BCPT Basic & Clinical Pharmacology & Toxicology

Basic & Clinical Pharmacology & Toxicology, 2012, **111**, 268–274 Doi: 10.1111/j.1742-7843.2012.00907.x

Toxinology of Venoms from Five Australian Lesser Known Elapid Snakes

Kyle Pycroft[1], Bryan G. Fry[2], Geoffrey K. Isbister[1,3], Sanjaya Kuruppu[4], Josie Lawrence[4], A. Ian Smith[4] and Wayne C. Hodgson[1]

[1]Monash Venom Group, Department of Pharmacology, Monash University, Clayton, Vic., Australia, [2]Venom Evolution Laboratory, School of Biological Sciences, University of Queensland, Brisbane, Qld, Australia, [3]Discipline of Clinical Pharmacology, University of Newcastle, Newcastle, NSW, Australia, and [4]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Vic., Australia

(Received 27 February 2012; Accepted 22 May 2012)

Abstract: Research into Australian elapid venoms has mainly focused on the seven genera of greatest clinical significance: *Acanthophis, Hoplocephalus*, *Notechis*, *Oxyuranus, Pseudechis*, *Pseudonaja* and *Tropidechis*. However, even small species represent a potential for causing severe clinical envenoming. Further, owing to taxonomic distinctiveness, these species are a potential source of novel toxins for use in drug design and development. This is the first study to characterize the venoms of *Cryptophis boschmai*, *Denisonia devisi*, *Echiopsis curta*, *Hemiaspis signata* and *Vermicella annulata*. MALDI analysis of each venom, over the range of 4–40 kDa, indicated components in the weight range for three finger toxins (6–8 kDa) and phospholipase A_2 (PLA_2; 12–14 kDA). Interestingly, *C. boschmai* venom was the only venom, which contained components > 25 kDa. All venoms (10 μg/ml) demonstrated *in vitro* neurotoxicity in the chick biventer cervicis nerve-muscle preparation, with a relative rank order of: *H. signata* ≥ *D. devisi* ≥ *V. annulata* = *E. curta* > *C. boschmai*. CSL polyvalent antivenom neutralized the inhibitory effects of *C. boschmai* venom but only delayed the inhibitory effect of the other venoms. All venoms displayed PLA_2 activity but over a wide range (i.e. 1–621 μmol/min./mg). The venoms of *C. boschmai* (60 μg/kg, i.v.), *D. devisi* (60 μg/kg, i.v.) and *H. signata* (60 μg/kg, i.v.) produced hypotensive effects *in vivo* in an anaesthetized rat preparation. *H. signata* displayed moderate pro-coagulant activity while the other venoms were weakly pro-coagulant. This study demonstrated that these understudied Australian elapids have varying pharmacological activity, with notable *in vitro* neurotoxicity for four of the venoms, and may produce mild to moderate effects following systemic envenoming.

Australia is unique in that the majority of snakes are venomous. The exact number of venomous Australian species continues to vary with the debate over taxonomy. However, it has been estimated that there are 90 species of Australian elapids, belonging to 26 genera [1], all of which are venomous [2]. A study that ranked the venoms of highly dangerous snakes from around the world, based on murine LD_{50} values, showed that the most potent venoms belonged to Australian snakes [3]. While this study included a number of Australian snake species, most research into the venoms of Australian snakes tends to focus on the seven genera of elapids of greatest clinical significance. These genera are *Acanthophis* (Death adders), *Notechis* (Tiger snakes), *Tropidechis* (Rough scale snake), *Hoplocephalus* (including Stephen's banded snake), *Oxyuranus* (Taipans), *Pseudechis* (Black snakes) and *Pseudonaja* (Brown snakes). The clinical effects of bites from these genera have been well defined [4]. Given the focus of research on the seven genera of elapids with the greatest clinical significance, there is a large gap in our understanding of many of Australia's other elapid venoms.

In the present study, we have investigated the venoms of five species: *Cryptophis boschmai*, *Denisonia devisi*, *Echiopsis curta*, *Hemiaspis signata* and *Vermicella annulata*. Currently, no research has been performed on the venoms of *C. boschmai*, *D. devisi* or *V. annulata*, while research into the venoms of *H. signata* and *E. curta* is extremely limited. Isbister *et al.* [5] reported on two cases of envenoming by *H. signata* in children, where the venom had mild procoagulative effects. Swindells *et al.* [6] reported on a suspected case of neurotoxic envenoming by *E. curta* in a dog. The animal tested positive to death adder venom using a snake venom detection kit (SVDK) and appeared to improve after death adder antivenom (CSL Ltd). However, envenoming occurred outside of the anticipated geographical range of death adders but where *E. curta* are endemic. Marshall reported on a human case of envenoming by *Notechis curtus* (now reclassified as *E. curta*) [7]. The patient did not require antivenom, but the SVDK returned a positive result to *Acanthophis* (i.e. death adder). *In vitro* coagulation tests were also performed, which showed that the venom had anticoagulant properties [7].

The aim of this study was to characterize venoms from some other Australasian elapids.

Materials and Methods

Venom collection and storage. All snakes were identified and venoms collected by Bryan Fry. *C. boschmai* were collected from Townsville, Queensland. *D. devisi* were collected from Glen Morgan, Queensland. *E. curta* were collected from Perth, Western Australia. *H. signata* were collected from Wollongong, New South Wales. *V. annulata* were

Author for correspondence: Wayne C. Hodgson, Monash Venom Group, Department of Pharmacology, Monash University, Clayton, Vic., Australia (fax: +61 3 99059327, e-mail wayne.hodgson@monash.edu).

