has an enzymatic activity dependent upon a single histidine residue (Sharp et al., 1989). This toxic component has direct anticoagulant, hemolytic, and myotoxic action and shares a significant degree of amino acid composition with myotoxic PLA_2s isolated from *P. australis*, notably Pa-11.

3.3. Phospholipase A_2 enzymes

Key among the toxic enzymes in all snake venoms are the phosphatidylcholine 2-acylhydrolases and consequently this is the area where the bulk of the research on Australian elapids has been concentrated. Based on primary structure and disulfide connectivity, venom PLA_2s worldwide are divided into three classes: class I are found in elapid venoms; class II in crotalid and viper venoms (as well as human pancreatic juices); and class III are found in *Heloderma* (Gila monsters and beaded lizards) and bee venoms (Arni and Ward, 1996).

The majority of the Australian elapid PLA_2s are basic, 118 amino acids, have seven disulfide bonds and molecular weights around 13 kDa (Table 5). The PLA_2s have two activities, a potent toxic activity in addition to the non-lethal enzymatic phospholipase activity, with the two active sites suspected to be located separate from each other. Of primary importance in the PLA_2s is the toxic activity of pre- or post-synaptic blockage of neuromuscular nerve transmission. In class I PLA_2s, there is a preserved structure, with the cysteine pattern almost invariably C–C–C–CC–C–C–C–C–C–C–C–C–C, disulfide spacing of 15:1:14:0:5:9:10:6:5:4:1:6:18 and cysteine connectivity of 1–8, 2–14, 3–5, 4–13, 6–12, 7–10, 9–11.

Despite a great deal homology existing in the sequences (Fig. 1) there is a wide range of enzymatic and toxic activity in the class I PLA_2s found in Australian elapids. Thus, Australian elapid venom class 1 PLA_2s can be further broken up

Table 5. Phospholipase A_2s with complete sequences

Source	Molecule	PLA_2 Type	Average MW*	Residues	*p*I*	References
Notechis scutatus	HT_e	H	14261.89	125	4.94	(Francis et al., 1995)
Notechis scutatus	Notechis II-1	X_2	13233.04	119	7.54	(Lind and Eaker, 1980)
Notechis scutatus	Notechis II-2	X_1	13013.71	118	7.54	(Ducancel et al., 1988)
Notechis scutatus	Notechis II-5	N	13676.58	119	7.74	(Halpert and Eaker, 1976)
Notechis scutatus	Notechis Ns	N	13621.54	119	7.56	(Chwetzoff et al., 1990)
Notechis scutatus	Notexin	N	13593.53	119	7.56	(Chwetzoff et al., 1990)
Oxyuranus s. scutellatus	Taipoxin-alpha	N	13828.88	119	8.61	(Lind and Eaker, 1982)
Oxyuranus s. scutellatus	Taipoxin-beta	X_2	13235.98	118	5.67	(Lind, 1982)
Oxyuranus s. scutellatus	Taipoxin-gamma	X_1	14603.24	133	3.88	(Fohlman et al., 1977)
Pseudechis australis	Pa-1G	M	13153.92	118	6.40	(Takasaki et al., 1990)
Pseudechis australis	Pa-3	M	13151.99	118	6.91	(Takasaki et al., 1990)
Pseudechis australis	Pa-5	M	13124.98	118	7.24	(Takasaki et al., 1990)
Pseudechis australis	Pa-9C	M	13298.18	118	7.52	(Takasaki et al., 1990)
Pseudechis australis	Pa-10A	M	13026.97	118	8.02	(Takasaki et al., 1990)
Pseudechis australis	Pa-11	M	12965.93	118	8.25	(Nishida et al., 1985)
Pseudechis australis	Pa-12C	M	12968.94	118	8.37	(Takasaki et al., 1990)
Pseudechis australis	Pa-12C isozyme	M	13008.96	118	8.37	(Takasaki et al., 1990)
Pseudechis australis	Pa-13	M	13213.19	118	8.02	(Nishida et al., 1985)
Pseudechis australis	Pa-15	M	13200.20	118	8.14	(Takasaki et al., 1990)
Pseudechis porphyriacus	Pseudexin-A	N	13110.02	117	8.14	(Schmidt and Middlebrook, 1989)
Pseudechis porphyriacus	Pseudexin-B	N	13016.12	117	8.02	(Schmidt and Middlebrook, 1989)
Pseudonaja textilis	Textilotoxin-A chain	N	13848.73	118	8.49	(Tyler et al., 1987a,b)
Pseudonaja textilis	Textilotoxin-B chain	X_1	13798.70	121	7.73	(Pearson et al., 1993)
Pseudonaja textilis	Textilotoxin-C chain	X_1	13010.57	118	4.31	(Pearson et al., 1993)
Pseudonaja textilis	Textilotoxin-D chain	X_1	14922.86	133	4.35	(Tyler et al., 1987a,b)

*Calculated by author from sequence. Types: H = Hemotoxic, M = Myotoxic, N = Neurotoxic, X_1 = Non-toxic, enzymatically active, X_2 = Non-toxic, non-enzymatically active.

