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The effects of antivenom on the in vitro neurotoxicity of venoms from the taipans *Oxyuranus scutellatus*, *Oxyuranus microlepidotus* and *Oxyuranus scutellatus canni*

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Abstract

The venoms of the inland (*Oxyuranus microlepidotus*), coastal (*O. scutellatus*) and Papuan (*O. s. canni*) taipans are among the most potent in the world. The present study compared the in vitro neurotoxic effects of these venoms and the protective effects of taipan antivenom. Venom (10 μg/ml) from all three snakes abolished nerve-mediated twitches of the chick biventer cervicis muscle preparation with the following rank order of potency (based on the time taken to inhibit 90% of the twitch response; t_{90}): *O. microlepidotus* (27 ± 3 min) > *O. scutellatus* (42 ± 3 min) = *O. S. canni* (48 ± 5 min). This inhibitory effect of all three venoms was primarily postsynaptic in origin as evidenced by the inhibition of responses to exogenous acetylcholine (ACh; 1 mM) and carbachol (CCh; 20 μM), but not potassium chloride (40 mM). In contrast, the presynaptic neurotoxins taipoxin (3 μg/ml) and paradoxin (3 μg/ml) abolished nerve-mediated twitches without producing a significant effect on contractile responses to exogenous agonists. Prior incubation of the tissue with taipan antivenom (1 unit/ml for 10 min) markedly attenuated the inhibitory effects of taipoxin (3 μg/ml) and paradoxin (3 μg/ml), as well as *O. scutellatus* (10 μg/ml) and *O. s. canni* (10 μg/ml) venom. However, in the presence of antivenom, *O. microlepidotus* venom (10 μg/ml) still abolished nerve-mediated twitches and responses to ACh and CCh. The results of the current study indicate that taipan antivenom, raised against *O. scutellatus venom*, is effective, in vitro, against the neurotoxic effects of venom from the Papuan and coastal taipans, as well as the presynaptic effects of venom from the inland taipan.

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However, the antivenom appears less effective against the postsynaptic effects of the latter. It is possible that inland taipan venom contains a component not neutralised by the antivenom which may contribute to the extreme potency of this venom. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

With murine LD_{50} (s.c. in 0.1% bovine serum albumin) values of 0.01 mg/kg (Broad et al., 1979), 0.064 mg/kg (Broad et al., 1979) and 0.0505 mg/kg (Sutherland, 1983) the Australian inland (*Oxyuranus microlepidotus*), Australian coastal (*O. scutellatus*) and Papuan (*O. s. canni*) taipans, respectively, are among the most venomous terrestrial snakes in the world. Systemic neurotoxicity, often resulting in respiratory paralysis, is a common feature in envenomed patients (Campbell, 1967; Connolly et al., 1995; Trevett et al., 1995a). The mainstay treatment for envenomation by these snakes is administration of CSL taipan antivenom which is extracted from horses immunised against venom of *O. scutellatus* (Lalloo et al., 1995; Trevett et al., 1995b; Warrell, 1996; White, 1996). However, there has been debate regarding the efficacy of taipan antivenom against *O. s. canni* venom (Trevett et al., 1995b; Lalloo et al., 1995) and there are only a few reported cases of envenomation, and subsequent antivenom administration, by *O. microlepidotus* (Mirtschin et al., 1984; Smith, 1992). The chick biventer cervicis muscle has been previously used to examine the neutralising potency of antivenoms against a range of venoms (Barfaraz and Harvey, 1994). The aim of the current study was to compare the in vitro neurotoxicity of the venoms from the three snakes and the efficacy of taipan antivenom against the neurotoxic action. In order to further elucidate the importance of the presynaptic neurotoxins isolated from the Australian taipans, we also examined the effect of the antivenom against the neurotoxic actions of taipoxin (*O. scutellatus;* Fohlman et al., 1976) and paradoxin (*O. microlepidotus;* Fohlman, 1979).

