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A pharmacological examination of venom from the Papuan taipan (*Oxyuranus scutellatus canni*)

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Abstract

The Papuan taipan (*Oxyuranus scutellatus canni*) is the third most venomous terrestrial snake in the world, however, little is know about the pharmacology of the venom. In the chick biventer cervicis muscle, venom (10 μg/ml) abolished nerve-mediated twitches (time to 90% inhibition (t_{90}) 44 ± 5 min, $n = 9$). This inhibition was unaffected by prior incubation of the venom with the phospholipase A inhibitor 4-bromophenacyl bromide (4-BPB; 0.72 mM) (t_{90} 48 ± 7 min, $n = 8$). The mouse phrenic nerve diaphragm preparation displayed greater sensitivity to venom (10 μg/ml) (t_{90} 25 ± 1 min, $n = 6$). In the chick biventer muscle, venom (10 μg/ml) significantly inhibited responses to acetylcholine (1 mM) and carbachol (20 μM), but not KCl (40 mM), indicating activity at post-synaptic nicotinic receptors. Venom (10 μg/ml) did not affect direct muscle stimulation. Venom (3–30 μg/ml) produced dose-dependant contractions of the guinea-pig ileum. Contractile responses were significantly inhibited by indomethacin (1 μM) or prior incubation of the venom with 4-BPB (0.72 mM) indicating involvement of a PLA component. In rat phenylephrine (0.3 μM) precontracted aortae, venom (3–100 μg/ml) produced endothelium-independent relaxation which was unaffected by prior incubation of venom (30 μg/ml) with 4-BPB (0.72 mM). In anaesthetised rats, 10 μg/kg (i.v.) venom produced rapid respiratory and cardiovascular collapse while 5 μg/kg (i.v.) venom produced only a small transient decrease in mean arterial blood pressure. Prior administration of 5 μg/kg (i.v.) venom enabled subsequent administration of 10 and 100 μg/kg (i.v.) venom without respiratory or cardiovascular collapse. Further work is required to identify specific toxins with the above pharmacological activity.

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1. Introduction

The Papuan (*Oxyuranus scutellatus canni*), Australian coastal (*O. s. scutellatus*) and Australian inland (*O. microlepidotus*) taipans constitute the most deadly group of terrestrial snakes in the world. The Papuan taipan is indigenous to southern, low-lying arid coastal regions of Irian Jaya and Papua New Guinea (Lalloo et al., 1995). With a mouse LD_{50} (s.c. in 0.1% bovine serum albumin) value of 0.0505 mg/kg (Sutherland, 1983), the Papuan taipan ranks as the third most venomous in the world (Broad et al., 1979). Despite being responsible for the vast majority of snake bites in the region (Lalloo et al., 1995; Trevett et al., 1995a), very little is known about the pharmacological properties of the venom from the Papuan taipan. Previous studies have focused largely on the clinical aspects and symptoms of envenomation (Connolly et al., 1995; Lalloo et al., 1995; Trevett et al., 1995a, b). In contrast, the pharmacological profiles of venom from the inland (Bell et al., 1998) and, in particular, coastal (Kamenskaya and Thesleff, 1974; Fohlman et al., 1976; Fohlman, 1979; Harris and Maltin, 1982; Lambeau et al., 1989; Possani et al., 1992; Zamudio et al., 1996) taipans have been reasonably well characterized.

Therefore, the aim of this study was to obtain a basic pharmacological profile of Papuan taipan venom in order to provide further insight into some of its components and their mechanism(s) of action.

2. Materials and methods

2.1. Venom preparation and storage

Freeze dried venom was obtained from Peter Mirtschin (Venom Supplies, Tanunda, South Australia). Venom was dissolved in 0.1% bovine serum albumin in saline (0.9% w/v) and stored at −20°C until required. Thawed solutions were kept on ice during experiments.

