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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. © 1998 Elsevier Science Ltd. All rights reserved.

### 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<sub>50</sub>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<sub>2</sub>s 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	<i>Hoplocephalus stephensi</i>
Kreffl's tiger snake	<i>Notechis ater ater</i>
Tasmanian King Island tiger snake	<i>Notechis ater humphreysi</i>
Peninsula tiger snake	<i>Notechis ater niger</i>
Western tiger snake	<i>Notechis ater occidentalis</i>
Chappell island tiger snake	<i>Notechis ater serventyi</i>
Inland taipan	<i>Oxyuranus microlepidotus</i>
Coastal taipan	<i>Oxyuranus scutellatus</i>
Red-bellied black snake	<i>Pseudechis porphyriacus</i>
Dugite	<i>Pseudonaja affinis</i>
Speckled brown snake	<i>Pseudonaja guttata</i>
Gwardar	<i>Pseudonaja nuchalis</i>
Eastern brown snake	<i>Pseudonaja textilis</i>
Rough-scaled snake	<i>Tropidechis carinatus</i>

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	<i>Acanthophis antarticus</i>
Lowland copperhead	<i>Austrelaps superbus</i>
Mulga	<i>Pseudechis australis</i>
Collett's snake	<i>Pseudechis colletti</i>
Spotted black snake	<i>Pseudechis guttatus</i>

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 <i>Pseudonaja</i> (brown snakes) species
Black snake monovalent	<i>Pseudechis australis</i> (Mulga) and likely <i>P. butleri</i> (Butler's snake/Spotted Mulga)
Death adder monovalent	All <i>Acanthophis</i> (Death adder) species
Sea snake monovalent	All members of the sea snake families Hydrophiidae and Laticaudidae
Taipan monovalent	All <i>Oxyuramus</i> (Taipan) species
Tiger snake monovalent	All species of <i>Austrelaps</i> , <i>Hoplocephalus</i> (banded/broad-headed/pale-headed snakes), <i>Notechis</i> (Tiger snakes), <i>Tropidechis carinatus</i> (Rough-scaled snakes) as well as all <i>Pseudechis</i> (Black snake) species except <i>P. australis</i> and perhaps <i>P. butleri</i> .

## 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<sub>2</sub>, 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<sub>2</sub> 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
<i>Notechis ater niger</i>	II	58	2	37, 23	(Williams and White, 1989)
<i>Notechis scutatus</i>	II	54	2	32, 23	(Tans et al., 1985)
<i>Oxyuranus scutellatus scutellatus</i>	III	200	2	110, 80	(Speijer et al., 1986)
<i>Pseudonaja affinis affinis</i>	III	80	unknown	unknown	(Williams et al., 1994)
<i>Pseudonaja textilis</i>	II	53	unknown	unknown	(Stocker et al., 1994)
<i>Pseudonaja textilis</i>	III	200	2	105, 65	(Masci et al., 1988)
<i>Tropidechis carinatus</i>	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<sub>1</sub>, A<sub>2</sub>, C, D) classified by the site at which they hydrolyze ester bonds of 3-*sn*-phosphoglycerides. Most venom phospholipases are phosphatidylcholine 2-acylhydrolase (PLA<sub>2</sub> 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<sub>2</sub>s. 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<sub>2</sub>s not only in primary structure and N-terminal sequence, but also in the physiological requirement of Ca<sup>2+</sup> 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<sub>2</sub> 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<sub>2</sub>s isolated from *P. australis*, notably Pa-11.

### 3.3. Phospholipase A<sub>2</sub> 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<sub>2</sub>s 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<sub>2</sub>s are basic, 118 amino acids, have seven disulfide bonds and molecular weights around 13 kDa (Table 5). The PLA<sub>2</sub>s 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<sub>2</sub>s is the toxic activity of pre- or post-synaptic blockage of neuromuscular nerve transmission. In class I PLA<sub>2</sub>s, there is a preserved structure, with the cysteine pattern almost invariably C–C–C–CC–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 of homology existing in the sequences (Fig. 1) there is a wide range of enzymatic and toxic activity in the class I PLA<sub>2</sub>s found in Australian elapids. Thus, Australian elapid venom class I PLA<sub>2</sub>s can be further broken up

Table 5. Phospholipase A<sub>2</sub>s with complete sequences

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

\*Calculated by author from sequence. Types: H = Hemotoxic, M = Myotoxic, N = Neurotoxic, X<sub>1</sub> = Non-toxic, enzymatically active, X<sub>2</sub> = Non-toxic, non-enzymatically active.