Phospholipase sequence alignments

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1.  --------NLAQFGFMIRCANGGSRSALDYADYGC
2.  --------NLLQFGFMIRCANRRSRPVWHYMDYGCYCGKGGSGTPVDDLDRCCQVHDECYGEAVRRFG
3.  --------NLVQFGKMIECAIRNRRPALDFMNYGCYCGKGGSGTPVDDLDRCCQVHDECYAEAEKHG-
4.  --------NLYQFGNMIQCANHGRRPTLAYADYGCYCGAGGSGTPVDELDRCCKAHDDCYGEAGKKG-
5.  --------NLVQFSNMIQCANHGSRPSLAYADYGCYCSAGGSGTPVDELDRCCKTHDDCYARATKSYS
6.  --------NLIQFANMIGCANHGSR
7.  --------NLIQFGNMIQCANKGSRPTRHYMDYGCYCGWGGSGTPVDELDRCCQTHDDCYGEAEKKG-
8.  --------NLIQFGNMIQCANKGSRPTRHYMDYGCYCGWGGSGTPVDELDRCCKVHDDCYGEAEKKG-
9.  --------NLIQFSNMIQCANKGSRPSLDYADYGCYCGWGGSGTPVDELDRCCKVHDDCYAEAGKKG-
10. --------NLIQFSNMIQCANKGSRPSLHYADYGCYCGWGGSGTPVDELDRCCKVHDDCYDQAGKKG-
11. --------NILQFRKMIQCANKGSRAAWHYLDYGCYCGPGGRGTPVDELDRCCKIHDDCYIEAGKDG-
12. --------NILQFRKMIQCANKGSRAAWHYLDYGCYCGPGGRGTPVDELDRCCKIHDDCYIEAGKDG-
13. --------NLIQFGNMIQCANKGSRPSLDYADYGCYCGWGGSGTPVDELDRCCQVHDNCYEQAGKKG-
14. --------NLIQFGNMIQCANKGSRPSLNYADYGCYCGWGGSGTPVDELDRCCQVHDNCYEQAGKKG-
15. --------NLIQFGNMIQCANKGSRPSLDYADYGCYCGWGGSGTPVDELDRCCQTHDNCYEQAGKKG-
16. --------NLYQFKNMIQCANKGSRSWLDYVNYGCYCGWGGSGTPVDELDRCCQTHDNCYDQAGKKG-
17. --------DLFQFGGMIGCANKGAYSWLSYVNYGCYCGWG
18. --------NLYQFGGMIQCANKGAYSWLSYVNYGCYCGWG
19. --------NLIQFKSIIECANRGSRRWLDYADYGCYCGWGGSGTPVDELDRCCKVHDECYGEAVKQG-
20. --------NLIQLSNMIKCAIPGSQPLF
21. --------NLIQLSNMIKCAIPGSRPLFQYTDYGCY
22. --------NLIQFSNMIKCAIPGSRPLFQYADYGCYCGPGGHGTPVDELDRCCKIHDDCYGEAGKKG-
23. --------NLIQFSNMIKCTIPGSQPLLDYANYGCYCGPGNNGTPVDDVDRCCQAHDECYDEASNHG-
24. --------NLVQFSYLIQCANHGRRPTWHYMDYGCYCGAGGSGTPVDELDRCCKIHDDCYDEAGKKG-
25. --------NLVQFSYLIQCANHGKRPTWHYMDYGCYCGAGGSGTPVDELDRCCKIHDDCYDEAGKKG-
26. --------NLVQFSYLIQCANHGRRPTRHYMDYGCYCGWGGSGTPVDELDRCCKIHDDCYSDAEKKG-
27. --------NLVQFSYLIRCANKYKRPGWHYANYGCYCGSGGRGTPVDDVDRCCQAHDKCYEDAEKLG-
28. --------DLVEFGFMIRCANRNSQPAWQYMDYGCYCGKRGSGTPVDDVDRCCQTHNECYDEAAKIPG
29. --------NLYQFGNMIQCANHGRRPTRHYMDYGCYCGKGGSGTPVDELDRCCQTHDDCYGEAEKLPA
30.                                                                     LPA
31. SELPQPSIDFEQFSNMIQCTIPCGSECLAYMDYGCYCGPGGSGTPIDDLDRCCKTHDECYAEAGKLSA
32. ASIPRPSLNIMLFGNMIQCTIPCEQSWLGYLDYGCYCGSGSSGIPVDDVDKCCKTHDECYYKAGQIPG
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Fig. 1. Cysteines aligned and sequences arranged in order of similarity. References are: (1) Taicatoxin (N-terminal fragment) (Possani et al., 1992), (2) Taipoxin alpha-chain (Lind and Eaker, 1982), (3) Taipoxin beta-chain (Lind and Eaker, 1982), (4) Notechis II-2 (Ducancel et al., 1988), (5) Notechis II-1 (Lind and Eaker, 1980), (6) *Austrelaps superbus* PLA_2 (Yuan et al., 1993) (7) Pa-1G (Takasaki et al., 1990), (8) Pa-3 (Takasaki et al., 1990), (9) Pa-5 (Takasaki et al., 1990), (10) Pa-10A (Takasaki et al., 1990), (11) Pa-13 (Nishida et al., 1985), (12) Pa-15 (Takasaki et al., 1990), (13) Pa-11 (Nishida et al., 1985), (14) Pa-12C (Takasaki et al., 1990), (15) Pa-12C (Isozyme) (Takasaki et al., 1990), (16) Pseudexin A (Schmidt and Middlebrook, 1989), (17) Acanthoxin-A_2 (N-terminal fragment) (Van Der Weyden et al., 1997), (18) Acanthoxin-A_1 (N-terminal fragment) (Van Der Weyden et al., 1997), (19) Pa-9C (Takasaki et al., 1990), (20) Notechis II-5b (N-terminal fragment) (Yang et al., 1991), (21) Pseudexin C (N-terminal fragment) (Schmidt and Middlebrook, 1989), (22) Pseudexin B (Schmidt and Middlebrook, 1989), (23) Textilotoxin-C chain (Pearson et al., 1993), (24) Notechis Ns (Chwetzoff et al., 1990), (25) Notexin (Chwetzoff et al., 1990), (26) Notechis II-5 (Halpert and Eaker, 1976), (27) Textilotoxin-A chain (Tyler et al., 1987a,b), (28) Textilotoxin-B chain (Pearson et al., 1993), (29) HTe (Francis et al., 1995), (30) HTg (fragment) (Francis et al., 1995), (31) Taipoxin gamma-chain (Fohlman et al., 1977), (32) Textilotoxin-D chain (Tyler et al., 1987a,b).

into five generalized types: hemotoxic; myotoxic; neurotoxic; non-toxic but enzymatically active; and non-toxic and non-enzymatically active. The activities of particular molecules are not necessarily restricted to a particular type but do act primarily in the manner indicated by the nomenclature. This homology of structure but diversity of action is a rich area of research.

3.3.1. Hemotoxic PLA_2s

The hemotoxic PLA_2s produce hemorrhage through blockage of factors in the coagulation cascade resulting in a disruption of hemostasis. These components themselves do not produce net anticoagulation through fibrinolysis but rather bind specifically to molecules essential to the coagulation processes.

In *Austrelaps superbus* (Lowland copperhead) several phospholipases have been isolated, including a 15 kDa phospholipase A_2 that has been shown to be inhibitor of human platelet aggregation (Yuan et al., 1993). Superbins I and II are two other anticoagulant PLA_2s from this species (Subburaju and Kini, 1997). Superbins I and II both demonstrated PLA_2 activity in addition to weak prothrombin time anticoagulation activity. These anticoagulants inhibit the extrinsic tenase complex of the coagulation cascade but not the prothrombinase complex. This study further demonstrated the collagen induced platelet aggregation produced by these venom factors.

Several hypotension and hemorrhage promoting, toxic acidic proteins, Ht_{a-l}, have been isolated from *N. scutatus*. Both Ht_e and its isoform Ht_g are PLA_2s that are slightly larger than the other PLA_2s, 14 kDa vs 12–13 kDa, and unusually run even higher (18–21 kDa) on gels (Francis et al., 1995). These toxins have a high degree of sequence homology with other Australian elapid venom PLA_2s at the beginning and end of the molecule but not in the middle forty three residues. In these middle residues, the sequence shares a significant degree of homology with the human pancreatic PLA_2s in the region corresponding to the 'pancreatic loop'. These components also have a neurological action. The neuropharmacology is blockage of neuromuscular transmission but not by acting selectively upon the nerve endings, as would be initially suspected, but upon both the nerve and muscle, producing both pre- and post-synaptic effects. Despite having similar physiological effects similar to that of the crotalid and viperid venom components there is little antigenic cross-reactivity (Francis et al., 1993).

3.3.2. Myotoxic PLA_2s

While elapids are not typically known for producing severe myonecrosis, this is not an unknown feature in bites by certain Australian elapids. The venom of *P. australis* differs markedly from that of other Australian elapids in that the primary toxins are not neurotoxic but rather myotoxic. The venom produces severe muscle damage and necrosis which are evidenced by an increase in serum creatine kinase as well as histological observations (Mebs et al., 1983; Sutherland, 1983; Dambisya et al., 1995). The pathological disruption of neuromuscular function produced by PLA_2s in the venom is characterized by pronounced morphological

damage to muscle fibers, motor nerve terminals and cytoplasmic organelles (Chen et al., 1994).

Numerous specific toxic phospholipase A_2s have been isolated from *P. australis* venom. PLA_2s Pa-1G, Pa-5, Pa-12C and Pa-15 are characterized as being homologous single chain postsynaptic-acting basic PLA_2s (with the exception of Pa-1G which is uniquely acidic) that damage muscle fibers resulting in a loss of muscle contractility (Geh et al., 1992a,b). Pa-1G and -5 are the two most enzymatically active and toxic of this group. Pa-3, Pa-8, Pa-9C, Pa-10F and Pa-12B are myotoxic PLA_2 isoenzymes that block K^+ conductance not through selective action on K^+ channels but secondary to membrane depolarization (Fatehi et al., 1994). Pa-10A, Pa-11 and Pa-13 are other lethal single chain PLA_2s, with Pa-10A and Pa-11 producing muscle paralysis through the reduction of acetylcholine release and direct blockage of muscle fiber contractility (Rowan et al., 1989). Pa-13 is much less bioactive but produces similar, but greatly reduced, effects.