2.2. Chick biventer cervicis nerve-muscle preparation

Tissues were dissected from chicks (4–10 days old) as described by 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; $CaCl_2$, 2.5; $NaHCO_3$, 25 and glucose, 11.1. The solution was maintained at 34°C and bubbled with carbogen (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. Prior to commencing the experiment *d*-tubocurarine (10 μM) was added to confirm selective stimulation of nerves. Responses to nerve stimulation were then reestablished. Contractile responses to submaximal concentrations of acetylcholine (ACh, 1 mM for 30 s), carbachol (CCh, 20 μM for 60 s) and KCl (40 mM for

30 s) were obtained in the absence of nerve stimulation. The preparations were then equilibrated for at least 30 min with continuous nerve stimulation before addition of venom. In all experiments, venom was left in contact with the preparation until responses to nerve stimulation were abolished, or for 2 h if twitch blockade did not occur. ACh (1 mM for 30 s), CCh (20 μM for 60 s) and KCl (40 mM for 30 s) were then readded. Times taken to block the amplitude of nerve mediated twitches by 50% (t_{50}) and 90% (t_{90}) were calculated in order to provide a quantitative measure of neurotoxicity. In additional experiments, the cervicis biventer muscle was directly stimulated by blocking neuromuscular transmission using *d*-tubocurarine (dTC; 10 μM) and by placing the electrodes directly over the belly of the muscle. The preparation was then electrically stimulated every 10 s (2 ms duration, supramaximal voltage).

2.3. Mouse isolated phrenic nerve diaphragm

Hemidiaphragms with intact phrenic nerves were dissected from male mice as described by Bülbring (1946). Preparations were attached to tissue holders with in-built electrodes and mounted in 10 ml organ baths containing carbogenated Krebs solution at 37°C. The phrenic nerve was stimulated every 10 s (0.2 ms duration, supramaximal voltage) using a Grass S88 stimulator. Preparations were allowed to equilibrate for at least 30 min before the addition of venom.

2.4. Guinea-pig isolated ileum

Segments of ileum (2–3 cm) from male guinea-pigs were mounted (1 g resting tension) on tissue holders in 15 ml organ baths containing carbogenated Krebs solution at 37°C.

2.5. Rat isolated aortic rings

Aortic rings (5 mm in length) from male rats were mounted (10 g resting tension) between two stainless steel hooks as described previously (James and Hodgson, 1995). Where indicated, the endothelium was removed by gently rubbing the intimal surface with thin wire. Tissues were placed into 15 ml organ baths containing carbogenated Krebs solution at 37°C. To confirm the presence or absence of endothelial cells, tissues were precontracted with a sub-maximal concentration of phenylephrine (0.3 μM) and the response to ACh (10 μM) observed. The presence of functional endothelial cells was indicated by subsequent relaxation (≥80% of precontraction) while the absence of endothelial cells was indicated by a lack of response to ACh.

2.6. Phospholipase A_2 (PLA_2) inhibition with 4-bromophenacyl bromide (4-BPB)

Inhibition of PLA_2 was achieved as described by Abe et al. (1977). Venom (1 mg/ml) was dissolved in sodium cacodylate buffer (0.1 M; pH 6.0) and then

either incubated with 4-BPB (0.72 mM final concentration) or vehicle (acetone) at 30°C for 12–16 h. Where required, ACh and PLA_2 were also incubated with 4-BPB and vehicle under the same conditions as negative and positive controls, respectively.

2.7. Anaesthetised rats

Male rats were anaesthetised with pentobarbitone sodium (70–100 mg/kg, i.p.). Venom or its vehicle was administered via a jugular vein cannula. Blood pressure was recorded via a Gould (P23) pressure transducer attached to a carotid artery cannula.

2.8. Analysis of results

In the chick biventer cervicis muscle and mouse phrenic nerve diaphragm, reponses to venom were expressed as percentage change of basal contractions. In the guinea-pig isolated ileum, all responses were expressed as a percentage of the maximal tissue response to ACh. In rat aortic rings, responses were expressed as percentage relaxation of the precontraction to phenylephrine (0.3 μM). All responses were recorded on a Grass Polygraph (Model 79D).

2.9. Drugs

The following drugs were used: acetylcholine chloride (Sigma); arachidonic acid (Sigma); Bay K 8644 (Sigma); bovine serum albumin (Sigma); 4-bromophenacyl bromide (Sigma); carbachol (Sigma); indomethacin (Sigma); *N*-nitro-L-arginine (Sigma); phenylephrine hydrochloride (ICN Pharmaceuticals); phospholipase A_2 (from *Naja mossambica mossambica*; Sigma).

Indomethacin was dissolved in 1% Na_2CO_3, phenylephrine was dissolved in catecholamine diluent (0.9% NaCl; 0.0156% $NaH_2PO_4.2H_2O$; 0.004% ascorbic acid), 4-bromophenacyl bromide was dissolved in acetone (0.0025% final bath concentration) and Bay K 8644 was dissolved in methanol. All other drugs were made up in distilled water with further dilutions in Krebs solution for in vitro experiments or in 0.9% saline (w/v) for in vivo experiments.