© 2012 The Authors
Basic & Clinical Pharmacology & Toxicology © 2012 Nordic Pharmacological Society

collected from Brisbane, Queensland. Venom was extracted, frozen at −80°C, freeze-dried and weighed before being made into 1 mg/ml solutions in milliQ water (Simplicity UV, Millipore Australia, North Ryde, NSW, Australia). Stock solutions were stored at −20°C until needed. Protein content of samples was determined using a Pierce BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA).

Mass spectrometry. Desalted samples were mixed 1:1 with Matrix, 10 mg/ml a-cyano-4-hydroxycinnamic acid (Laser BioLabs, Sophia-Antipolis, France) in 50% Acetonitrile in 0.1% TFA and spotted onto a 4700 MALDI target plate. The plate was loaded into a 4700 Proteomics ToF/ToF analyser (Applied Biosystems, Foster City, CA, USA). Proteins were analysed in linear mode with a mass range of 4–40 kDa and a focus mass of 13 kDa. Laser shots were fired randomly across the sample well and the summed spectrum consists of spectra collected at the rate of 2500 shots/spectrum.

Chick isolated biventer cervicis nerve-muscle preparation. Male chicks, aged 4–10 days old, were killed by CO_2 asphyxiation and exsanguination. The biventer cervicis nerve-muscle preparations were dissected, then mounted under 1 g tension in 5 ml organ baths containing physiological salt solution (NaCl, 118.4 mM; KCl, 4.5 mM, $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 25 mM; and glucose, 11.1 mM) at 34°C, bubbled with carbogen (95% O_2; 5% CO_2) as previously described [8]. Indirect twitches were evoked by electrical stimulation of the motor nerves using a Grass S88 stimulator (0.2 ms duration, 0.1 Hz, supramaximal V) (Grass Technologies, West Warwick, RI, USA). In the absence of electrical stimulation, responses to acetylcholine (ACh, 1 mM for 30 sec.; Sigma-Aldrich, St. Louis, MO, USA), carbachol (CCh, 20 μM for 60 sec.; Sigma) and potassium chloride (KCl, 40 mM for 30 sec.; 8) were obtained. After thorough washing, electrical stimulation was recommenced and the preparation allowed to equilibrate for 30 min. Venom (10 or 30 μg/ml) was added to the organ bath and twitch height recorded for 1 hr or until twitches were abolished. Where indicated, CSL polyvalent antivenom (5 U/ml; each unit (U) of antivenom will neutralize the effects of at least 10 μg venom; CSL Ltd, Parkville, VIC, Australia) was added 10 min. prior to the addition of venom. Contractile responses to ACh, CCh and KCl were obtained (as described previously) at the conclusion of the experiment.

Anaesthetized rats. Male Sprague-Dawley rats (250–380 g) were anaesthetized with pentobarbitone sodium (80–100 mg/kg, i.p., supplemented as required; ilium). A midline incision was made in the cervical region and cannulae inserted into the trachea (artificial respiration if required), jugular vein and carotid artery. Arterial blood pressure was recorded from the carotid cannula via a Gould P23 pressure transducer connected to a PowerLab system (ADInstruments, Bella Vista, NSW, Australia). Venom was administered via the right jugular vein.

PLA_2 assay. Phospholipase A_2 (PLA_2) activity was determined using a secretory PLA_2 colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. This assay uses the 1,2-dithio analogue of diheptanoyl phosphatidylcholine, which serves as a substrate for PLA_2 enzymes. Bee venom is used as a positive control. The venoms of *C. boschmai*, *D. devisi*, *E. curta*, *H. signata* or *V. annulata* (10 μl of 5 or 50 μg/ml) were added to wells in duplicate. Colour changes were monitored at 414 nm every 30 sec. for 10 min. on a Fusion α plate reader (PerkinElmer, Waltham, MA, USA). PLA_2 activity was expressed as micromoles of phosphatidylcholine hydrolysed per minute per milligram of enzyme.

Procoagulation assay. Cryptophis boschmai, *D. devisi*, *E. curta, V. annulata* (15.6–1000 μg/ml) or *H. signata* (0.156–10 μg/ml) venoms were placed in a 96-well microtitre plate (100 μl/well). Tris-buffered saline (TBS) (100 μl/well) was used as a control. Frozen plasma was allowed to thaw and then warmed to 37°C. $CaCl_2$ (75 μl/ml of plasma) was added to the plasma and then immediately added to each well (100 μl/well). Kinetic absorbance was measured at 37°C every 30 sec. for 30 min. at 340 nm on a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Analysis of results and statistics. Statistics were performed using GraphPad PrismTM (v5.03; GraphPad Software, La Jolla, CA, USA). For neurotoxicity experiments, twitch height was measured at regular intervals and responses expressed as a percentage of the twitch height immediately preceding administration of venom. Student's unpaired *t*-test was used to determine statistical significance of concentration dependency and antivenom efficacy. Paired *t*-tests were used to determine the statistical significance of the post-venom responses to exogenous agonists. For the anaesthetized rat experiments, blood pressure recorded from the carotid artery was converted to mean arterial pressure (MAP), where MAP = diastolic pressure + ⅓ pulse pressure (where pulse pressure equals systolic pressure minus diastolic pressure). One-way ANOVA with a Bonferroni-corrected *post hoc* test was used to determine statistical significance of the cardiovascular effects of venoms compared with saline.