Like *P. australis*, the myolytic effects of *Pseudechis colletti* (Collett's snake) can be diagnosed not only through studies but also by testing for an sharp increase in serum creatine kinase, with these two species being the Australian elapids typically producing this increase which is more commonly seen in species of *Crotalus* (Rattlesnakes) (Mebs et al., 1983). The phospholipase A_2s isolated from this specie have been shown to induce myodegeneration and necrosis with subsequent myoglobinuria and myoglobinuric nephropathy (Weinstein et al., 1992). The primary structures of the two phospholipase A_2s responsible for myoglobinuria has been shown to have strong homology with that of a similarly acting enzyme from *Enhydrina schistosa* (Beaked sea snake) (Mebs and Samejima, 1980).

3.3.3. Neurotoxic PLA_2s

The neurotoxic PLA2s are usually single chain but multi-chain complexes have also been isolated. These venom components produce neurological effects not through the enzymatic esterase activity but rather through direct blockage.

3.3.3.1. Single chain neurotoxic PLA_2. The single chain neurotoxic PLA_2s from Australian elapids are almost exclusively basic and have a potent presynaptic activity. Notexin is the principle neurotoxic and myonecrotic phospholipase A_2 in *Notechis* species and is representative of single chain neurotoxic PLA_2s found in Australian elapids. In addition to having the non-lethal esterase activity seen in many other PLA_2s, this PLA_2 is a presynaptic acetylcholine release blocker with a very potent and specific myotropic activity for the vertebrate neuromuscular system in a manner similar to ceruleotoxin from *Bungarus fasciatus* (Banded Krait) (Ho and Lee, 1983). Notexin does not block the acetylcholine release directly, but rather reduces the amount of available intracellular acetylcholine through the calcium stimulated mobilization of vesicular acetylcholine (Mollier et al., 1990a,b). Notexin differs from similarly bioactive isoform notechis Ns only in a Lys substitution for Arg at position 16 (Chwetzoff et al., 1990). The presynaptic acetyl-

choline blocking notechis II-5 is strongly homologous with notexin yet is much less toxic but has a higher specific phospholipase activity (Halpert and Eaker, 1976; Kannan et al., 1981). Scutatoxin isoforms A and B are other toxic PLA_2s isolated *N. scutatus* with a neurotoxicity comparable that of notexin and over seven times that of the weakly toxic notechis II-5 (Francis et al., 1991). Further, the scutatoxin isoforms are immunologically similar to both and have phospholipase activity falling in-between the two yet are less basic than either notexin or notechis II-5.

The pseudexins are the primary toxic phospholipase A_2s isolated from *P. porphyriacus* venom and are putatively presynaptic blockers. As is seen in a great many of the other PLA_2s isolated from Australian elapids, there is a great range in toxic activities among the isoforms of these basic PLA_2s; pseudexin A is only moderately toxic while pseudexin B is potently toxic and significant differences exist at key residues (Schmidt and Middlebrook, 1989).

Two significant phospholipase A_2s isolated from *O. s. scutellatus* are OS_1 and OS_2. These toxic monochain PLA_2s have been quite useful in characterizing mammalian phospholipase A_2 receptors by binding to PLA_2 receptors that bee (*Apis mellinifer*) venom PLA_2s are unable to bind. OS_1 binds to M-type (bee insensitive) neuronal PLA_2 receptors but not to N-type (bee sensitive) receptors while OS_2, however, is capable of binding to both M- and N-type PLA_2 receptors (Lambeau et al., 1990; Lambeau et al., 1991a,b; Fossier et al., 1995; Gandolfo et al., 1996).

Acanthophis (Death adder) species have long thought to be unique among Australian elapids in the lack of PLA_2s in the venoms. Recently, however, PLA_2s have been purified. Acanthoxin (isoforms A1 and A2) and Acanthin from *A. antarticus* (Common death adder) and Praelongin from *A. praelongus* (Northern death adder) (Kini (unpublished results) 1997; Van Der Weyden et al., 1997). While the basic PLA_2s Acanthoxin A1 and A2 are highly enzymatic and share a significant degree of N-terminal sequence homology with toxic PLA_2s, they are only moderately toxic. Interestingly, the N-terminal sequences of A1 and A2 are closest to the moderately neurotoxic, basically charged pseudexin A and the myotoxic, neutral Pa-9C.

3.3.3.2. Multi-chain neurotoxic PLA_2s. The multi-chain PLA_2 toxins take on two forms, composed of only phospholipases (taipoxin and textilotoxin) or also containing smaller peptidic components (taicatoxin). The principle neurotoxin of *O. s. scutellatus* is taipoxin, an extremely potent blocker of the presynaptic release of acetylcholine. Taipoxin is composed of three phospholipase-related peptide chains: alpha, beta (the identical beta-1 and beta-2) and gamma with the molecular weight of the complete molecule being 45 600 daltons and while all chains are PLA_2-like, there is a range in activities. Despite a high degree of homology between the alpha and beta subunits, only the alpha-chain is an extremely potent blocker of the presynaptic release of acetylcholine (Fohlman et al., 1977; Lind, 1982; Lind and

Eaker, 1982). Paradoxin from *O. microlepidotus* is a presynaptic neurotoxic complex of 47 kDa that shares significant homology with taipoxin (Fohlman, 1979).

Taicatoxin is a another, but quite different multi-chain neurotoxin from *O. s. scutellatus*. This highly basically charged toxin complex is composed of three non-covalently linked subunits (alpha, beta, and gamma, in ratios of 1:1:4) that contains as one of the components a highly toxic PLA_2 that is essential for complete activity of the complex (Possani et al., 1992). This toxin does not affect the low threshold calcium channel currents or have any effect on potassium or sodium channels but is a potent voltage dependent, reversible blocker of high threshold calcium channel currents by binding to the extracellular face of the channel (Brown et al., 1987).

Of the *Pseudonaja* (Brown snake) species, only *P. textilis* has been closely studied. Textilotoxin is a potent presynaptic neuromyotoxin with phospholipase A_2 activity and causes a presynaptic blockade of neuromuscular transmission involving a disruption of the regulatory mechanism that controls acetylcholine release (Su et al., 1983; Wilson et al., 1995). This 623 amino acid toxin has been shown to have the most complex structure and highest lethality of any identified snake neurotoxin (Tyler et al., 1987a). The structure has been determined to be composed of five non-covalently linked subunits (two of which (D) are identical). Subunits A is the lethal subunit, being potently neurotoxic yet has only low phospholipase activity (Pearson et al., 1993). Subunit A of textilotoxin is not antigenically similar to subunits B, C, or D, having instead a high degree of structural and immunological homology with notexin from tiger snake venom (Pearson et al., 1991a,b).