2.10. Statistics

Paired Student's *t*-test was used to compare agonist or venom responses before and after treatment in the same animal or preparation. Comparison of agonist or venom responses between preparations was made by a Student's unpaired *t*-test. A

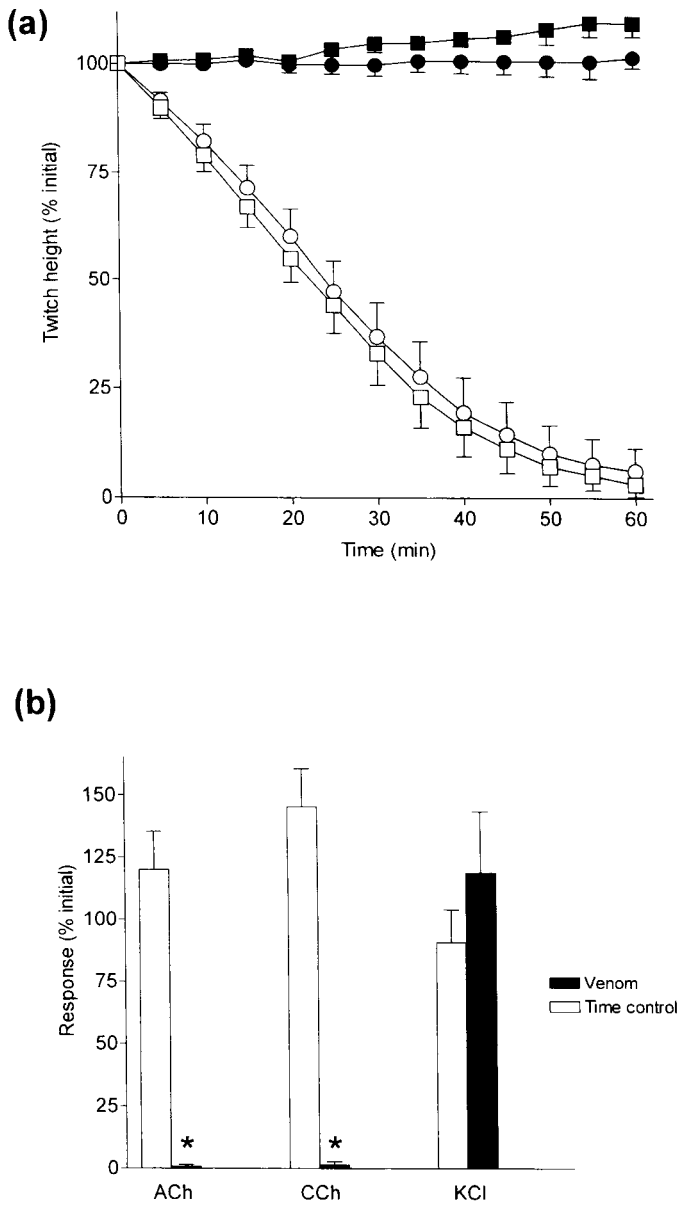

Fig. 1. (a) The effect of venom (10 μg/ml; $n=9$; □), venom (10 μg/ml) incubated with 4-BPB (0.72 mM; $n=8$; ○) or time ($n=5$; ●) on nerve-mediated twitches and venom (10 μg/ml; $n=4$; ■) on direct muscle stimulation of the chick biventer cervicis muscle preparation. (b) The effect of venom (10 μg/ml; $n=9$) on responses of the chick biventer cervicis muscle preparation to acetylcholine (ACh, 1 mM), carbachol (CCh, 20 μM) or potassium chloride (KCl, 40 mM) (*$P < 0.05$ compared to time control, 1-way ANOVA).