All animal experiments were approved by the Monash University animal ethics committee.

Results

Mass spectrometry.

MALDI analysis of each venom, over the range of 4–40 kDa, showed them to have the greatest intensity (in this mode of analysis) for components at molecular weights in the weight range for three finger toxins (3FTx; 6–8 kDa) and PLA_2 (12–14 kDA) [9] (fig. 1). Only *C. boschmai* venom had components with molecular weights greater than 25 kDA. 3FTx were dominant in *D. devisii, E. curta* and *V. annulata*, while PLA_2 dominated in *C. boschmai* and *H. signata* both still possessed appreciable amounts of 3FTx. Peaks were evident at lesser intensity for other toxin types previous identified as present in Australian elapid venoms [9,10]: natriuretic peptide (3–4 kDa), kunitz peptide (5–6 kDa), lectin (14–19 kDa), NGF (18–19 kDa), vespryn (18–19 kDa), CRiSP (23–25 kDa) and kallikrein-type serine protease (25–28 kDA).

Chick biventer cervicis nerve-muscle preparation.

All venoms (10 or 30 μg/ml, n = 4–5) abolished indirect twitches in the chick biventer preparation (fig. 2). In addition, all venoms significantly attenuated contractile responses to the nicotinic agonists ACh and CCh, without significantly affecting the response to KCl (fig. 3). The time taken to produce neuromuscular blockade, as demonstrated by inhibition of twitches, was quantified by determination of t_{90} values. These showed the following rank order of potency at 10 μg/ml: *H. signata* ≥ *D. devisi* ≥ *V. annulata* = *E. curta* > *C. boschmai* (table 1). The inhibition of twitch height by the venoms (10 μg/ml) was significantly delayed by the prior addition of CSL polyvalent antivenom (5 U/ml) (fig. 2). Antivenom also

© 2012 The Authors
Basic & Clinical Pharmacology & Toxicology © 2012 Nordic Pharmacological Society

