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SOME PHARMACOLOGICAL STUDIES OF VENOM FROM THE INLAND TAIPAN (*OXYURANUS MICROLEPIDOTUS*)

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K. L. Bell, S. K. Sutherland and W. C. Hodgson. Some pharmacological studies of venom from the inland taipan (*Oxyuranus microlepidotus*). *Toxicon* **36,** 63–74, 1998.—The present study was designed to obtain a basic pharmacological profile of venom from the inland taipan (*Oxyuranus microlepidotus*). Venom (0.05–50 μg/ml) produced dose-dependent contractions in guinea-pig ileum, which could not be reproduced upon second administration. The cyclooxygenase inhibitor indomethacin (1 μM), a preceding anaphylactic response induced by egg albumin and inactivation of phospholipase A_2 (PLA_2) by incubation with 4-bromophenacyl bromide (1.8 mM) all significantly inhibited responses to venom (0.5 μg/ml). Venom (0.5 μg/ml) caused inhibition of stimulation-induced contractions in the prostatic segment of rat vas deferens which was not significantly affected by the α_2-adrenoceptor antagonist idazoxan (0.3 μM). Venom (10 μg/ml) caused time-dependent inhibition of the rat electrically stimulated phrenic nerve–diaphragm preparation, positive inotropic and chronotropic responses in rat isolated atria and relaxation in rat endothelium-denuded and -intact isolated aortae. In endothelium-intact aortae, the nitric oxide synthase inhibitor *N*-nitro-L-arginine (NOLA, 0.1 mM) significantly inhibited the response to venom (10 μg/ml). Venom (50 μg/kg, i.v.) caused an immediate drop in blood pressure followed by cardiovascular collapse in anaesthetised rats. Venom (10 μg/kg, i.v.) caused a gradual fall in blood pressure which was sometimes accompanied by a temporary cessation of respiration. A PLA_2 assay detected the presence of PLA_2 in the venom. These results suggest that the venom contains a component capable of causing the synthesis of arachidonic acid metabolites and a component capable of relaxing vascular smooth muscle. The inhibitory effect on the phrenic nerve–diaphragm is probably due to the previously identified neurotoxin (paradoxin).

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INTRODUCTION

The inland taipan (*Oxyuranus microlepidotus*) is one of three species in the *Oxyuranus* genus, along with the coastal taipan (*O. scutellatus scutellatus*), which is found along the north-eastern coast of Australia, and the Papuan taipan (*O. scutellatus canni*), which is found in parts of Papua New Guinea. The inland taipan is believed to have the most toxic snake venom in the world (Sutherland, 1994). The LD_{50} of *O. microlepidotus* venom has been determined to be 0.025 mg/kg (s.c., in mice) when diluted in saline and 0.010 mg/kg when diluted in 0.1% bovine serum albumin (Broad *et al.*, 1979). The inland taipan is found in the area between Cooper Creek, Diamantina River and Georgina River in far south-western Queensland and north-eastern South Australia. However, there is recent evidence to suggest that the area of distribution may be much greater (Read, 1994).

There have been four documented cases of people being bitten by the inland taipan. All were herpetologists, and all survived owing to the correct administration of first aid and antivenom (Pearn *et al.*, 1995). Problems resulting from inland taipan envenomation include paralysis, coagulopathy, thrombocytopenia, rhabdomyolysis and renal function impairment (Covacevich *et al.*, 1988).

Fohlman (1979) completed a comparative study on the venoms of *O. microlepidotus* and *O. s. scutellatus* and found the two venoms to be biochemically similar. Both were shown to contain a direct prothrombin activator and a presynaptic neurotoxin (paradoxin and taipoxin, respectively). *Oxyuranus microlepidotus* venom also exhibits high alkaline phosphomonoesterase activity, high phospholipase A_2 (PLA_2) activity and high hyaluronidase activity. Moreover, only moderate 5′ nucleotidase and low protease, phosphodiesterase and L-amino acid oxidase activity were detected. In addition, no acetylcholinesterase or arginine esterase activity was observed (Tan and Ponnudurai, 1990).

Despite the extreme lethality of *O. microlepidotus* venom, little is known about its pharmacological activity. Therefore, the aim of the present study was to obtain a basic pharmacological profile of venom from the inland taipan. These studies will provide further insight into the mechanism of action of *O. microlepidotus* venom.

MATERIALS AND METHODS

Snake collection

Inland taipans were collected and venom prepared by Mr P. Mirtschin (Venom Supplies, South Australia). Snakes were collected from Goyders Lagoon in north-eastern South Australia. Snakes had a snout vent length ranging from 1300 to 1780 mm and a tail length of 220–320 mm.