2. Materials and methods

2.1. Venoms and toxins

Freeze dried snake venoms, paradoxin and taipoxin were obtained from Peter Mirtschin (Venom Supplies, Tanunda, South Australia). *O. scutellatus*, *O. microlepidotus* and *O. s. canni* venoms were obtained from pools of 8, 6 and 8 snakes, respectively. Venom and toxins were dissolved in 0.1% bovine serum albumin (BSA). Venoms, toxins and stock solutions were stored at -20°C until required.

2.2. Chick biventer cervicis preparation

Chick isolated biventer cervicis muscle preparations were set up as described previously (Ginsborg and Warriner, 1960). Preparations were mounted under 1 g resting tension in 10 ml organ baths containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$; $NaHCO_3$, 25 and glucose, 11.1. The solution was maintained at 34°C and bubbled with 95% O_2 and 5% CO_2. Motor nerves were stimulated every 10 s (0.2 ms duration, supramaximal voltage) using a Grass S88 stimulator. Nerve stimulation was confirmed using *d*-tubocurarine (10 μM). Contractile responses to submaximal concentrations of acetylcholine (ACh, 1 mM for 30 s), carbachol (20 μM for 60 s) and potassium chloride (KCl, 40 mM for 30 s) (Harvey et al., 1994) were obtained in the absence of nerve stimulation prior to the addition of venom or antivenom, and again at the conclusion of the experiment. Antivenom (1 unit/ml) was administered 10 min prior (Barfaraz and Harvey, 1994) to the venom or neurotoxin where indicated.

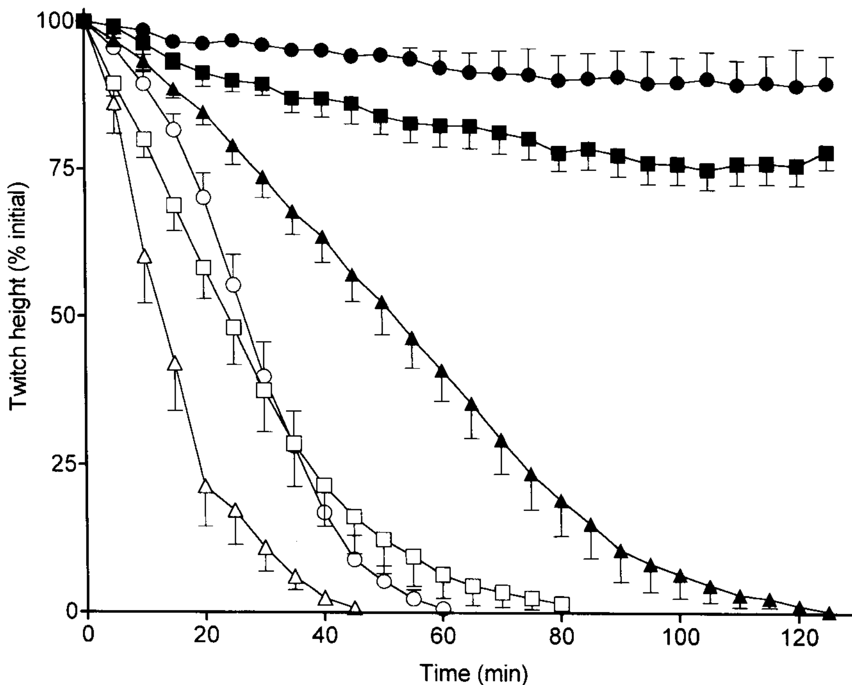

Fig. 1. The effect of *Oxyuranus microlepidotus* (inland taipan) (without antivenom, open triangle, $n = 11$; with antivenom, filled triangle, $n = 6$), *O. scutellatus* (coastal taipan) (without antivenom, open circle, $n = 10$; with antivenom, filled circle, $n = 5$) or *O. s. canni* (Papuan taipan) (without antivenom, open square, $n = 11$; with antivenom, filled square, $n = 8$) venoms (10 μg/ml) on nerve-mediated twitches of the chick biventer cervicis preparation.