3.3.4. Non-toxic, enzymatic PLA_2s

These components are devoid of direct toxic activity but still retain the non-lethal enzymatic activity. Notechis II-2, isolated from *N. scutatus,* contains all the residues predicted for enzymatic activity in snake venom PLA_2s and exhibit an esterase activity selective for neutral phospholipids, yet is non-lethal (Bouchier et al., 1991). Notechis II-5b, also from *N. scutatus*, notexin is non-toxic and has only weak enzymatic activity despite having significant homology with notechis II-5 and (Yang et al., 1991). Another non-toxic component with only weak phospholipase activity is the acidic gamma-chain of taipoxin from *O. s. scutellatus*, unique in having eight disulfide bonds (Fohlman et al., 1976). Textilotoxin subunits B, C, and D, from *P. textilis,* are not neurotoxic and have very low phospholipase activity yet are essential for the neurotoxicity of textilotoxin (Tyler et al., 1987a,b; Pearson et al., 1991a,b, 1993). Significant differences exist between the three subunits not only in structure but also in *p*I; textilotoxin B is basic while C and D are both strongly acidic and (Pearson et al., 1991a,b). Interestingly, subunit D shows a significant degree of homology with the gamma-subunit of taipoxin, but has only one extra cysteine, rather than the two extra in taipoxin-gamma. Subunit D is present as a homodimer enabled by having the extra cysteine. Pseudexin C from *P. porphyriacus* is another enzymatically

active basic charged PLA_2 that is non-toxic despite sequential similarity with the toxic pseudexins A and B (Schmidt and Middlebrook, 1989).

3.3.5. Non-toxic, non-enzymatic PLA_2s

These components are lacking not only in toxic activity but also in the non-lethal enzymatic activity. These components thus are useful in elucidating the active sites of the PLA_2 molecules through the comparisons of primary structures. The toxins notechis II-1, from *N. scutatus,* and the beta chain of taipoxin, from *O. s. scutellatus*, not only are devoid of toxic activity but are also lacking in enzymatic activity (Lind and Eaker, 1980; Lind, 1982). Sequentially, notechis II-1 is between enzymatically active, non-toxic, basic notechis II-2 and the enzymatically active, myotoxic, acidic Pa-1G while taipoxin-beta is between the highly toxic alpha-subunit of taipoxin and the non-toxic but enzymatically active notechis II-1.

3.3.6. Variations in PLA_2s

By studying the variations in PLA2 primary structures and pharmacology, the precise residues upon which bioactivity is dependent upon can be worked out. This information may then be potentially useful for drug design and development.

Further evidence indicating that the lethal neurotoxic site and the myotoxic enzymatic site of notexin, and by implication possibly for all the snake venom PLA_2s, are distinct for each comes from chemical modification of Trp-110 significantly decreasing the neurotoxicity of the molecule but without affecting enzymatic activity (Mollier et al., 1989a). Further, modification of Tyr-7 and Tyr-77 results in only a 35.8% decrease in catalytic activity but in a dramatic decrease in lethal toxicity (Yang and Chang, 1991). Antibody binding studies have shown that the antigenic domain specific for notexin, with no cross-reactivity for other venom PLA_2s, exists at the C-terminal portion of the protein (Mollier et al., 1989b). However, results suggest that it is the N-terminal alpha-amino group that is essential for the phospholipase A_2 activity and lethal toxicity of notexin (Yang and Chang, 1990). The nonenzymatic, 119 residue notechis II-1 contains the conserved amino acid sequences found in a diverse spectrum of elapid venom phospholipase A_2s, as well as in pancreatic PLA_2s, with the notable exception of the ubiquitous glycine at position 30 being replaced by a serine, perhaps explaining the loss of enzymatic toxicity (Lind and Eaker, 1980).

There is a definite geographical variation in the venom composition of *Notechis* species and subspecies. Venom from the southern range of *N. scutatus* contains notechis II-5 in three times the amount of notexin (Yang et al., 1991). Further, *N. scutatus* populations collected from the Victorian region contain significant amounts of notexin yet notechis II-5 is entirely absent and in its place is the weakly active notechis II-5b. A comparison of the venoms of *N. ater ater* (Krefft's tiger snake), *N. a humphreysi* (Tasmanian tiger snake), *N. a serventyi* (Chapel island tiger snake), and *N. s. scutatus* showed variations in elution profiles, but with all venoms showing notexin-like proteins except for *N. a. serventyi* (John and

Kaiser, 1990). *N. a. serventyi* was shown to be lacking in notechis II-5, notechis Ns, and notexin and as a result the least toxic of the *N. ater* (Black tiger snake) subspecies, being on average half as toxic as the other subspecies and almost three times less toxic than *N. scutatus* (Broad et al., 1979; Chwetzoff et al., 1990; John and Kaiser, 1990).

3.4. Phospholipase B enzymes

Snake venom phospholipase Bs found in Australian elapids hydrolyze phosphatidylcholine to fatty acids and glycerophosphorylcholine. A phospholipase B has been isolated from the venom of *A. superbus* which is partially responsible for the venom induced hemolysis (Bernheimer et al., 1986). *P. colletti* bites produce myoglobinuria and have a relatively high direct hemolytic activity, with hemolysis resulting from hydrolysis of phosphatidylcholine by a phospholipase B found in the venom (Bernheimer et al., 1986). This phospholipase B is a 35 kDa homodimer and is strongly hemolytic towards human and rabbit erythrocytes, but not for bovine or ovine erythrocytes, and is partially responsible for the myoglobinuria seen in bites from this species (Bernheimer et al., 1987).

4. Low-molecular weight peptides

4.1. Neurotoxins

The alpha neurotoxins found in Australian elapids are postsynaptic blocking short- or long-chain neurotoxins. These toxins are similar in action, binding with high affinity to skeletal nicotinic acetylcholine receptors, but differ in size, from an average of slightly above sixty amino acids vs an average of seventy three amino acids, and having either four or five disulfide bridges respectively. These structural differences are due to the short chain neurotoxins having a primary structure similar to the long chains but with the later having C-terminal extension containing a fifth cysteine bond (Dufton and Harvey, 1989).

4.1.1. Short chain neurotoxins

In the short chain neurotoxins worldwide, there are typically eight cysteines in the pattern C–C–C–C–C–CC–C with disulfide spacing of 9–13:6:16–21:13:10:0:4 and cysteine connectivity of 1–3, 2–4, 5–6, 7–8. The short chain neurotoxins isolated thus far from Australian elapids are homologous, basically charged (Table 6) postsynaptic blockers of neuromuscular transmission with a great deal of sequential homology (Fig. 2).

The short-chain neurotoxin toxin Aa-c from *A. antarticus* is a lethal toxin homologous with other elapid short-chain neurotoxins, particularly from the sea snakes (Kim and Tamiya, 1981a). Toxin Pa-A from *P. australis* is a typical short-chain neurotoxin which produces peripheral paralysis by blocking neuromuscular

Table 6. Short chain neurotoxins with complete sequences

Source	Molecule	Average MW*	Residues	*p*I*	References
Acanthophis antarcticus	Aa-c	6879.99	62	8.50	(Kim and Tamiya, 1981a,b)
Oxyuranus s. scutellatus	Taipan toxin 1	6733.58	62	8.42	(Zamudio et al., 1996)
Oxyuranus s. scutellatus	Taipan toxin 2	6789.79	62	8.42	(Zamudio et al., 1996)
Pseudechis australis	Pa-A	6757.69	62	8.15	(Takasaki, 1989)

*Calculated by author from sequence.

transmission at the postsynaptic site through binding to the nicotinic acetylcholine receptor and, like Aa-c, shows a great deal of homology with other long chain neurotoxins (Takasaki and Tamiya, 1985). However, both of these toxins contain an extra cysteine residue at the N-terminal which may have tertiary structural implications.

Taipan toxin 1 and 2 from *O. s. scutellatus* are short-chain alpha-neurotoxins that inhibit the binding of bungarotoxin to nicotinic acetylcholine receptors in skeletal muscles but not to central neuronal nicotinic receptors (Zamudio et al., 1996). This is in contrast to erabutoxin a and b from *Laticauda semifasciata* (Broad-banded blue sea snake) which block the binding of alpha-bungarotoxin to this receptor. However, like alpha-bungarotoxin and the erabutoxins, these neurotoxins do not do not inhibit the binding of nicotine to high-affinity nicotine receptors in brain.