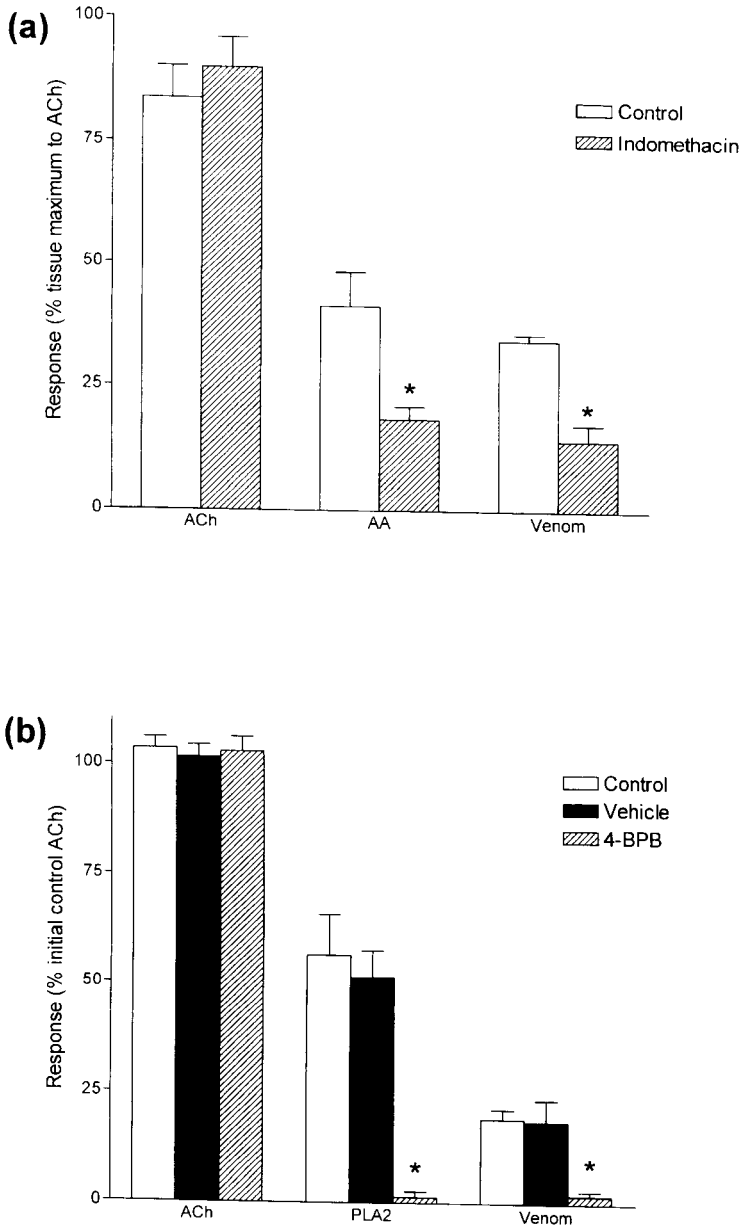

Fig. 2. (a) Effect of indomethacin (1 μM) on responses of the guinea-pig ileum to arachidonic acid (AA, 20 μM), acetylcholine (ACh; 5 μM) or venom (10 μg/ml). $^*P < 0.05$, paired t-test, $n = 7$. (b) Effect of 4-BPB (0.72 mM) on responses of the guinea pig ileum to ACh (3–10 μM), phospholipase A_2 (PLA_2, 0.33 μg/ml) or venom (10 μg/ml). $^*P < 0.05$, unpaired t-test, $n = 6$. All responses were compared to control responses obtained in the absence of either indomethacin or 4-BPB.

one way ANOVA was used for multiple comparisons. Data are expressed as mean ±SE with statistical significance indicated wherever $P < 0.05$.

3. Results

3.1. Chick isolated biventer cervicis muscle

Papuan taipan venom (10 μg/ml) abolished nerve mediated twitches in the chick biventer cervicis muscle preparation (Fig. 1a; $t_{50} = 27 \pm 3$ min, $t_{90} = 44 \pm 5$ min). Control experiments indicated no significant inhibition of twitch height with time (Fig. 1a). Venom (10 μg/ml) significantly inhibited contractile responses to ACh (1 mM) and CCh (20 μM) while having no effect upon responses to KCl (40 mM; $P > 0.05$, ANOVA, Fig. 1b). However, responses to direct stimulation of muscle were not significantly affected by venom (10 μg/ml, Fig. 1a).

The phospholipase A inhibitor 4-BPB (0.72 mM) had no significant effect on the inhibitory response of venom on nerve-mediated twitches in the chick biventer cervicis muscle (Fig. 1a; $t_{50} = 26 \pm 4$ min, $t_{90} = 48 \pm 7$ min).