2.3. Drugs

The following were purchased from Sigma–Aldrich Pty Ltd: acetylcholine chloride, bovine serum albumin, *d*-tubocurarine chloride and carbachol. All drugs were made up in distilled water. Taipan antivenom (Batch number 0548-04801) was obtained from CSL Ltd (Parkville, Victoria, Australia).

2.4. Data analysis and statistics

Responses to venoms/toxins on nerve-mediated twitch responses were expressed as percentage inhibition of basal twitches obtained prior to the addition of venoms/toxins. Contractile responses to agonists in the presence of venoms/toxins were expressed as a percentage of the response obtained prior to the addition of venoms/toxins. Differences between venoms/toxins were compared using 1-way ANOVA with $P < 0.05$ indicating statistical significance.

3. Results

3.1. Chick biventer cervicis

All three venoms (10 μg/ml) abolished nerve-mediated twitch responses in the chick biventer cervicis muscle preparation (Fig. 1). However, time to 90% inhibition (t_{90}) was significantly less for *O. microlepidotus* venom (27 ± 3 min) compared to *O. scutellatus* and *O. s. canni* (42 ± 3 min and 48 ± 5 min, respectively; $P < 0.05$; $n = 10$–11) venoms. In addition, all three venoms (10 μg/ml) significantly inhibited contractile responses to ACh (1 mM) and CCh (20 μM) while not significantly affecting responses to potassium chloride (KCl, 40 mM) (Fig. 2a). Prior incubation (10 min) of the tissues with antivenom (1 unit/ml) markedly attenuated the inhibition of nerve-mediated twitch responses (Fig. 1) and agonist-induced contractile responses (Fig. 2b) to *O. scutellatus* and *O. s. canni* venom. In contrast, antivenom (1 unit/ml) significantly delayed (t_{90} 90 ± 3 min vs 27 ± 3 min; $P < 0.05$) but did not prevent the effect of *O. microlepidotus* venom upon nerve-mediated twitches (Fig. 1). Similarly, in the presence of antivenom (1 unit/ml), responses to ACh and CCh were still significantly inhibited by *O. microlepidotus* venom (Fig. 2b). Antivenom (1 unit/ml) alone had no significant effect on twitch height (data not shown).

The presynaptic neurotoxins, taipoxin (3 μg/ml) (t_{90} 126 ± 12 min; $n = 4$) and paradoxin (3 μg/ml) (t_{90} 129 ± 8 min; $n = 7$), abolished nerve-mediated twitch responses of the chick biventer cervicis preparation (Fig. 3). There was no significant difference between the inhibitory effects of the toxins. However, the inhibitory effect of the toxins was significantly slower compared to their respective whole venoms ($P < 0.05$). Prior incubation of the tissues with antivenom (1 unit/ml) almost abolished the neurotoxic effect of the toxins (Fig. 3). Taipoxin (3 μg/ml) or paradoxin (3 μg/ml), either in the presence (1 unit/ml) or absence of