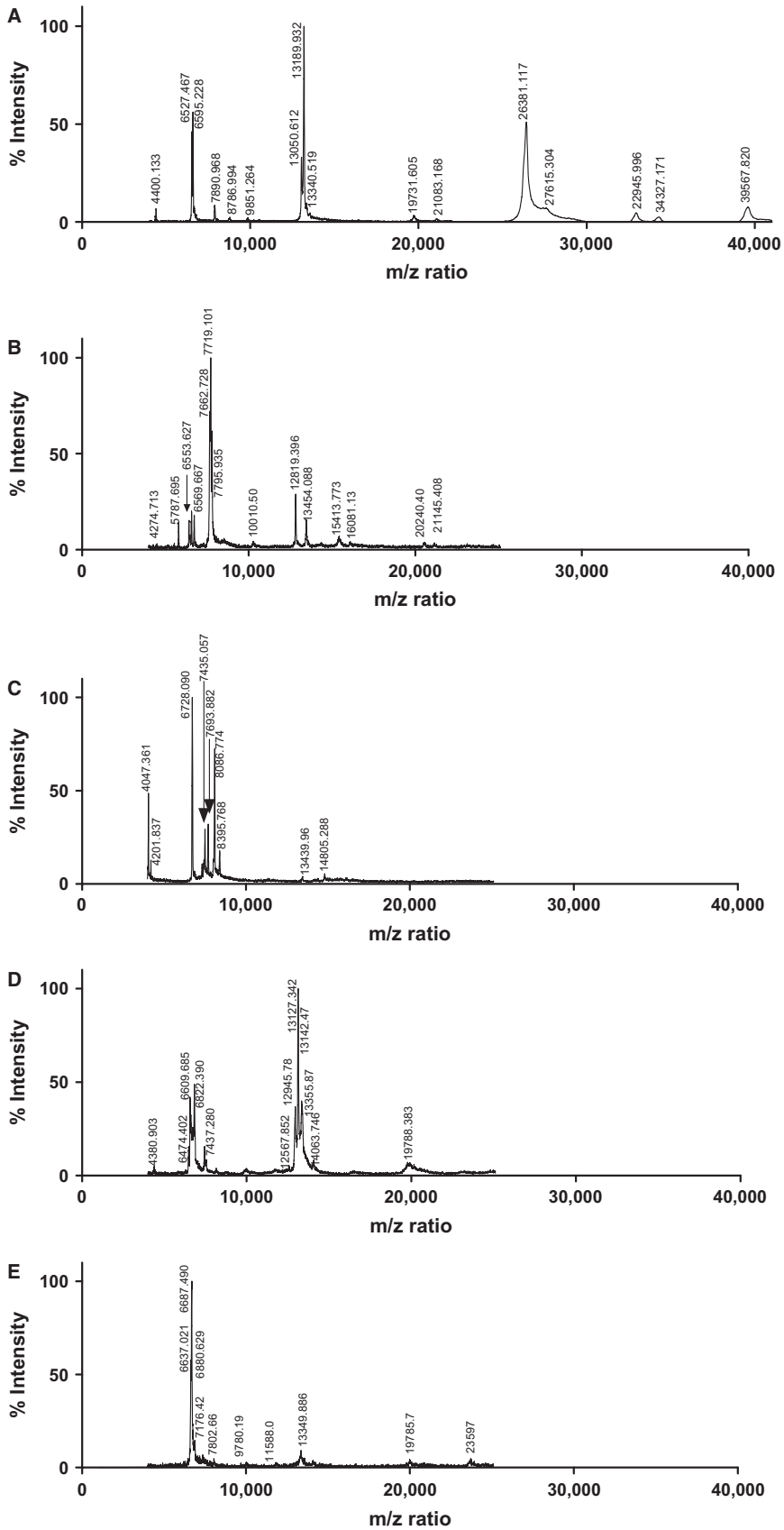

Fig. 1. MALDI mass spectrometry results, shown over the range 4–40 kDa, for (A) *Cryptophis boschmai*, (B) *Denisonia devisi*, (C) *Echiopsis curta*, (D) *Hemiaspis signata* and (E) *Vermicella annulata* venoms.

© 2012 The Authors
Basic & Clinical Pharmacology & Toxicology © 2012 Nordic Pharmacological Society

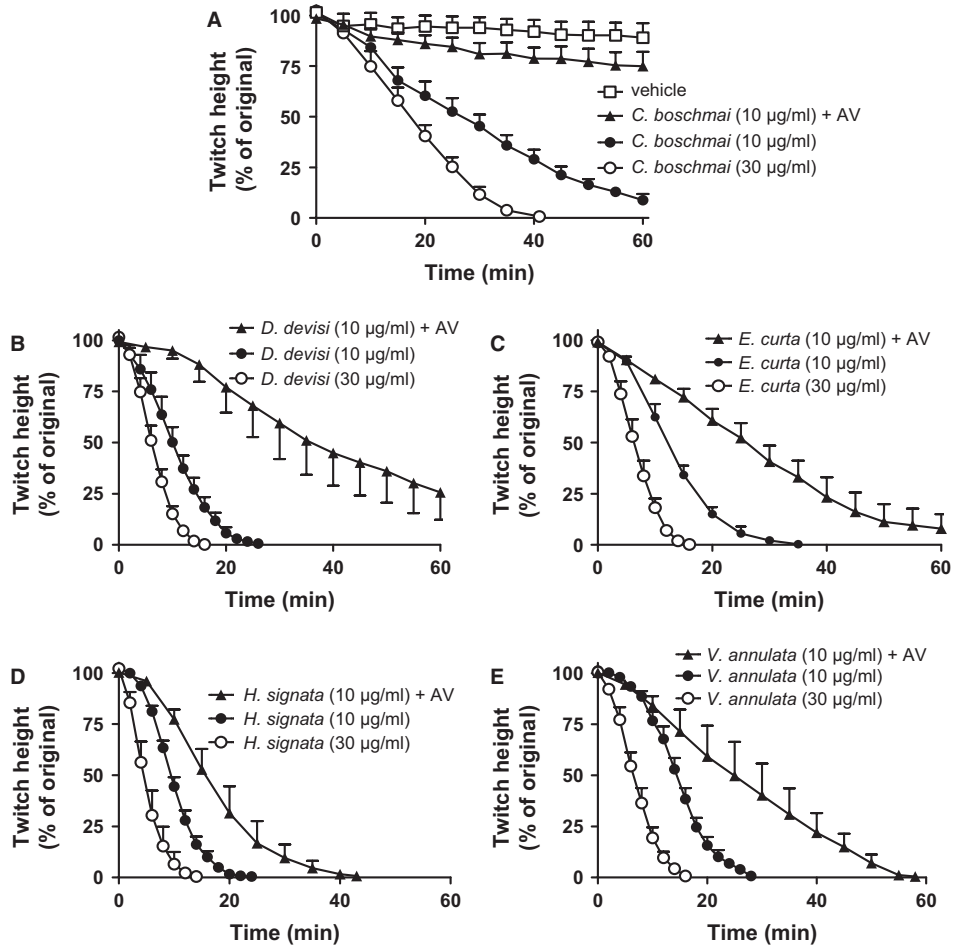

Fig. 2. The effects of (A) *Cryptophis boschmai*, (B) *Denisonia devisi*, (C) *Echiopsis curta*, (D) *Hemiaspis signata* or (E) *Vermicella annulata* venom (10 or 30 µg/ml; n = 4–5) or venom (10 µg/ml) in the presence of polyvalent antivenom (5 U/ml; n = 4–6) on indirect twitches of the chick biventer cervicis nerve-muscle preparation.

prevented the inhibition of contractile responses to ACh and CCh by *C. boschmai* venom (10 µg/ml) (fig. 3A) but not by the other venoms used (fig. 3B–B–B–B–E).

Cardiovascular effects in anesthetized rats.

Cryptophis boschmai and *D. devisi* venoms (60 µg/kg, i.v.) caused a significant decrease in the mean arterial pressure (MAP) of anaesthetized rats (fig. 4). This effect was transient with the MAP returning to pre-venom values within 15 min. *H. signata* (60 µg/kg, i.v.) venom produced a small but not statistically significant pressor response followed by a significant depressor response with MAP returning to pre-venom values within 15 min. (fig. 4). *V. annulata* and *E. curta* venoms (60 µg/kg, i.v.) had no significant effect on MAP, compared with saline injection (data not shown), in anaesthetized rats (fig. 4).