3.2. Mouse isolated phrenic nerve diaphragm

Venom (10 μg/ml) abolished nerve-mediated twitch responses in the mouse phrenic nerve diaphragm preparation ($t_{50} = 17 \pm 1$ min, $t_{90} = 25 \pm 1$ min, $n = 6$).

3.3. Guinea-pig isolated ileum

Venom (3 30 μg/ml) caused concentration-dependent contractile responses in the guinea-pig isolated ileum (19 ± 5 53 ± 17% of tissue maximum; $n = 3$–4 for each concentration). A submaximal venom concentration (10 μg/ml) was chosen for subsequent experiments using this preparation. The response was rapid in onset (i.e., time to maximum contraction approximately 30 s) and reversible by washing of the tissue (i.e., tension of preparation returned to basal level).

The cyclooxygenase inhibitor indomethacin (1 μM) significantly attenuated the contractile responses to arachidonic acid (20 μM) and venom (10 μg/ml), but not ACh (5 μM), in the guinea-pig isolated ileum ($P < 0.05$, $n = 7$, Student's unpaired t-test; Fig. 2a).

Incubation with 4-BPB (0.72 mM) resulted in significant inhibition of the contractile responses to venom (10 μg/ml) and phospholipase A_2 (0.33 μg/ml), but not ACh (3–10 μM), compared to vehicle and time control responses ($P < 0.05$, $n = 7$, Student's unpaired t-test; Fig. 2b).

3.4. Rat isolated aortic rings

Venom (3–100 μg/ml) caused relaxation of endothelium-intact aortic rings pre-contracted with phenylephrine (PE; 0.3 μM; 20 ± 11–93 ± 12% of precontraction;