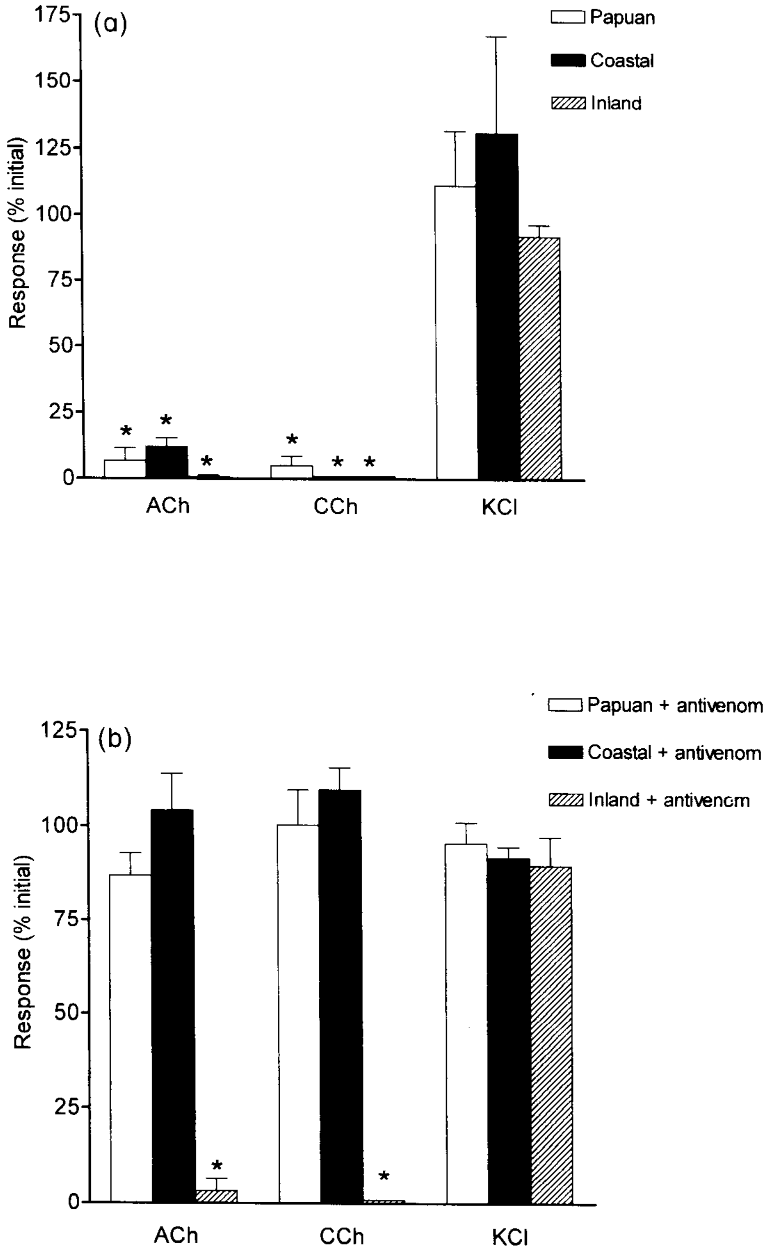

Fig. 2. (a) The effect of venoms (10 μg/ml) on responses of the chick biventer cervicis preparation to acetylcholine (ACh, 1 mM), carbachol (CCh, 20 μM) or potassium chloride (KCl, 40 mM) ($^*P < 0.05$ compared to initial response); (b) the effect of venoms, in the presence of antivenom, on responses of the chick biventer cervicis preparation to ACh (1 mM), CCh (20 μM) or KCl (40 mM) ($^*P < 0.05$ compared to initial response).