### Phospholipase sequence alignments

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1. -----NLAQFGFMIRCANNGSRSALDYADYGC
2. -----NLLQFGFMIRCANRRSRPVWHYMDYGCYCGKGGSGTPVDDLDRCCQVHDECYGEAVRRFG
3. -----NLVQFGKMIECAIRNRRPALDFMNYGCYCGKGGSGTPVDDLDRCCQVHDECYAEAEKHG-
4. -----NLYQFGNMIQCANHGRRPTLAYADYGCYCGAGGSGTPVDELDRCCKAHDDCYGEAGKKG-
5. -----NLVQFSNMIQCANHGRSRPSLAYADYGCYCSAGGSGTPVDELDRCCKTHDDCYARATKSYS
6. -----NLIQFANMIGCANHGSR
7. -----NLIQFGNMIQCANKGSRPTRHYMDYGCYCGWGGSGTPVDELDRCCQTHDDCYGEAEKKG-
8. -----NLIQFGNMIQCANKGSRPTRHYMDYGCYCGWGGSGTPVDELDRCCKVHDDCYGEAEKKG-
9. -----NLIQFSNMIQCANKGSRPSLDYADYGCYCGWGGSGTPVDELDRCCKVHDDCYAEAGKKG-
10. -----NLIQFSNMIQCANKGSRPSLHYADYGCYCGWGGSGTPVDELDRCCKVHDDCYDQAGKKG-
11. -----NLIQFRKMIQCANKGSRAAWHYLDYGCYCGPGGRGTPVDELDRCCKIHDDCYEAGKDG-
12. -----NLIQFRKMIQCANKGSRAAWHYLDYGCYCGPGGRGTPVDELDRCCKIHDDCYEAGKDG-
13. -----NLIQFGNMIQCANKGSRPSLDYADYGCYCGWGGSGTPVDELDRCCQVHDDNCEYQAGKKG-
14. -----NLIQFGNMIQCANKGSRPSLNYADYGCYCGWGGSGTPVDELDRCCQVHDDNCEYQAGKKG-
15. -----NLIQFGNMIQCANKGSRPSLDYADYGCYCGWGGSGTPVDELDRCCQTHDDNCEYQAGKKG-
16. -----NLYQFKNMIQCANKGSRSWLDYVNYGCYCGWGGSGTPVDELDRCCQTHDDNCEYDQAGKKG-
17. -----DLFQFGGMIGCANKGAYSWLSYVNYGCYCGWG
18. -----NLYQFGGMIQCANKGAYSWLSYVNYGCYCGWG
19. -----NLIQFKSIIECANRGSRRWLDYADYGCYCGWGGSGTPVDELDRCCKVHDECYGEAVKQG-
20. -----NLIQLSNMIKCAIPGSQPLF
21. -----NLIQLSNMIKCAIPGSRPLFYTYDYGCY
22. -----NLIQFSNMIKCAIPGSRPLFYADYGCYCGPGGHGTPVDELDRCCKIHDDCYEAGKKG-
23. -----NLIQFSNMIKCTIPGSQPLLDYANYGCYCGPGNNGTPVDDVDRCCQAHDECYDEASNHG-
24. -----NLVQFSYLIQCANHGRRPTWHYMDYGCYCGAGGSGTPVDELDRCCKIHDDCYDEAGKKG-
25. -----NLVQFSYLIQCANHGRRPTWHYMDYGCYCGAGGSGTPVDELDRCCKIHDDCYDEAGKKG-
26. -----NLVQFSYLIQCANHGRRPTWHYMDYGCYCGWGGSGTPVDELDRCCKIHDDCYSDAEKKG-
27. -----NLVQFSYLIRCANKYKRPWHYANYGCYCGSGGRGTPVDDVDRCCQAHDDCYEDAELKLG-
28. -----DLVEFGFMIRCANRNSQPAWQYMDYGCYCGKRGSGTPVDDVDRCCQTHNECYDEAAKIPG
29. -----NLYQFGNMIQCANHGRRPTWHYMDYGCYCGKGGSGTPVDELDRCCQTHDDCYGEAEKLEPA
30.
LPA
31. SELPQPSIDFEQFSNMIQCTIPCGSECLAYMDYGCYCGPGGSGTPIDDLDRCCKTHDECYAEAGKLSA
32. ASIPRPSLNIMLFGNMIQCTIPCEQSWLGLYDYGCYCGSGSGSIPVDDVDKCCKTHDECYKAGQIPG