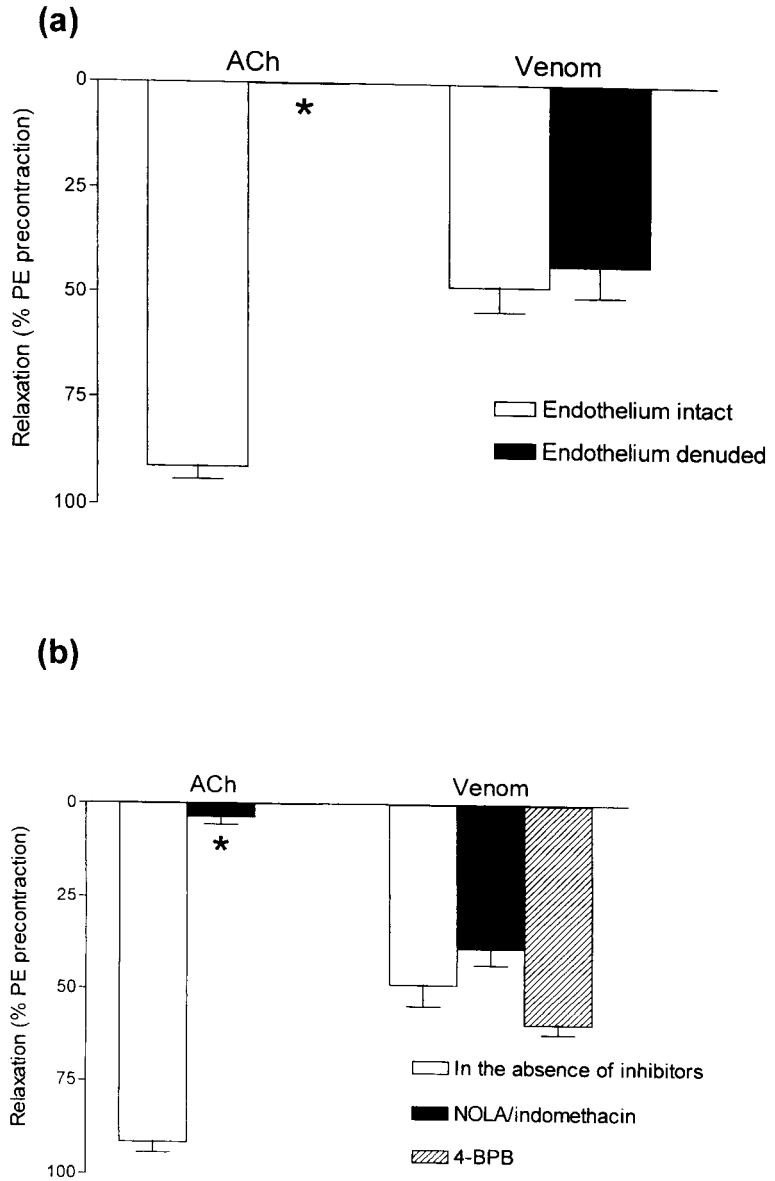

Fig. 3. (a) The effect of acetylcholine (ACh, 10 μM) or venom (30 μg/ml) on precontracted (PE 0.3 μM) endothelium-intact and endothelium-denuded aortic rings from rats ($n=5$). $^*P < 0.05$ significant difference between intact and denuded rings, unpaired t-test. (b) The effect of the combination of NOLA (0.1 mM) and indomethacin (10 μM) on responses to venom (30 μg/ml) or acetylcholine (ACh, 10 μM) and prior incubation of venom with 4-BPB in precontracted (PE 0.3 μM) endothelium-intact aortae ($n=8$). $^*P < 0.05$ significant difference from response in the absence of NOLA and indomethacin, unpaired t-test.

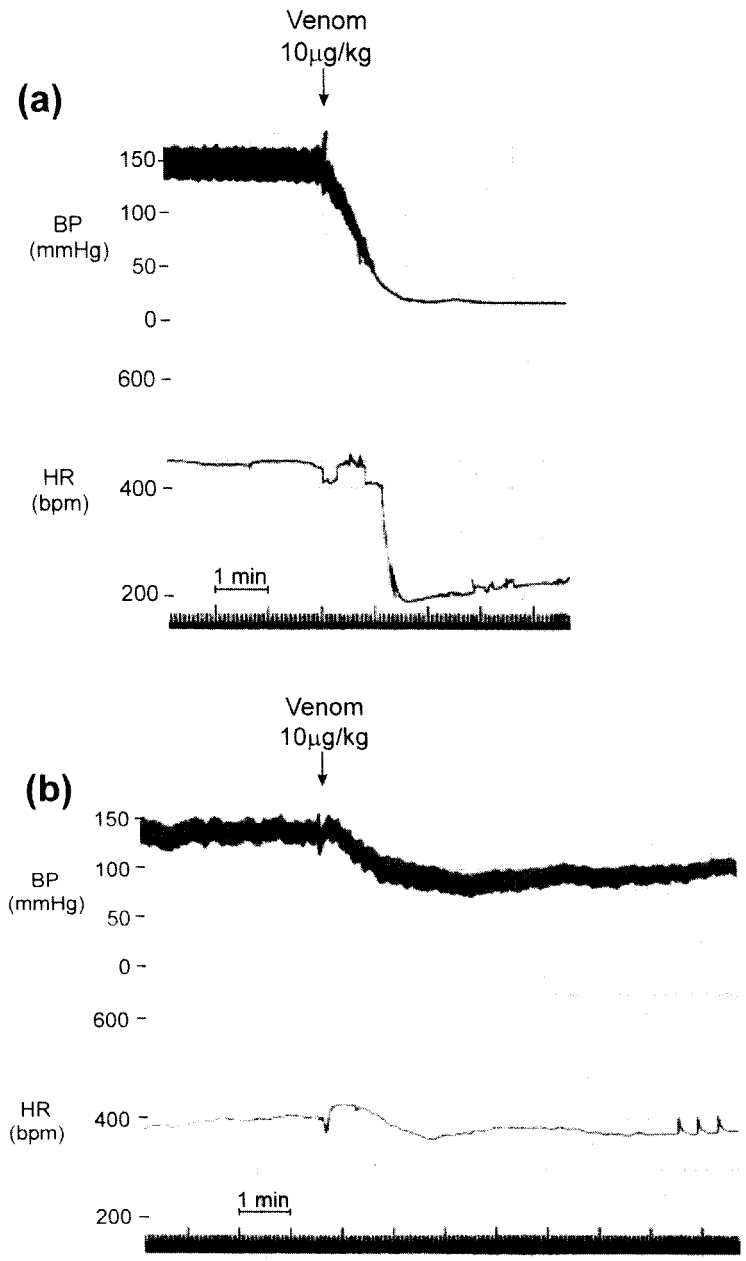

Fig. 4. (a) Trace showing the response to venom (10 μg/kg, i.v.) in an anaesthetised rat. (b) Trace showing the response to venom (10 μg/kg, i.v.) in an anaesthetised rat after previous administration of 5 μg/kg (i.v.) venom.