4.1.2. Long chain neurotoxins

The long chain neurotoxins worldwide typically have cysteine pattern of C–C–C–C–C–C–C–CC–C with disulfide spacing of 9:6:5:3:10:3:10:0:4 and cysteine connectivity of 1–3, 2–4, 5–6, 7–8, and 9–10. Like the short chain neurotoxins, the long chain neurotoxins are potent postsynaptic blockers of neuromuscular transmission but are not all basic (Table 7) and have much more variance in sequence (Fig. 3). Despite this, there still exists a significant degree of homology between the components.

The long chain neurotoxins isolated from *A. antarticus* differ from other elapid neurotoxins by having a valine at the N-terminus. Acanthopin-d does not differ otherwise from other blockers of postsynaptic neuromuscular transmission

Short chain neurotoxin alignments

```
1.  MTCCNQQSSQPKTTTICAGGESSCYKKTWSDHRGSRTERGCGCPHVKPGIKLTCCKTDECNN
2.  MQCCNQQSSQPKTTTTCPGGVSSCYKKTWRDHRGTIIERGCGCPRVKPGIRLICCKTDECNN
3.  MTCYNQQSSEAKTTTTCSGGVSSCYKKTWSDIRGTIIERGCGCPSVKKGIERICCRTDKCNN
4.  MTCYNQQSSEAKTTTTCSGGVSSCYKKTWSDGRGTIIERGCGCPSVKKGIERICCRTDKCNN
```

Fig. 2. Cysteines aligned and sequences arranged in order of similarity. References are: (1) Pa-A (Takasaki, 1989), (2) Aa-c (Kim and Tamiya, 1981a,b), (3) Taipan toxin 2 (Zamudio et al., 1996), (4) Taipan toxin 1 (Zamudio et al., 1996).

Table 7. Long chain neurotoxins with complete sequences

Source	Molecule	Average MW*	Residues	*p*I*	References
Acanthophis antarcticus	Aa-b	8135.47	73	8.24	(Kim and Tamiya, 1981a,b)
Acanthophis antarcticus	Aa-e	8761.31	79	8.95	(Tyler et al., 1997)
Acanthophis antarcticus	Acanthopin-d	8386.92	74	9.54	(Sheumack et al., 1990)
Notechis scutatus	Notechis III-4	8060.42	73	8.08	(Halpert et al., 1979)
Pseudechis australis	Pa-ID	7760.88	68	6.16	(Takasaki and Tamiya, 1985)
Pseudonaja textilis	Pseudonajatoxin-b	7761.02	71	7.32	(Tyler et al., 1987a,b)

*Calculated by author from sequence.

(Sheumack et al., 1990). The two toxin Aa-e isoforms (1 and 2) are identical but elute at different spots due to the presence of a Pro-Pro the C-terminal tail which allows the tail-sequence to adopt two different conformations (Tyler et al., 1997). Toxin Aa-b differs further with other elapid neurotoxins with the ubiquitous Lys-23 being replaced by Arg (Kim and Tamiya, 1981b).

Notechis III-4 from *N. scutatus* does not differ significantly from other long chain neurotoxins but is markedly absent from the black form of *N. scutatus* found in the Lake Alexandria region as well as *N. ater* subspecies (Halpert et al., 1979).

Pa-ID from *P. australis* is a non-lethal long-chain neurotoxin homologous to lethal elapid long-chain neurotoxins, with the loss of lethality likely due to distinctive structural deviations (Takasaki, 1989). The invariant Tyr-22 is replaced by a cysteine while the invariant functional residues Val/Ala-49 and Lys/Arg-50 are replaced by Arg and Met (the loss of positive charge at position 50 being significant) respectively. In addition, the often seen Gly-17, Ala-43, Ser-59, and Phe/His-66 are replaced by a Glu, Thr, Thr, and Val, respectively.

In *O. scutellatus*, alpha-subunit of taicatoxin affects channel gating of calcium channels and can form a dimer with the serine protease component of taicatoxin that is bioactive but not lethal without the phospholipase component (Brown et al., 1987; Possani et al., 1992).

Long chain neurotoxin sequence alignments

```
1. VICYRGYNNPQT----CPPGENVCFTRTWCDAFCSSRGKVVELG-CAATCPIVKSYNEVK-CCSTDKCNPFPVRPRRPP
2. VICYVGYNNPQT----CPPGGNVCFTKTWCDARCHQLGKRVEMG-CATTCPKVNRGVDIK-CCSTDKCNPFPKTTPPWK
3. LICYMGPKTPRT----CPRGQNLCYTKTWCDAFCSSRGKVVELG-CAATCPIAKSYEDVT-CCSTDNCNPFPVRPRHPP
4. VICYRKYTNNVKT---CPDGENVCYTKMWCDGFCTSRGKVVELG-CAATCPIRKPGNEVK-CCSTNKCNHPPKRKKRRP
5. RTCFITPDVKSKP---CPPGQEVCYTETWCDGFCGIRGKRVELG-CAATCPTPKKTGIDIQCCSTDDCNTFPLRP
6. RRCFITPNVRSER---CPPGQEVCFTKTX-DG
7. LTCYKGRDRSSET---CRSEQELCCTKTWCDQWCQDRGPRLEMG-CTATCPRRMPGLDFT-CCTTDNCNPVPT
```

Fig. 3. Cysteines aligned and sequences arranged in order of similarity. References are: (1) Aa-b (Kim and Tamiya, 1981a,b), (2) Aa-e (Tyler et al., 1997), (3) Notechis III-4 (Halpert et al., 1979), (4) Acanthopin-d (Sheumack et al., 1990), (5) Pseudonajatoxin-b (Tyler et al., 1987a,b), (6) Taicatoxin-8 kDa subunit (Possani et al., 1992), (7) Pa-ID (Takasaki and Tamiya, 1985).

Pseudonajatoxin-b from *P. textilis* is another highly lethal postsynaptic neurotoxin that shows a high degree of homology with other elapid long-chain neurotoxins but also has substitutions for Lys-23, having Glu in its place. (Tyler et al., 1987b).

4.2. Enzyme inhibitors

The gamma subunit of taicatoxin form *O. s. scutellatus* is a 7 kDa serine protease inhibitor found to be distinct from all other toxins isolated from elapids and was determined to be related (64%) to a chymotrypsin inhibitor from *Vipera ammodytes* (European long-nosed viper) (Possani et al., 1992). The unique primary structure (Fig. 4) and potent inhibitory activity makes this component very interesting to study. Another 7 kDa serine protease inhibitor has also been isolated from *P. textilis* and has been shown to be an inhibitor of the serine protease plasmin (Willmott et al., 1995). The pharmacological action of this inhibitor differs from the standard action of a slow, tight binding mechanism of small protein serine protease inhibitors. Rather, this molecule acts as a single-stage competitive inhibitor of both plasmin and trypsin.

5. Future directions

Despite the inordinate toxicity of the Australian elapids, most have been understudied and little is known about the clinical pathology of the whole venom or the bioactivity of many individual venom components. The bulk of the research thus far has been directed towards only a handful of species in particular: *Acanthophis antarticus* (Common death adder), *Notechis scutatus* (Mainland tiger snake), *Oxyuranus scutellatus scutellatus* (Coastal taipan), *Pseudechis australis* (Mulga snake), and *Pseudonaja textilis* (Eastern brown snake). Further, research has been concentrated heavily in one area (PLA_2s). In fact, the most toxic snake in the world, *Oxyuranus microlepidotus*, is very poorly characterized and it is still unknown at this point what makes this snake so much more potently toxic than *O. s. scutellatus* or any another snake for that matter. Further research needs to be dedicated towards these unique animals and in areas other than the traditional ones.