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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<sub>2</sub> (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<sub>2</sub> (N-terminal fragment) (Van Der Weyden et al., 1997), (18) Acanthoxin-A<sub>1</sub> (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) HTc (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,  $Ht_{a-1}$ , have been isolated from *N. scutatus*. Both  $Ht_c$  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<sub>2</sub>s have been isolated from *P. australis* venom. PLA<sub>2</sub>s Pa-1G, Pa-5, Pa-12C and Pa-15 are characterized as being homologous single chain postsynaptic-acting basic PLA<sub>2</sub>s (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<sub>2</sub> isoenzymes that block K<sup>+</sup> conductance not through selective action on K<sup>+</sup> channels but secondary to membrane depolarization (Fatehi et al., 1994). Pa-10A, Pa-11 and Pa-13 are other lethal single chain PLA<sub>2</sub>s, 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<sub>2</sub>s 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<sub>2</sub>s 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<sub>2</sub>s

The neurotoxic PLA<sub>2</sub>s 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<sub>2</sub>.** The single chain neurotoxic PLA<sub>2</sub>s from Australian elapids are almost exclusively basic and have a potent presynaptic activity. Notexin is the principle neurotoxic and myonecrotic phospholipase A<sub>2</sub> in *Notechis* species and is representative of single chain neurotoxic PLA<sub>2</sub>s found in Australian elapids. In addition to having the non-lethal esterase activity seen in many other PLA<sub>2</sub>s, this PLA<sub>2</sub> 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<sub>2</sub>s isolated from *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<sub>2</sub>s isolated from *P. porphyriacus* venom and are putatively presynaptic blockers. As is seen in a great many of the other PLA<sub>2</sub>s isolated from Australian elapids, there is a great range in toxic activities among the isoforms of these basic PLA<sub>2</sub>s; 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<sub>2</sub>s isolated from *O. s. scutellatus* are OS<sub>1</sub> and OS<sub>2</sub>. These toxic monochain PLA<sub>2</sub>s have been quite useful in characterizing mammalian phospholipase A<sub>2</sub> receptors by binding to PLA<sub>2</sub> receptors that bee (*Apis mellinifer*) venom PLA<sub>2</sub>s are unable to bind. OS<sub>1</sub> binds to M-type (bee insensitive) neuronal PLA<sub>2</sub> receptors but not to N-type (bee sensitive) receptors while OS<sub>2</sub>, however, is capable of binding to both M- and N-type PLA<sub>2</sub> 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<sub>2</sub>s in the venoms. Recently, however, PLA<sub>2</sub>s have been purified. Acanthoxin (isoforms A1 and A2) and Acanthin from *A. antarcticus* (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<sub>2</sub>s Acanthoxin A1 and A2 are highly enzymatic and share a significant degree of N-terminal sequence homology with toxic PLA<sub>2</sub>s, 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<sub>2</sub>s.** The multi-chain PLA<sub>2</sub> 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<sub>2</sub>-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 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<sub>2</sub> 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<sub>2</sub> 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). Subunit 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<sub>2</sub>s

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<sub>2</sub>s 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 pI; 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<sub>2</sub> 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<sub>2</sub>s

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<sub>2</sub> 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<sub>2</sub>s