$n = 3$–5 for each concentration). A submaximal venom concentration (30 μg/ml) was chosen for subsequent experiments using this preparation. While responses to the endothelium-dependent relaxant ACh (10 μM) were abolished by endothelium denudation, responses to venom (30 μg/ml) were not significantly different from responses in endothelium-intact aortas ($P > 0.05$, $n = 5$, unpaired t-test; Fig. 3a).

Neither 4-BPB (0.72 mM) nor the combination of the nitric oxide synthase inhibitor NOLA (0.1 mM) and indomethacin (10 μM) inhibited relaxation induced by venom (30 μg/ml) in endothelium-intact aortic rings precontracted with PE while the latter abolished responses to ACh ($P < 0.05$, $n = 8$; Fig. 3b).

Venom (30 μg/ml) relaxed (64 ± 5% of precontraction; $n = 9$) endothelium-denuded aortic rings precontracted with the L-type calcium channel opener, Bay K 8644 (100 nM).

3.5. *Anaesthetised rat*

Administration of venom (10 μg/kg, i.v.) produced an immediate decrease in blood pressure and heart rate, followed by total respiratory collapse within 1–2 min (Fig. 4a). Artificial respiration (1 ml/140 g body wt; 50 strokes/min; Ugo Basile 7025 rodent respirator), either prior to or after venom administration, was without effect (data not shown).

Administration of a lower dose of venom (5 μg/kg, i.v.) produced a small decrease in mean arterial pressure (19 ± 4 mmHg, $n = 6$). Subsequent administration of venom (10 and 100 μg/kg, i.v.) to the same rat failed to produce respiratory collapse, or notable respiratory paralysis, but caused slightly larger transient decreases in mean arterial pressure (10 μg/kg, 34 ± 6 mmHg, $n = 6$; 100 μg/kg, 28 ± 2 mmHg, $n = 3$; Fig. 4b).

4. Discussion

The results obtained from this study present, for the first time, a pharmacological profile of the venom from the Papuan taipan (*O. s. canni*). Respiratory paralysis caused by neurotoxins is a major clinical feature of taipan envenomation (White, 1996). Therefore, initial studies of the effects of the venom were undertaken using the chick biventer cervicis muscle preparation. It was observed that Papuan taipan venom produced time-dependent inhibition of electrically evoked, nerve-mediated twitches and abolished the responses of the preparation to the nicotinic agonists, acetylcholine and carbachol but not to potassium chloride. These results are consistent with the presence of a postsynaptic neurotoxin(s). Postsynaptic neurotoxins have been shown to bind with high affinity to nicotinic acetylcholine receptors where they competitively antagonise the actions of acetylcholine (Stroud et al., 1990). Postsynaptic neurotoxins have been isolated from the venom of *O. scutellatus* (Zamudio et al., 1996) and identified in the venom of *O. microlepidotus* (Hodgson and Rowan, 1997).

The presynaptic neurotoxins taipoxin and paradoxin have been isolated from the venoms of coastal and inland taipans respectively (Fohlman et al., 1976; Fohlman, 1979), thus it is likely that the venom from the Papuan taipan contains a similar neurotoxin. Presynaptic neurotoxins inhibit electrically evoked twitches without affecting responses to cholinergic agonists. In the current series of experiments, any presynaptic effect would be masked by the postsynaptic effect, thus making it impossible to confirm the presence of a presynaptic neurotoxin.

Taipoxin and paradoxin have been classified as phospholipase A_2 (PLA_2) neurotoxins. In order to determine whether any PLA_2 activity contributed to the neurotoxic activity of Papuan taipan, the venom was incubated with the phospholipase A inhibitor 4-BPB. However, 4-BPB failed to significantly affect venom-induced twitch inhibition in the chick biventer cervicis muscle.

At the concentrations utilised, Papuan taipan venom was devoid of any significant myotoxic activity as indicated by the failure of the venom to significantly affect the responses of the preparation to potassium chloride or to affect twitch height in muscle-stimulated preparations. These results are consistent with clinical observations, where envenomed patients have not displayed symptoms of venom myotoxicity such as myoglobinurea (Lalloo et al., 1995). Interestingly, the presynaptic neurotoxin from the closely related coastal taipan, has been found to be highly myotoxic in vitro (Harris and Maltin, 1982).

The mouse phrenic nerve diaphragm preparation is often used in conjunction with the chick biventer cervicis muscle preparation to study venom neurotoxins. The former preparation showed greater sensitivity to Papuan taipan venom neurotoxins than the chick biventer cervicis muscle as indicated by comparing t_{50} and t_{90} values.