Venom preparation and storage

Freeze-dried venom was obtained from milkings in February 1996 and stored at −20°C. Stock solutions of 1 mg/ml or 100 μg/ml were made by dissolving venom in 0.1% bovine serum albumin in 0.9% saline. Aliquots (100–200 μl) of these solutions were stored at −20°C until the day of experimentation, when they were thawed and kept on ice. Siliconised glassware was used to minimise loss of activity due to venom components adhering to the glass.

Guinea-pig isolated ileum

Segments of ileum (1.5–2.5 cm) from Dunkin–Hartley guinea-pigs (300–750 g) were mounted (1 g resting tension) on tissue holders, in isolated organ baths, as described previously (Hopkins *et al.*, 1996). Tissues were bathed in Krebs solution (37°C), bubbled with carbogen (95% O_2 and 5% CO_2), of the following composition (mM): NaCl 118.4; KCl 4.7; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25; glucose 11.1; $CaCl_2$ 2.5.

Egg albumin-sensitised ileum

Dunkin–Hartley or Monash strain guinea-pigs (350–750 g) were injected with chicken egg albumin (200 mg/ml, 0.5 ml i.p. and 0.5 ml s.c.), dissolved in 0.9% saline, 3 weeks before the experiments, and 2 weeks before the experiments further injections (10 mg/ml, 0.5 ml i.p. and 0.5 ml s.c.) were given to increase sensitivity. The ileum was prepared and experiments were conducted under the same conditions as described above.

Rat isolated vas deferens

Epididymal and prostatic vasa deferentia from male Wistar or Sprague–Dawley rats (350–550 g) were attached to tissue holders as described previously (Hopkins *et al.*, 1996). The preparations were mounted (1 g resting tension) in isolated organ baths containing Krebs solution (32°C) bubbled with carbogen. Prostatic segments were field-stimulated (100 V supramaximal voltage, 0.2 Hz and 0.3 msec) using a Grass SD9 stimulator.

Rat isolated phrenic nerve–diaphragm

Hemidiaphragms, with phrenic nerves intact, from Wistar or Sprague–Dawley rats (300–500 g) were attached to tissue holders as originally described by Bulbring (1946). The tissues were then mounted (1 g resting tension) in isolated organ baths containing Holmans solution (37°C), bubbled with carbogen, of the following composition (mM): NaCl 120; KCl 5; $NaHCO_3$ 25; NaH_2PO_4 1; $MgSO_4$ 1; glucose 11; sucrose 10; $CaCl_2$ 2.5. The phrenic nerve was stimulated (3–10 V supramaximal voltage, 0.2 Hz, 0.3 msec) using a Grass SD9 stimulator and isometric twitches were measured.

Rat isolated spontaneously beating atria

Atria from Wistar rats (350–550 g) were attached to tissue holders as described previously (Hopkins *et al.*, 1996). The tissues were mounted (0.75 g resting tension) in isolated organ baths containing Krebs solution (32°C) bubbled with carbogen.

Rat isolated aortic rings

Aortic rings (5 mm in length) from Wistar rats (350–500 g) were mounted (10 g resting tension) between two stainless-steel hooks as described previously (James and Hodgson, 1995). Where indicated, endothelial cells were removed by gently rubbing the intimal surface with thin wire. Tissues were placed in isolated organ baths containing Krebs solution (37°C) bubbled with carbogen. To confirm the presence or absence of endothelial cells a submaximal concentration of phenylephrine (0.3 μM) was added to the bath and, at the plateau of the response, acetylcholine (ACh, 10 μM) was added. The presence of functional endothelial cells was indicated by subsequent relaxation ($\geq$80% of precontraction) while the absence of endothelial cells was indicated by a lack of response to ACh.

Inhibition of PLA_2 with 4-bromophenacyl bromide

PLA_2 inhibition was achieved according to the method of Abe *et al.* (1977). Venom (5 mg) was dissolved in sodium cacodylate buffer (5 ml, 0.1 M, pH 6.0) and 4-bromophenacyl bromide (4-BPB, 50 μl, 36 mM) was added to give a final 4-BPB concentration of 1.8 mM. This solution was then incubated at 30°C for 12 hr. Agonists were also incubated in the presence of 4-BPB or vehicle as described above.

Analysis of results

In ileum a maximal response to ACh (0.1–0.3 mM) was obtained and all subsequent responses were represented as a percentage. In the atria and stimulated vas deferens, responses were expressed as percentage potentiation or inhibition of tissue contractions. Five contractions immediately before addition of the drug or venom, and five contractions after the maximum effect, were measured and the mean response was determined. In the unstimulated vas deferens, maximal responses to phenylephrine (0.1–0.5 mM) were obtained and all subsequent responses expressed as a percentage. In endothelium-intact aortae, all responses were expressed as a percentage of the relaxation to ACh (10 μM). In endothelium-denuded aortae, all responses were expressed as a percentage of the precontraction to phenylephrine (0.3 μM).