By studying the variations in PLA<sub>2</sub> 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<sub>2</sub>s, 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<sub>2</sub>s, 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<sub>2</sub> 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<sub>2</sub>s, as well as in pancreatic PLA<sub>2</sub>s, 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* (Kreffl'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. superbis* 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. antarcticus* 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	pI*	References
<i>Acanthophis antarcticus</i>	Aa-c	6879.99	62	8.50	(Kim and Tamiya, 1981a,b)
<i>Oxyuramus s. scutellatus</i>	Taipan toxin 1	6733.58	62	8.42	(Zamudio et al., 1996)
<i>Oxyuramus s. scutellatus</i>	Taipan toxin 2	6789.79	62	8.42	(Zamudio et al., 1996)
<i>Pseudechis australis</i>	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-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. antarcticus* 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. **MTCCNQQSSQPKTTTTICAGGESSCYKKTWSDHRGSRTERGCGCPHVKPGIKLTCCKTDECNN**
2. **MQCCNQQSSQPKTTTTICPGGVSSCYKKTWRDHRGTIIERGCPCPRVKPGIRLICCCTDECNN**
3. **MTCYNQQSSEAKTTTTICSGGVSSCYKKTWSDIRGTIIERGCPCPSVKKGIERICCRTRDKCNN**
4. **MTCYNQQSSEAKTTTTICSGGVSSCYKKTWSDGRGTIIERGCPCPSVKKGIERICCRTRDKCNN**

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) Taipian toxin 2 (Zamudio et al., 1996), (4) Taipian toxin 1 (Zamudio et al., 1996).

Table 7. Long chain neurotoxins with complete sequences

Source	Molecule	Average MW*	Residues	pI*	References
<i>Acanthopis antarcticus</i>	Aa-b	8135.47	73	8.24	(Kim and Tamiya, 1981a,b)
<i>Acanthopis antarcticus</i>	Aa-e	8761.31	79	8.95	(Tyler et al., 1997)
<i>Acanthopis antarcticus</i>	Acanthopin-d	8386.92	74	9.54	(Sheumack et al., 1990)
<i>Notechis scutatus</i>	Notechis III-4	8060.42	73	8.08	(Halpert et al., 1979)
<i>Pseudechis australis</i>	Pa-ID	7760.88	68	6.16	(Takasaki and Tamiya, 1985)
<i>Pseudonaja textilis</i>	Pseudonajatotoxin-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

- VICYRGYNNPQT---CPPGENVCFTRTWCDAFCSRRGKVVVELG--CAATCPIVKSYSYNEVK--CCSTDKCNPFPPVRRRPP
- VICYVGYNNPQT----CPPGGNVCFTKTWCDARCHQLGKRVEMG--CATTCPKVNRGVDIK--CCSTDKCNPFPPKTPPWK
- LICYMGPKTPRT---CPRGQNLCTYKTKWCDAFCSRRGKVVVELG--CAATCPIAKSYEDVT--CCSTDNKNPFPPVRRPHPP
- VICYRKYTNNVKT---CPDGENVCYTKMWCDFGCTSRGKVVVELG--CAATCPIRKPGEVVK--CCSTNKNHPKRRKRRP
- RTCFITPDVKSKEP---CPPGQEVCTETWCDGFCGIRGKRVVELG--CAATCPTPKKTGIDIQCCSTDDCNTFFPLRP
- RRCFITPNVRSER---CPPGQEVCFPTKTX--DG
- LTCYKGRDRSSET---CRSEQLCCTKTWCDQWCQDRGPRLEMG--CTATCPRRMPGLDFT--CCTTDNKNPVPT

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) Pseudonajatotoxin-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 from *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 antarcticus* (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<sub>2</sub>S). 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 antarcticus* 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

KDRPKFCHLPPKPGPCRAAI PRFYYNPHSKQCEKFTYGGCHGNANKFKTPDCECNYTCLGVSL

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

(*Acanthophis antarcticus*, *Notechis scutatus*, *Pseudechis australis* and *Pseudonaja textilis*) and only one (*A. antarcticus*) 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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