A number of snake venoms have been shown to cause contraction of smooth muscle from guinea pigs (Sket and Gubensek, 1976; Bell et al., 1999), therefore, we examined the effects of Papuan taipan venom on the guinea-pig isolated ileum. Previous studies have revealed two different mechanisms underlying snake venom-induced contraction of ileum smooth muscle. Osman et al. (1976) reported that contractile responses to venom from the Cobra (*Naja*) were mediated by the release of ACh, while studies of venom from the inland taipan (Bell et al., 1998) and sand viper (*Vipera ammodytes*) (Sket and Gubensek, 1976) suggest the involvement of PLA_2 and cyclooxygenase metabolites. PLA_2 acts by liberating arachidonic acid from membrane phospholipids, leading to the synthesis of mediators such as prostaglandins or leukotrienes. In the present study, prior incubation of venom with the PLA inhibitor, 4-BPB, abolished the contractile response of the guinea-pig ileum to Papuan taipan venom. In addition, venom responses of the ileum were significantly inhibited by the cyclooxygenase inhibitor, indomethacin, suggesting that these responses are most likely mediated by a venom PLA_2 component, leading to the synthesis of cyclooxygenase metabolites such as prostaglandins.

The effect of Papuan taipan venom on blood vessels was examined using isolated vascular preparations. Although venoms from some snakes such as the rhinoceros horned viper (*Bitis nasicornis*) have been known to cause contraction

of aortic strips (Tilmisany et al., 1986), in the present study, venom produced relaxation in both endothelium-intact and endothelium-denuded precontracted aortic rings. Venom induced relaxation was not altered by prior incubation of the venom with 4-BPB or by the presence of the nitric oxide synthase inhibitor, NOLA, in combination with indomethacin. These results indicate that this effect was independent of the nitric oxide pathway and was not mediated by cyclooxygenase metabolites.

Possani et al. (1992) isolated an L-type calcium channel blocker (taicatoxin) from the closely related coastal taipan which leads to the suggestion that a similar toxin may occur in the Papuan taipan venom. In partial support of this hypothesis, we showed that Papuan taipan venom relaxed endothelium-denuded rat aortas precontracted with the L-type calcium channel agonist, Bay K 8644. Similar results have been observed using venom from the inland taipan (Bell et al., 1999). Our results however, do not conclusively support the presence of a calcium channel blocking component and further studies are warranted to substantiate this claim.

Further studies of the effects of venom on the cardiovascular system were carried out in anaesthetised rats. Venom (10 μg/kg, i.v.), when administered on its own, produced an immediate drop in blood pressure and total respiratory collapse of the animal within 1–2 min. Interestingly, following the administration of 5 μg/kg (i.v.) of venom the previously lethal dose of venom (10 μg/kg, i.v.) produced only a slight and transient decrease in mean arterial pressure (MAP). Likewise, 100 μg/kg (i.v.) caused only a transient decrease in MAP following the initial dose of the venom. Although the mechanism for this effect has yet to be determined, previous studies suggest that venom-induced hypotension may be mediated through a PLA_2 component of the venom. PLA_2 fractions isolated from both the sand viper (*Vipera ammodytes*) (Sket and Gubensek, 1976) and cobra (*Naja naja*) (Cicala and Cirino, 1993) have been shown to cause hypotension in anaesthetised rats.

In conclusion, it appears that Papuan taipan venom is highly neurotoxic and able to produce rapid twitch inhibition in different neuromuscular preparations. Although the presence of presynaptic neurotoxin(s) cannot be confirmed, venom-induced twitch inhibition appears to be caused by a postsynaptic neurotoxin(s) which does not have PLA_2 activity. Similarly, venom-induced relaxation of isolated aortic rings appears not to be mediated via a PLA_2 component, although the exact mechanism of this action is still unknown. In contrast, the contractile response in ileal smooth muscle appears to be mediated by a venom PLA_2 component. Furthermore, data from the present study, along with those from previous studies, suggests that the venom-induced depressor response observed in anaesthetised rats may be mediated through a venom PLA_2 component. While the present study presents a basic pharmacological profile of Papuan taipan venom, there is still much scope for further investigation. The fractionation of whole venom will allow us to isolate and identify the pharmacologically active constituents of the venom.

Acknowledgements

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