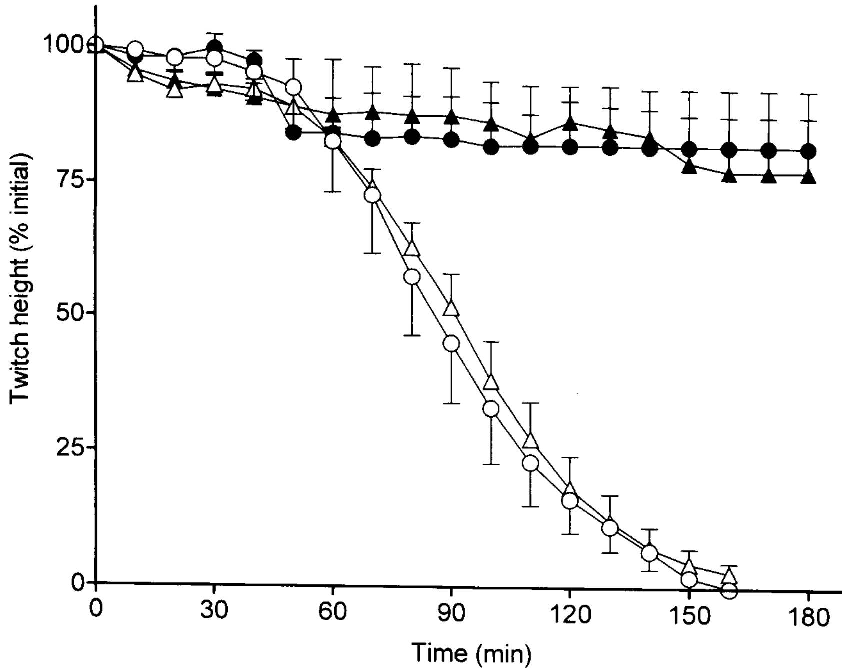

Fig. 3. The effect of taipoxin (3 μg/ml) (without antivenom, open circle, $n=4$; with antivenom, filled circle, $n=3$) or paradoxin (3 μg/ml) (without antivenom, open triangle, $n=7$; with antivenom, filled triangle, $n=5$) on nerve-mediated twitches of the chick biventer cervicis preparation.

antivenom, had no effect upon contractile responses elicited by ACh, CCh or KCl (data not shown).

4. Discussion

We have previously reported that the venoms of the Papuan (Crachi et al., 1999) and Australian inland (Hodgson and Rowan, 1997) taipans produce postsynaptic blockade of nerve-mediated twitches of skeletal muscle preparations in vitro. In addition, postsynaptic neurotoxins have been isolated from the venom of the Australian coastal taipan (Zamudio et al., 1996). However, the neurotoxic effects of these venoms have not been compared. The results of the current study confirm that the venoms of the taipans *O. microlepidotus*, *O. scutellatus* and *O. s. canni* display potent postsynaptic neurotoxic activity. Interestingly, *O. microlepidotus* venom was significantly more potent in producing postsynaptic blockade. This finding may partially explain the marked difference in previously reported LD_{50} values between *O. microlepidotus* venom and its counterparts (Broad et al., 1979; Sutherland, 1983). In contrast, the timeframe of the

presynaptic neurotoxic effects of paradoxin and taipoxin is almost identical supporting their close structural similarities (Fohlman, 1979).

It has been reported that the longer the delay between envenomation by *O. s. canni* and the administration of antivenom, the greater the risk and severity of neurotoxicity (Lalloo et al., 1995). Clinically, it has been shown that taipan antivenom given within 4 h of *O. s. canni* envenoming decreases the time for resolution of neurotoxicity. However, after this time period the efficacy of antivenom is markedly reduced (Trevett et al., 1995b). It has been suggested that CSL antivenom may be less effective in neutralising *O. s. canni* venom than *O. scutellatus* venom (Lalloo et al., 1995). The results of the current study show that antivenom raised against the coastal taipan is effective at preventing the in vitro neurotoxic effects of Papuan taipan venom but, despite delaying the neurotoxic activity, relatively ineffective against venom from the Australian inland taipan. This finding suggests that Papuan and coastal taipan venoms may be more closely related in composition than venom from the inland taipan. The latter venom may contain a neurotoxic component unique to this particular subspecies or, at least, a neurotoxic component which is not targeted by the available antivenom. Given that the antivenom effectively blocked the action of paradoxin in this preparation, it is unlikely that this neurotoxin is responsible for the greater toxicity of inland taipan venom. In addition, the antivenom did not abolish the inhibitory effect of inland taipan venom on contractile responses to skeletal muscle nicotinic receptor agonists, suggesting that this venom contains a postsynaptic neurotoxin which is markedly different from those contained in the venoms from the other two snakes. It is possible that this postsynaptic neurotoxin(s) contributes to the high lethality of this venom. These findings may warrant research into the development of an antivenom raised specifically against the venom of the Australian inland taipan.

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