In all tissues, when antagonists/inhibitors were used they were in contact with the tissue for an initial period of 45 min. After the addition of each agonist, the tissue was washed and the antagonist reapplied for 3–5 min before the addition of the next agonist or venom concentration. Wherever possible, agonists were used as positive (i.e. significantly inhibited by the antagonist) and negative (i.e. not significantly inhibited by the antagonist) controls to ensure the antagonist/inhibitor was both effective and selective at the chosen concentration.

Anaesthetised rat blood pressure

Male Wistar rats (300–450 g) were anaesthetised with sodium pentobarbitone (100 mg/kg, i.p.). Drugs or venom were administered via a jugular vein cannula and flushed through with 0.2 ml of 0.9% saline. Blood pressure was measured via a carotid artery cannula which was connected to a Gould–Statham pressure transducer (P23).

Pulse pressure was defined as the difference between systolic and diastolic blood pressure, and mean arterial pressure (MAP) defined as diastolic blood pressure plus one-third of the pulse pressure. All responses were expressed as changes in MAP.

PLA_2 assay

PLA_2 activity of the venom (100 μg/ml) was determined using a radial diffusion assay in which PLA_2 hydrolyses chicken egg yolk phospholipids in an agarose gel following the method of Habermann and Hardt (1972). A standard curve for PLA_2 was conducted over a range of 0–1000 units PLA_2/ml by measuring the diameter of clearing around wells (indicating hydrolysis of phospholipids by PLA_2) in the agarose gel.

Drugs

The following drugs were used: acetylcholine chloride, arachidonic acid, atropine sulfate, bovine serum albumin, 4-BPB, chicken egg albumin, histamine dihydrochloride, 5-hydroxytryptamine (5-HT) creatine sulfate, indomethacin, isoprenaline sulfate, *N*-nitro-L-arginine (NOLA), noradrenaline bitartrate, PLA_2 (from *Naja mossambica mossambica*), propranolol hydrochloride (Sigma Chemical Co., St Louis, MO, U.S.A.), clonidine hydrochloride (Boehringer Ingelheim, Artarmon, NSW, Australia), idazoxan hydrochloride (Reckitt and Coleman, Kingston-upon-Hull, U.K.), mepyramine maleate (May and Baker, Dagenham, U.K.) and phenylephrine hydrochloride (ICN Pharmaceuticals, Plainview, NY, U.S.A.).

Except where indicated, stock solutions were made up in distilled water with subsequent dilutions in Krebs solution for *in vitro* use and in 0.9% (w/v) saline for *in vivo* experiments. Arachidonic acid was dissolved and stored in hexane. When needed, the hexane was evaporated with nitrogen gas and the arachidonic acid redissolved in 1% Na_2CO_3. Stock solutions of indomethacin were dissolved in 1% Na_2CO_3. Stock solutions and subsequent dilutions of isoprenaline, noradrenaline and phenylephrine were prepared in catecholamine diluent (0.9% NaCl, 0.0156% $NaH_2PO_4.2H_2O$, 0.004% ascorbic acid). 4-BPB was dissolved in acetone (0.0025% final bath concentration).

Statistics

Paired Student's *t*-tests were used to compare agonist responses before and after antagonists/inhibitors in the same animal/tissue. Venom responses were not repeatable, therefore only one addition of venom was made to each preparation and these were compared by unpaired Student's *t*-tests. A two-way analysis of variance (ANOVA) was used for multiple comparisons. Data are expressed as mean $\pm$ S.E. Statistical significance was indicated when $P < 0.05$.

RESULTS

Guinea-pig isolated ileum

Venom produced dose-dependent contractile responses in ileum [expressed as percentage of tissue maximum (n = 5–10): 0.05 μg/ml, 17 $\pm$ 4%; 0.1 μg/ml, 24 $\pm$ 6%; 0.5 μg/ml, 25 $\pm$ 4%; 5 μg/ml, 32 $\pm$ 6%; 10 μg/ml, 39 $\pm$ 5%; 50 μg/ml, 32 $\pm$ 5%). A concentration (i.e. 0.5 μg/ml) producing approximately 65% of the maximum venom response was chosen for further experiments in this preparation. Responses to venom (0.5 μg/ml) were characterised by a lapse of approximately 20 sec after addition, followed by a contraction which reached maximum within 1–2 min and took a further 2–3 min to return to baseline [Fig. 1(a)].