PLA_2 assay.

High PLA_2 activity was displayed by *H. signata* (620.8 µmol/min./mg), *D. devisi* (576.7 µmol/min./mg) and *C. boschmai* (344.6 µmol/min./mg) venoms, while much lower PLA_2 activity was for *V. annulata* (69.1 µmol/min./mg). *E. curta* displayed almost no PLA_2 activity (1.1 µmol/min./mg) (table 1).

Pro-coagulation assay.

The venoms of *C. boschmai*, *D. devisi*, *E. curta* and *V. annulata* all displayed extremely weak coagulopathic properties with MCC_5 (i.e. the minimum concentration required to begin clotting plasma within 5 min.) of 500, 1000, 125 and 250 µg/ml, respectively. In contrast, *H. signata* displayed moderate activity with a MCC_5 of 1.25 µg/ml (table 1).

Discussion

Most research into Australian snake venoms has been focussed on the seven genera of greatest clinical importance: *Acanthophis* (death adders), *Hoplocephalus* (including Stephen's banded snake), *Notechis* (tiger snakes), *Oxyuranus* (taipans), *Pseudechis* (Black snakes), *Pseudonaja* (Brown snakes) and *Tropidechis* (Rough scale snake). The venoms of snakes from these genera contain well-characterized neurotoxins, myotoxins and coagulant toxins that cause significant clinical effects in human

© 2012 The Authors
Basic & Clinical Pharmacology & Toxicology © 2012 Nordic Pharmacological Society

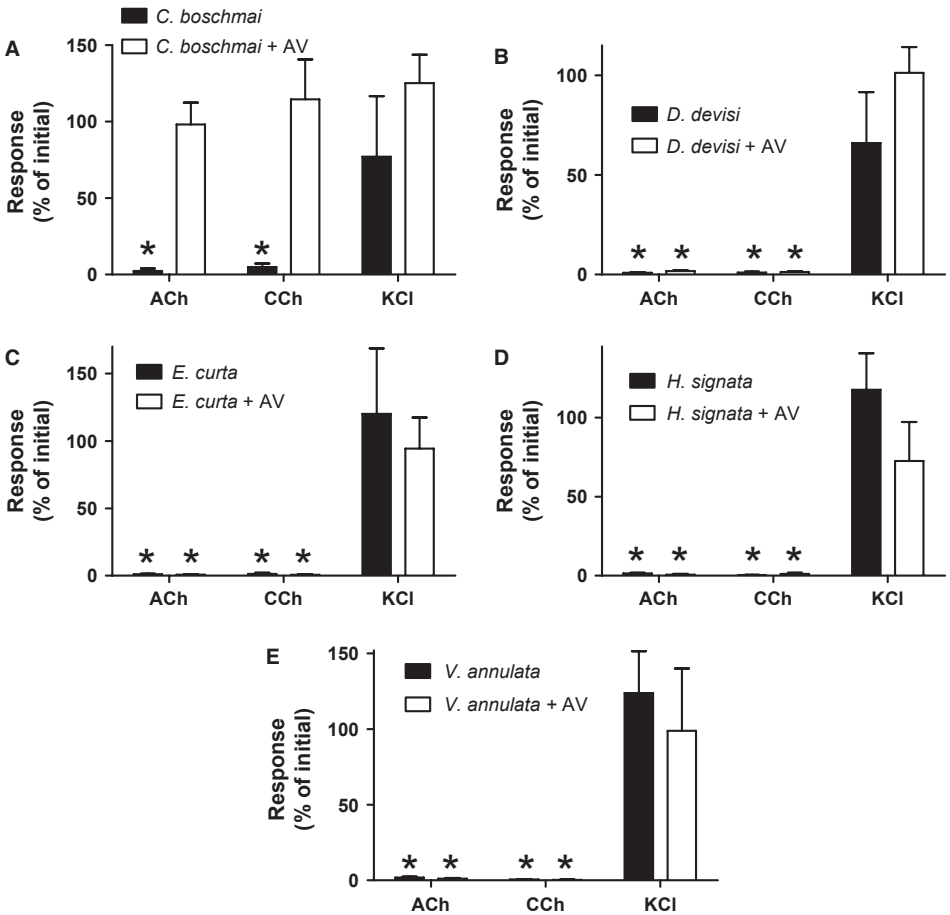

Fig. 3. The effects of (A) *Cryptophis boschmai*, (B) *Denisonia devisi*, (C) *Echiopsis curta*, (D) *Hemiaspis signata* or (E) *Vermicella annulata* venom (10 μg/ml) in the presence or absence of polyvalent antivenom (5 U/ml) on contractile responses to acetylcholine (1 mM), carbachol (20 μM) or KCl (40 mM) in the chick biventer cervicis nerve-muscle preparation (n = 4–6). *$p < 0.05$, significantly different from pre-venom response, paired *t*-test.

Table 1.
A comparison of the neurotoxic, PLA_2 and coagulopathic activities of the snake venoms.