Of the short-chain neurotoxins, only two species (*Acanthophis antarticus and O. s. scutellatus)* have yielded components and only two each. Of the long-chain neurotoxins, only four species have had these molecules isolated from the venoms

Primary structure of taicatoxin-gamma

KDRPKF**C**HLPPKPGP**C**RAAIPRFYYNPHSKQ**C**EKFIYGG**C**HGNANKFKTPDE**C**NYT**C**LGVSL

Fig. 4. Primary structure of gamma-subunit of taicatoxin (Possani et al., 1992).

Table 8. Suspected toxic species with notably understudied venoms

Common name	Scientific name	Suspected toxicity
Black-headed death adder	*Acanthophis abditus*	lethal
Barkly tableland death adder	*Acanthophis hawkei*	lethal
Northern death adder	*Acanthophis praelongus*	lethal
Desert death adder	*Acanthophis pyrrhus*	lethal
Pygmy copperhead	*Austrelaps labialis*	potentially dangerous
Highland copperhead	*Austrelaps ramsayi*	lethal
Papuan whip snake	*Demansia papuanensis*	potentially dangerous
Black whip snake	*Demansia vestigiata*	potentially dangerous
Ornamental snake	*Denisonia maculata*	potentially dangerous
Lake Cronin snake	*Echiopsis atriceps*	potentially dangerous
Bardick snake	*Echiopsis curta*	potentially dangerous
Pale-headed snake	*Hoplocephalus bitorquatus*	potentially dangerous
Broad-headed snake	*Hoplocephalus bungaroides*	potentially lethal
Stephen's banded snake	*Hoplocephalus stephensi*	potentially lethal
Butler's snake	*Pseudechis butleri*	potentially lethal
Ingram's brown snake	*Pseudonaja ingrami*	lethal
Ringed brown snake	*Pseudonaja modesta*	potentially dangerous
Small-eyed snake	*Rhinoplocephalus nigrescens*	potentially dangerous

(*Acanthophis antarticus, Notechis scutatus, Pseudechis australis* and *Pseudonaja textilis*) and only one (*A. antarticus*) has had more than one isolated. Of the other types of low-molecular weight compounds, only one (taicatoxin-gamma from *O. s. scutellatus*) has been isolated. It is illogical that these components are present in only a handful of species and in such scarcity.

Further, there are still species with potentially dangerous venoms that the action of the crude venom is not known let alone individual components (Table 8). As more of these species are encountered through encroachment upon habitat or increased keeping in captivity, bites will occur with potentially fatal outcomes simply because of lack of data regarding the venom.

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References

Arni, R. K., Ward, R. J., 1996. Phospholipase A_2 – Structural review. Toxicon 34 (8), 827–841.

Bernheimer, A. W., Weinstein, S. A., Linder, R., 1986. Isoelectric analysis of some Australian elapid snake venoms with special reference to phospholipase B and hemolysis. Toxicon 24 (8), 841–849.

Bernheimer, A. W., Linder, R., Weinstein, S. A., Kim, K. S., 1987. Isolation and characterization of a phospholipase B from venom of Collett's snake, *Pseudechis colletti*. Toxicon 25 (5), 547–554.

Bouchier, C., Boyot, P., Tesson, F., Tr'emeau, O., Bouet, F., Hodgson, D., Boulain, J. C., M'Enez, A., 1991. Notechis 11′2, a non-toxic phospholipase A_2 from the venom of *Notechis scutatus scutatus*. Eur. J. Biochem. 202 (2), 493–500.

Broad, A. J., Sutherland, S. K., Coulter, A. R., 1979. The lethality in mice of dangerous Australian and other snake venom. Toxicon 17, 661–664.

Brown, A. M., Yatani, A., Lacerda, A. E., Gurrola, G. B., Possani, L. D., 1987. Neurotoxins that act selectively on voltage-dependent cardiac calcium channels. Circ. Res. 61 (4(2)), I6–9.

Chen, S. Z., Gopalakrishnakone, P., Gwee, M. C., 1994. Pharmacological effects and pathological changes induced by the venom of *Pseudechis australis* in isolated skeletal muscle preparations. Toxicon 32 (3), 303–315.

Chester, A., Crawford, G. P. M., 1982. *In vitro* coagulant properties of venoms from Australian snakes. Toxicon 20 (2), 501–504.

Chwetzoff, S., Mollier, P., Bouet, F., Rowan, E. G., Harvey, A. L., M'Enez, A., 1990. On the purification of notexin. Isolation of a single amino acid variant from the venom of *Notechis scutatus scutatus*. FEBS Lett. 261 (2), 226–230.

Dambisya, Y. M., Lee, T. L., Gopalakrishnakone, P., 1995. Anticoagulant effects of *Pseudechis australis* (Australian king brown snake) venom on human blood: A computerized thromboelastography study. Toxicon 33 (10), 1378–1382.

Ducancel, F., Guignery, F.-G., Bouchier, C., M'Enez, A., Boulain, J. C., 1988. Complete amino acid sequence of a PLA_2 from the tiger snake *Notechis scutatus scutatus* as deduced from a complementary DNA. Nucleic Acids Res. 16 (18), 9049.

Dufton, M. J., Harvey, A. L., 1989. The long and the short of neurotoxins. TiPS 10, 258–259.

Fatehi, M., Rowan, E. G., Harvey, A. L., Harris, J. B., 1994. The effects of five phospholipases A_2 from the venom of king brown snake, *Pseudechis australis*, on nerve and muscle. Toxicon 32 (12), 1559–1572.

Fohlman, J., Eaker, D., Karlsson, E., Thesleff, S., 1976. Taipoxin, an extremely potent presynaptic neurotoxin from the venom of the Australian snake taipan (*Oxyuranus s. scutellatus*). Isolation, characterization, quaternary structure and pharmacological properties. Eur. J. Biochem. 68 (2), 457–469.

Fohlman, J., Lind, P., Eaker, D., 1977. Taipoxin, an extremely potent presynaptic snake venom neurotoxin. Elucidation of the primary structure of the acidic carbohydrate-containing taipoxin-subunit, a prophospholipase homolog. FEBS Lett. 84 (2), 367–371.

Fohlman, J., 1979. Comparison of two highly toxic Australian snake venoms: The Taipan (*Oxyuranus s. scutellatus*) and the fierce snake (*Parademansia microlepidotus*). Toxicon 17 (2), 170–172.

Fossier, P., Lambeau, G., Lazdunski, M., Baux, G., 1995. Inhibition of ACh release at an Aplysia synapse by neurotoxic phospholipases A_2: Specific receptors and mechanisms of action.. J. Physiol. 489 (1), 29–40.

Francis, B., Coffield, J. A., Simpson, L. L., Kaiser, I. I., 1995. Amino acid sequence of a new type of toxic phospholipase A_2 from the venom of the Australian tiger snake (*Notechis scutatus scutatus*). Arch. Biochem. Biophys. 318 (2), 481–488.

Francis, B., John, T. R., Seebart, C., Kaiser, I. I., 1991. New toxins from the venom of the common tiger snake (*Notechis scutatus scutatus*). Toxicon 29 (1), 85–96.

Francis, B., Williams, E. S., Seebart, C., Kaiser, I. I., 1993. Proteins isolated from the venom of the common tiger snake (*Notechis scutatus scutatus*) promote hypotension and hemorrhage. Toxicon 31 (4), 447–458.