Common name	Species	t_{90} @ 10 μg/ml (min.)	PLA_2 activity (μmol/min./mg)	MMC_5 (μg/ml)
Common death adder (NSW)	*Acanthophis antarcticus*	10.2 ± 0.6 [24]	ND	ND
Curl snake	*Suta suta*	13 ± 1 [13]	ND	ND
Marsh snake	***Hemiaspis signata***	**15.8 ± 1.1**	**620.8**	**1.25**
De Vi's banded snake	***Denisonia devisi***	**18.3 ± 1.4**	**576.7**	**1000**
Tiger snake	*Notechis scutatus*	21.7 ± 1.6 [25]	ND	ND
Bandy-bandy	***Vermicella annulata***	**22.0 ± 0.9**	**69.1**	**250**
Bardick snake	***Echiopsis curta***	**22.3 ± 1.8**	**1.1**	**125**
Australian copperhead	*Austrelaps superbus*	26 ± 3 [25]	ND	ND
Inland taipan	*Oxyuranus microlepidotus*	27 ± 3 [26]	ND	ND
Carpentaria snake	***Cryptophis boschmai***	**59.0 ± 1.0**	**344.6**	**500**
Black whip snake	*Demansia papuensis*	83.5 ± 8.2 [12]	ND	ND
Brown-headed snake	*Glyphodon tristis*	99.7 ± 7.7 [11]	ND	ND

ND, not determined as part of this study.
NB: the neurotoxic activity of some other Australian snake venoms has been included as a comparison.
Venoms from present study in bold.

beings. Snakes from most of the other genera of Australian elapids are thought to be of limited clinical importance owing to the rarity of bites and/or the lack of clinical effects following envenoming. The study of these venoms is limited by the difficulties associated with obtaining sufficient quantity of venom from these snakes because they are rare. However, we have

© 2012 The Authors
Basic & Clinical Pharmacology & Toxicology © 2012 Nordic Pharmacological Society

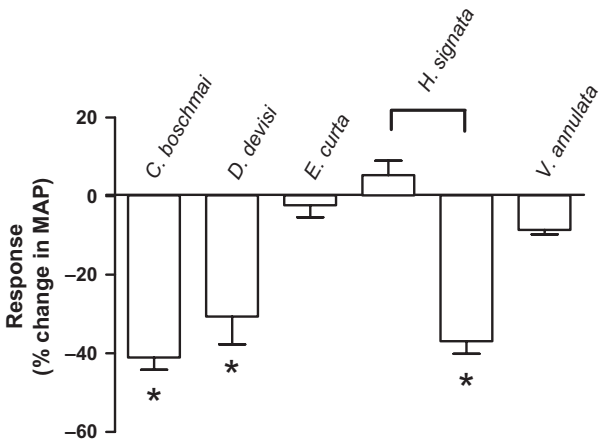

Fig. 4. The effect of *Cryptophis boschmai*, *Denisonia devisi*, *Echiopsis curta*, *Hemiaspis signata* or *Vermicella annulata* venom (60 μg/kg i.v., n = 4–6) on the mean arterial pressure of anaesthetized rats. *$p < 0.05$, significantly different from saline, one-way ANOVA.

started to investigate the venoms of Australian elapids not belonging to the seven main genera including *Suta, Demansia* and *Glyphodon* [9,11–13]. The present study is the first to examine the pharmacological and biochemical properties of the venoms of *C. boschmai*, *D. devisi*, *E. curta*, *H. signata* and *V. annulata*.

Mass spectrometry data indicated the presence of components with molecular weights in the range expected of 3FTx (i.e. 6–8 kDa). These toxins, which are also known as α-neurotoxins, act as reversible/pseudo-irreversible antagonists at the skeletal muscle nicotinic receptor. Their presence in the venoms is supported by *in vitro* data which indicated that all these venoms displayed post-synaptic neurotoxic activity. In the chick biventer cervicis preparation, 4 (i.e. *D. devisi*, *E. curta*, *H. signata* and *V. annulata*) of the five snake venoms were highly neurotoxic with t_{90} values comparable with venoms from the tiger snake, copperhead and inland taipan. This is likely to be mainly post-synaptic neurotoxicity given that responses to exogenous nicotinic receptor agonists were also inhibited. Interestingly, the venom of *C. boschmai* displayed much weaker neurotoxicity, which may correlate with the different MALDI-TOF profile for this venom, which indicated a wider spread of components and, perhaps, the presence of less 3FTx.

These data raise the issue as to why marked neurotoxicity is not seen in envenomed human beings. Contributing factors may include low venom yield, small fang size or species differences in the selectivity of 3FTx for skeletal muscle nicotinic receptors. Indeed, based on the collection of venom for the current study, venom yields from individual 'milkings' of *C. boschmai* and *H. signata* are in the range of 1–2 mg while larger specimens of the other species may yield up to 10 mg (Fry, personal communication). These yields are certainly at the lower end of the spectrum for Australian elapids [14]. Alternatively, the lack of neurotoxicity in envenomed human beings may be due to a similar phenomenon as the 'Brown snake paradox' whereby human envenoming by Brown snakes is rarely associated with neurotoxicity despite the presence of post- and pre-synpatic neurotoxins in the venom. Investigating this phenomenon, we have recently shown that Brown snake (*Pseudonaja textilis*) venom is highly potent in the chick biventer cervicis preparation, owing to the activity of 3FTx, but the presynaptic neurotoxin present in the venom (i.e. textilotoxin) has low potency and does not contribute significantly to the activity in this preparation [15]. This questions the importance of post-synaptic neurotoxins in human envenoming, in particular, short-chain α-neurotoxins. This observation is supported by earlier work indicating that the nicotinic receptors in human skeletal muscle were relatively resistant to the 3FTx in the venom of the Australian elapid *Pseudechis colletti* (Collett's snake) [16]. Interestingly, neurotoxicity also appears to be absent following human envenoming by this species [17], reinforcing that caution must be employed when extrapolating the results of experimental data to the clinical situation.

Given the rapid onset of the effects of post-synaptic neurotoxins, the presence of slower acting pre-synaptic neurotoxins in these venoms could not be determined. The mass spectrometry data indicated the presence of components in the molecular weight range of PLA_2 (i.e. 12–14 kDa), which may have been pre-synaptic neurotoxins. However, this would require fractionation of the venoms to further investigate components within the appropriate molecular weight range.

We also examined the efficacy of CSL polyvalent snake antivenom against the *in vitro* neurotoxicity of the different snake venoms. The concentration of antivenom used (i.e. 5 U/ml) prevented the neurotoxic effects of Tiger snake (*Notechis scutatus*) venom (10 μg/ml; data not shown), confirming the efficacy of the antivenom that is raised against venoms from *Notechis*, *Pseudechis*, *Pseudonaja*, *Acanthophis* and *Oxyuranus* sp. Interestingly, polyvalent antivenom attenuated, but did not prevent, the neurotoxicity produced by all venoms with greatest efficacy against the venom of *C. boschmai*, which we identified as the venom with the weakest activity. These data may indicate that there is some cross-neutralization between toxins in these elapid venoms and the neurotoxins in the genera that the antivenom is raised against [18].

The cardiovascular effects of the venoms, as indicated by changes in blood pressure of anaesthetized rats, were relatively weak or absent. After preliminary screening, a dose of 60 μg/kg (i.v.) was chosen for comparison between the venoms. Three of the venoms (i.e. *C. boschmai*, *D. devisi* and *H. signata)* caused a rapid hypotensive effect which may indicate the presence of direct or indirectly acting vasodilator components in the venom or components which act on cardiac or vascular tissue, consistent with the presence of molecular weights indicative of natriuretic peptides isolated from other Australian venoms and shown to be potently hypotensive [19]. The presence of molecular weight components in the range consistent with kallikrein-type serine proteases may also contribute to the hypotensive response induced by *C. boschmai* venom. However, the dose of the venoms required to produce a fall in blood pressure was relatively high when compared to our previous work with taipan or box jellyfish (*Chironex fleckeri*) venoms, which both produce cardiovascular collapse at a much lower doses (e.g. 10 μg/kg, i.v.; 20,21).

© 2012 The Authors
Basic & Clinical Pharmacology & Toxicology © 2012 Nordic Pharmacological Society

However, an interesting relationship was observed between PLA_2 activity and cardiovascular effects. Consistent with the mass spectrometry results of components in the known molecular weight range for PLA_2 toxins, *C. boschmai*, *D. devisi* and *H. signata* all expressed high PLA_2 activity and also produced significant changed in MAP in anaesthetized rats. Also consistent with only low level of PLA_2 weight components, *V. annulata* expressed a lesser amount of PLA_2 activity and produced a small, albeit insignificant, change in MAP. Similarly, *E. curta* expressed very little PLA_2 activity and did not cause a change in MAP. It is possible that the PLA_2 enzymes, or the by-products of their activity, are responsible for the cardiovascular effects.

Only *H. signata* had significant procoagulant activity compared with the other four venoms. However, it was still much less potent than the other Australasian elapid venoms (e.g. MCC_5 0.0005–0.02 μg/ml; 22,23). This is consistent with *H. signata* causing very mild clinical coagulopathy in only one previously reported case [5].

In conclusion, this study provides the first characterization of venoms from *C. boschmai*, *D. devisi*, *E. curta*, *H. signata* and *V. annulata*. It provides some explanation as to why bites by these snakes have not been responsible for severe envenoming. Although they cause significant neurotoxicity in an avian muscle, this may be only due to post-synaptic neurotoxins and not relevant in human envenoming. Only *H. signata* venom causes mild coagulopathy, which is consistent with clinical reports.

Acknowledgements

GKI was funded by an NHMRC Clinical Career Development Award (ID300785). BGF was funded by the Australia & Pacific Science Foundation, the Australian Research Council (with WCH) and the University of Queensland.