Gandolfo, G., Lambeau, G., Lazdunskim, M., Gottesmann, C., 1996. Effects on behavior and EEG of single chain phospholipases A_2 from snake and bee venoms injected into rat brain: Search for a functional antagonism. Pharmacol. Toxicol. 78 (5), 341–347.

Geh, S. L., Rowan, E. G., Harvey, A. L., 1992. A comparison of the neuromuscular effects of two phospholipases A_2 from the venom of the Australian king brown snake. Recent Adv. Toxinol. Res., (World Conf. Anim., Plant Microb. Toxin), 10th, Natl. Univ. Singapore, Venom Toxin Res. Group, Singapore, Singapore.

Geh, S. L., Rowan, E. G., Harvey, A. L., 1992b. Neuromuscular effects of four phospholipases A_2 from the venom of *Pseudechis australis*, the Australian king brown snake. Toxicon 30 (9), 1051–1057.

Halpert, J., Eaker, D., 1976. Isolation and amino acid sequence of a neurotoxic phospholipase A from the venom of the Australian tiger snake *Notechis scutatus scutatus*. J. Biol. Chem. 251 (23), 7343–7347.

Halpert, J., Fohlman, J., Eaker, D., 1979. Amino acid sequence of a postsynaptic neurotoxin from the venom of the Australian tiger snake *Notechis scutatus scutatus*. Biochimie 61 (5–6), 719–723.

Ho, C. L., Lee, C. Y., 1983. Mode of neuromuscular blocking action of caeruleotoxin. Toxicon 21 (2), 301–307.

John, T. R., Kaiser, I. I., 1990. Comparison of venom constituents from four tiger snake (*Notechis*) subspecies. Toxicon 28 (9), 1117–1122.

Kannan, K. K., Sinh, S., Ramanadham, M., Eaker, D., 1981. Structure of presynaptic toxins: Crystallization and preliminary X-ray diffraction data on Notechis II-5, a presynaptic toxin phospholipase. J. Biosci. 3 (1), 29–31.

Kim, H. S., Tamiya, N., 1981a. The amino acid sequence and position of the free thiol group of a short-chain neurotoxin from common-death-adder (*Acanthophis antarcticus*) venom. Biochem. J. 199 (1), 211–218.

Kim, H. S., Tamiya, N., 1981b. Isolation, properties and amino acid sequence of a long-chain neurotoxin, *Acanthophis antarcticus* b, from the venom of an Australian snake (the common death adder, *Acanthophis antarcticus*). Biochem. J. 193 (3), 899–906.

Lambeau, G., Schmid, A.-A., Lazdunski, M., Barhanin, J., 1990. Identification and purification of a very high affinity binding protein for toxic phospholipases A_2 in skeletal muscle. J. Biol. Chem. 265 (16), 9526–9532.

Lambeau, G., Barhanin, J., Lazdunski, M., 1991a. Identification of different receptor types for toxic phospholipases A_2 in rabbit skeletal muscle. FEBS. Lett. 293 (1–2), 29–33.

Lambeau, G., Lazdunski, M., Barhanin, J., 1991b. Properties of receptors for neurotoxic phospholipases A_2 in different tissues. Neurochem. Res. 16 (6), 651–658.

Lind, P., Eaker, D., 1980. complete amino-acid sequence of a non-neurotoxic, non-enzymatic phospholipase A_2 homolog from the venom of the Australian tiger snake *Notechis scutatus scutatus*. Eur. J. Biochem. 111 (2), 403–409.

Lind, P., Eaker, D., 1982. Amino-acid sequence of the alpha-subunit of taipoxin, an extremely potent presynaptic neurotoxin from the Australian snake taipan (*Oxyuranus s. scutellatus*). Eur. J. Biochem. 124 (3), 441–447.

Lind, P., 1982. Amino acid sequence of the beta 1 isosubunit of taipoxin, an extremely potent presynaptic neurotoxin from the Australian snake taipan (*Oxyuranus s. scutellatus*). Eur. J. Biochem. 128 (1), 71–75.

Marsh, N. A., Fyffe, T. L., Benner, E. A., 1997. Isolation and partial characterisation of a prothrombin-activating enzyme from the venom of the Australian Rough-scaled snake (*Tropidechis carinatus*). Toxicon 35 (4), 563–571.

Masci, P. P., Whitaker, A. N., de, J.-J., 1988. Purification and characterization of a prothrombin activator from the venom of the Australian brown snake, *Pseudonaja textilis textilis*. Biochem. Int. 17 (5), 825–835.

Mebs, D., Samejima, Y., 1980. Purification, from Australian elapid venoms, and properties of phospholipases A which cause myoglobinuria in mice. Toxicon 18 (4), 443–454.

Mebs, D., 1982. Studies on hemoglobinuria produced by venom from the Australian snake *Tropidechis carinatus* (Rough-scaled snake). Toxicon 20 (6), 1085–1088.

Mebs, D., Ehrenfeld, M., Samejima, Y., 1983. Local necrotizing effect of snake venoms on skin and muscle: Relationship to serum creatine kinase. Toxicon 21 (3), 393–404.

Mollier, P., Chwetzoff, S., Bouet, F., Harvey, A. L., M'Enez, A., 1989a. Tryptophan 110, a residue involved in the toxic activity but not in the enzymatic activity of notexin. Eur. J. Biochem. 185 (2), 263–270.

Mollier, P., Chwetzoff, S., Frachon, P., M'Enez, A., 1989b. Immunological properties of notexin, a potent presynaptic and myotoxic component from venom of the Australian tiger snake *Notechis scutatus scutatus*. FEBS. Lett. 250 (2), 479–482.

Mollier, P., Brochier, G., Gaudry-Talarmain, Y. M., 1990a. The action of notexin from tiger snake venom (*Notechis scutatus scutatus*) on acetylcholine release and compartmentation in synaptosomes from electric organ of *Torpedo marmorata*. Toxicon 28 (9), 1039–1052.

Mollier, P., Chwetzoff, S., M'Enez, A., 1990b. A monoclonal antibody recognizing a conserved epitope in a group of phospholipases A_2. Mol. Immunol. 27 (1), 7–15.

Nishida, S., Terashima, M., Tamiya, N., 1985. Amino acid sequences of phospholipases A_2 from the venom of an Australian elapid snake (King brown snake, *Pseudechis australis*). Toxicon 23 (1), 87–104.

Pearson, J. A., Tyler, M. I., Howden, M. E., 1991a. Immunological relationships between the subunits of textilotoxin and rabbit antisera raised against textilotoxin and some snake venoms. Toxicon 29 (3), 375–378.

Pearson, J. A., Tyler, M. I., Retson, K. V., Howden, M. E. H., 1991b. Studies on the subunit structure of textilotoxin, a potent presynaptic neurotoxin from the venom of the Australian common brown snake (*Pseudonaja textilis*). 2. The amino acid sequence and toxicity studies of subunit D. Biochim. Biophys. Acta 1077 (2), 147–150.

Pearson, J. A., Tyler, M. I., Retson, K. V., Howden, M. E., 1993. Studies on the subunit structure of textilotoxin, a potent presynaptic neurotoxin from the venom of the Australian common brown snake (*Pseudonaja textilis*). 3. The complete amino-acid sequences of all the subunits. Biochim. Biophys. Acta 1161 (2–3), 223–229.

Possani, L. D., Martin, B. M., Yatani, A., Mochca-Morales, J., Zamudio, F. Z., Gurrola, G. B., Brown, A. M., 1992. Isolation and physiological characterization of taicatoxin, a complex toxin with specific effects on calcium channels. Toxicon 30 (11), 1343–1364.