References

1 Wilson S, Swan G. Venomous land snakes. In: Savage A (ed.). A Complete Guide to Reptiles of Australia, 1st edn. New Holland, Sydney, NSW, 2003;392–447.
2 Fry BG. Structure-function properties of venom components from Australian elapids. Toxicon 1999;**37**:11–32.
3 Broad AJ, Sutherland SK, Coulter AR. The lethality in mice of dangerous Australian and other snake venom. Toxicon 1979;**17**:661–4.
4 Isbister GK. Snake bite: a current approach to management. Aust Prescr 2006;**29**:125–9.
5 Isbister GK, Dawson AH, Whyte IM. Two cases of bites by the black-bellied swamp snake (*Hemiaspis signata*). Toxicon 2002;**40**:317–9.
6 Swindells KL, Russel NJ, Angles JM, Foster SF. Four cases of snake envenomation responsive to death adder antivenom. Aust Vet J 2006;**84**:22–9.
7 Marshall LR. Positive cross reactivity of Bardick snake (*Notechis curtus*) venom with Death adder (*Acanthophis antarcticus*) antivenom and negative reactivity with Tiger (*Notechis scutatus*) antivenom. Copeia 1985;**1**:257–9.
8 Harvey AL, Barfaraz A, Thomson E, Faiz A, Preston S, Harris JB. Screening of snake venoms for neurotoxic and myotoxic effects using simple *in vitro* preparations from rodents and chicks. Toxicon 1994;**32**:257–65.
9 Fry BG, Wüster W, Ramjan SFR, Jackson T, Martelli P, Kini RM. Analysis of Colubroidea snake venoms by liquid chromatography with mass spectrometry: evolutionary and toxinology implications. Rapid Comm Mass Spec 2003;**17**:2047–62.
10 Birrell GW, Earl ST, Wallis TP, Masci PP, de Jersey J, Gorman JJ *et al.* The diversity of bioactive proteins in Australian snake venoms. Mol Cell Prot 2007;**6**:973–86.
11 Kuruppu S, Fry BG, Hodgson WC. Presynaptic neuromuscular activity of venom from the brown-headed snake (*Glyphodon tristis*). Toxicon 2005;**45**:383–8.
12 Kuruppu S, Fry BG, Hodgson WC. *In vitro* neurotoxic and myotoxic effects of the venom from the black whip snake (*Demansia papuensis*). Clin Exp Pharmacol Physiol 2006;**33**:364–8.
13 Kuruppu S, Robinson S, Fry BG, Hodgson WC. The *in vitro* neurotoxic and myotoxic effects of the venom from the *Suta* genus (curl snakes) of elapid snakes. Basic Clin Pharmacol Toxicol 2007; **101**:407–10.
14 Mirtschin PJ, Dunstan N, Hough B, Hamilton E, Klein S, Lucas J *et al.* Venom yields from Australian and some other species of snakes. Ecotoxicology 2006;**15**:531–8.
15 Barber CM, Isbister GK, Hodgson WC. Solving the 'Brown snake paradox': in vitro characterisation of Australasian snake presynaptic neurotoxin activity. Toxicol Lett 2012;**210**:318–23.
16 Hart A, Scott-Davey T, Harris J. Venom of Collett's snake (*Pseudechis colletti*) blocks the binding of α-bungarotoxin to acetylcholine receptors at chick but not human neuromuscular junctions: a histochemical study. Toxicon 2008;**52**:647–50.
17 Isbister GK, Hooper MR, Dowsett R, Maw G, Murray L, White J. Collett's snake (*Pseudechis colletti*) envenoming in snake handlers. QMJ 2006;**99**:109–15.
18 Isbister GK, O'Leary MA, Hagan J, Nichols K, Jacoby T, Davern K *et al.* Cross-neutralisation of Australian brown snake, taipan and death adder venoms by monovalent antibodies. Vaccine 2010;**28**:798 –802.
19 Fry BG, Wickramaratana J, Lemme S, Beuve A, Garbers D, Hodgson WC *et al.* Novel natriuretic peptides from the venom of the inland taipan (*Oxyuranus microlepidotus*): isolation, chemical and biological characterization. Biochem Biophys Res Comm 2005;**327**:1011–5.
20 Crachi MT, Hammer LW, Hodgson WC. A pharmacological examination of venom from the Papuan taipan (*Oxyuranus scutellatus canni*). Toxicon 1999;**37**:1721–34.
21 Ramasamy S, Isbister GK, Seymour JE, Hodgson WC. Pharmacologically distinct cardiovascular effects of box jellyfish (*Chironex fleckeri*) venom and a tentacle-only extract in rats. Toxicol Lett 2005;**155**:219–26.
22 Isbister GK, Woods D, Alley S, O'Leary MA, Seldon M, Lincz LF. Endogenous thrombin potential as a novel method for the characterization of procoagulant snake venoms and the efficacy of antivenom. Toxicon 2010;**56**:75–85.
23 Isbister GK, Scorgie FE, O'Leary MA, Seldon M, Brown SGA, Lincz LF. Factor deficiencies in venom-induced consumption coagulopathy resulting from Australian elapid envenomation: Australian Snakebite Project (ASP-10). J Thromb Haem 2010; **8**:2504–13.
24 Fry BG, Wickramaratna JC, Jones A, Alewood PF, Hodgson WC. Species and regional variations in the effectiveness of antivenom against the in vitro neurotoxicity of death adder (*Acanthophis*) venoms. Toxicol Appl Pharmacol 2001;**175**:140–8.
25 Hodgson WC, Eriksson CO, Alewood PF, Fry BG. Comparison of the *in vitro* neuromuscular activity of venom from three Australian snakes (*Hoplocephalus stephensi*, *Austrelaps superbus* and *Notechis scutatus*): efficacy of tiger snake antivenom. Clin Exp Pharmacol Physiol 2003;**30**:127–32.
26 Crachi MT, Hammer LW, Hodgson WC. The effects of antivenom on the in vitro neurotoxicity of venoms from the taipans *Oxyuranus scutellatus, Oxyuranus microlepidotus* and *Oxyuranus scutellatus canni*. Toxicon 1999;**37**:1171–8.

© *2012 The Authors*
Basic & Clinical Pharmacology & Toxicology © 2012 Nordic Pharmacological Society