Rosing, J., Tans, G., 1992. Structural and functional properties of snake venom prothrombin activators. Toxicon 30 (12), 1515–1527.

Rowan, E. G., Harvey, A. L., Takasaki, C., Tamiya, N., 1989. Neuromuscular effects of three phospholipases A_2 from the venom of the Australian king brown snake *Pseudechis australis*. Toxicon 27 (5), 551–560.

Schmidt, J. J., Middlebrook, J. L., 1989. Purification, sequencing and characterization of pseudexin phospholipases A_2 from *Pseudechis porphyriacus* (Australian red-bellied black snake). Toxicon 27 (7), 805–818.

Sharp, P. J., Berry, S. L., Spence, I., Howden, M. E., 1989. A basic phospholipase A from the venom of the Australian king brown snake (*Pseudechis australis*) showing diverse activities against membranes. Comp. Biochem. Physiol. B 92 (3), 501–508.

Sheumack, D. D., Spence, I., Tyler, M. I., Howden, M. E., 1990. The complete amino acid sequence of a post-synaptic neurotoxin isolated from the venom of the Australian death adder snake *Acanthophis antarcticus*. Comp. Biochem. Physiol. B 95 (1), 45–50.

Speijer, H., Govers, R.-J. W., Zwaal, R. F., Rosing, J., 1986. Prothrombin activation by an activator from the venom of *Oxyuranus scutellatus* (Taipan snake). J. Biol. Chem. 261 (28), 13258–13267.

Speijer, H., Govers, R.-J. W., Zwaal, R. F., Rosing, J., 1987. Platelet procoagulant properties studied with snake venom prothrombin activators. Thromb. Haemost. 57 (3), 349–355.

Stocker, K., Hauer, H., Muller, C., Triplett, D. A., 1994. Isolation and characterization of Textarin, a prothrombin activator from eastern brown snake (*Pseudonaja textilis*) venom. Toxicon 32 (10), 1227–1236.

Su, M. J., Coulter, A. R., Sutherland, S. K., Chang, C. C., 1983. The presynaptic neuromuscular blocking effect and phospholipase A_2 activity of textilotoxin, a potent toxin isolated from the venom of the Australian brown snake, *Pseudonaja textilis*. Toxicon 21 (1), 143–151.

Subburaju, S., Kini, R. M., 1997. Isolation and purification of superbins I and II from *Austrelaps superbus* (Copperhead) snake venom and their anticoagulant and antiplatelet effects. Toxicon 35 (8), 1239–1250.

Sutherland, S. K., 1983. Australian animal toxins. Oxford University Press, Melbourne.

Takasaki, C., Tamiya, N., 1982. Isolation and properties of lysophospholipases from the venom of an Australian elapid snake, *Pseudechis australis*. Biochem. J. 203 (1), 269–276.

Takasaki, C., Tamiya, N., 1985. Isolation and amino acid sequence of a short-chain neurotoxin from an Australian elapid snake, *Pseudechis australis*. Biochem. J. 232 (3), 367–371.

Takasaki, C., 1989. Amino acid sequence of a long-chain neurotoxin homologue, Pa ID, from the venom of an Australian elapid snake, *Pseudechis australis*. J. Biochem. Tokyo 106 (1), 11–16.

Takasaki, C., Yutani, F., Kajiyashiki, T., 1990. Amino acid sequences of eight phospholipases A_2 from the venom of Australian king brown snake, *Pseudechis australis*. Toxicon 28 (3), 329–339.

Tan, N. H., Ponnudurai, G., 1990. A comparative study of the biological properties of Australian elapid venoms. Comp. Biochem. Physiol. C 97 (1), 99–106.

Tans, G., Govers, R.-J. W., van, R.-J. L., Rosing, J., 1985. Purification and properties of a prothrombin activator from the venom of *Notechis scutatus scutatus*. J. Biol. Chem. 260 (16), 9366–9372.

Tyler, M. I., Barnett, D., Nicholson, P., Spence, I., Howden, M. E., 1987a. Studies on the subunit structure of textilotoxin, a potent neurotoxin from the venom of the Australian common brown snake (*Pseudonaja textilis*). Biochim. Biophys. Acta 915 (2), 210–216.

Tyler, M. I., Spence, I., Barnett, D., Howden, M. E., 1987b. Pseudonajatoxin b: Unusual amino acid sequence of a lethal neurotoxin from the venom of the Australian common brown snake, *Pseudonaja textilis*. Eur. J. Biochem. 166 (1), 139–143.

Tyler, M., Retson-Yip, K. V., Gibson, M. K., Barnett, D., Howe, E., Stocklin, R., 1997. Isolation and amino acid sequence of a new long-chain neurotoxin with two chromatographic isoforms (Ae-e1 and Ae-e2) from the venom of the Australian death adder (*Acanthophis antarticus*). Toxicon 35 (4), 555–562.

Van Der Weyden, L., Hains, P., Morris, M., Broady, K., 1997. Acanthoxin, a toxic phospholipase A_2 from the venom of the common death adder (*Acanthophis antarticus*). Toxicon 35 (8), 1315–1325.

Weinstein, S. A., Bell, R. C., Brown, C. R., II, Fletcher, J. E., Smith, L. A., 1992. Pathologic changes induced by phospholipase A_2 isolated from the venom of Collett's snake, *Pseudechis colletti*: A light and electron microscopic study. Toxicon 30 (2), 171–185.

Williams, V., White, J., 1989. Purification and properties of a procoagulant from Peninsula tiger snake (*Notechis ater niger*) venom. Toxicon 27 (7), 773–779.

Williams, V., White, J., Mirtschin, P. J., 1994. Comparative study on the procoagulant from the venom of Australian brown snakes (Elapidae; *Pseudonaja spp.*). Toxicon 32 (4), 453–459.

Willmott, N., Gaffney, P., Masci, P., Whitaker, A., 1995. A novel serine protease inhibitor from the Australian brown snake, *Pseudonaja textilis textilis*: Inhibition kinetics. Fibrinolysis 9, 1–8.

Wilson, H. I., Nicholson, G. M., Tyler, M. I., Howden, M. E., 1995. Induction of giant miniature end-plate potentials during blockade of neuromuscular transmission by textilotoxin. Naunyn–Schmiedeberg's Arch. Pharmacol. 352 (1), 79–87.

Yang, C. C., Chang, L. S., 1990. The N-terminal amino group essential for the biological activity of notexin from *Notechis scutatus scutatus* venom. Biochim. Biophys. Acta. 1040 (1), 35–42.

Yang, C. C., Chang, L. S., Wu, F. S., 1991. Venom constituents of *Notechis scutatus scutatus* (Australian tiger snake) from differing geographic regions. Toxicon 29 (11), 1337–1344.

Yang, C. C., Chang, L. S., 1991. Dissociation of lethal toxicity and enzymic activity of notexin from *Notechis scutatus scutatus* (Australian tiger snake) venom by modification of tyrosine residues. Biochem. J. 280 (3), 739–744.

Yuan, Y., Jackson, S. P., Mitchell, C. A., Salem, H. H., 1993. Purification and characterisation of a snake venom phospholipase A_2: A potent inhibitor of platelet aggregation. Thromb. Res. 70 (6), 471–481.

Zamudio, F., Wolf, K. M., Martin, B. M., Possani, L. D., Chiappinelli, V. A., 1996. Two novel alpha-neurotoxins isolated from the taipan snake, *Oxyuranus scutellatus*, exhibit reduced affinity for nicotinic acetylcholine receptors in brain and skeletal muscle. Biochemistry 35 (24), 7910–7916.