Venom samples were stored at a range of temperatures (–20°C, –4°C, 20°C and 37°C) for three different time periods (24, 48 and 72 hr) to determine whether temperature or duration of storage had any effect on contractile responses to venom in ileum. It was found that no significant loss of activity occurred in the venom (0.5 μg/ml) at any of the temperatures for up to 72 hr (n = 3, data not shown).

The contractile response to venom (0.5 μg/ml) was significantly attenuated (31 $\pm$ 8% vs 2 $\pm$ 1%) upon subsequent administration (n = 5, $P < 0.05$, Student's paired *t*-test).

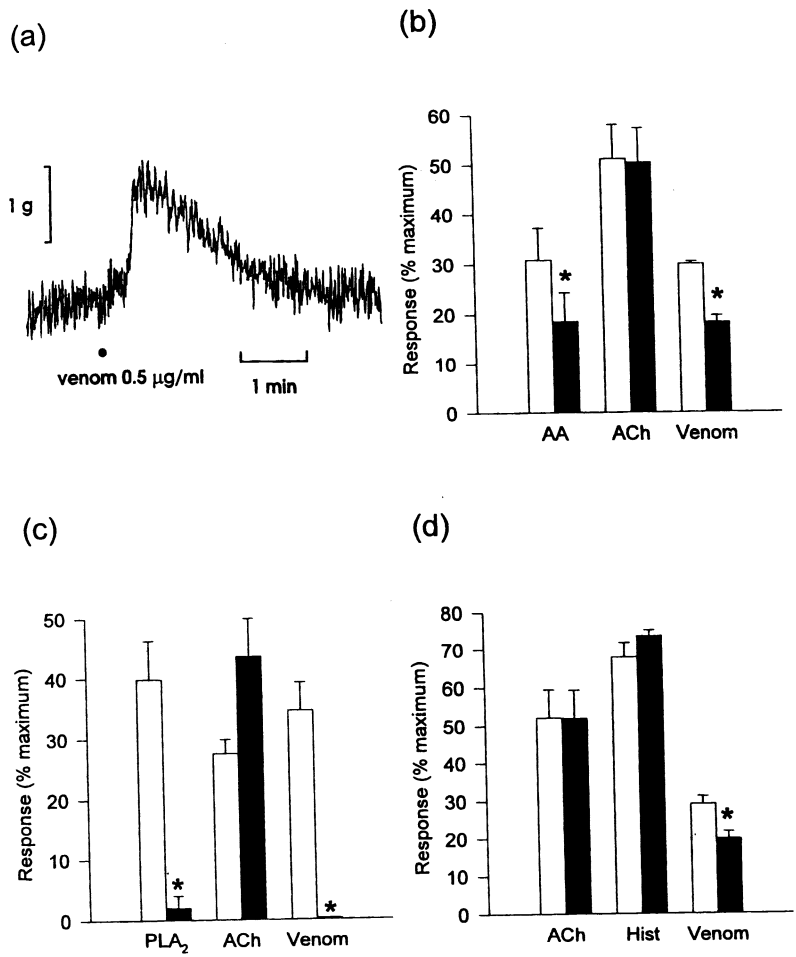

Fig. 1. (a) Trace showing response to venom (0.5 μg/ml) in guinea-pig ileum. Effect of (b) indomethacin (1 μM) or (c) 4-BPB (1.8 mM) on responses to venom (0.5 μg/ml, n = 3–6), acetylcholine (ACh, 1 μM, n = 6), arachidonic acid (AA, 2 μM, n = 6) or PLA_2 (0.32 U/ml, n = 3). *P < 0.05, significantly different from response in controls. (d) Response of sensitised ileum to venom (0.5 μg/ml, n = 5), histamine (2 μM, n = 5) or ACh (1 μM, n = 5). Open bars represent control values, filled bars represent treatment effect. *P < 0.05, significantly different from sensitised ileum without anaphylactic response.

However, the response to venom (0.5 μg/ml) was not significantly affected by the muscarinic receptor antagonist atropine (0.1 μM), the histamine receptor antagonist mepyramine (0.5 μM) or 5-HT receptor desensitisation with 5-HT (0.1 mM) (data not shown, n = 4–6).

Responses to venom (0.5 μg/ml) were significantly inhibited by the cyclooxygenase inhibitor indomethacin [1 μM, Fig. 1(b)] and the non-specific phospholipase A inhibitor 4-BPB [1.8 mM, Fig. 1(c)].

There was no significant difference between responses to ACh (0.1 μM) or histamine (1 μM) before and after the *in vitro* induction of an anaphylactic response, in egg-albu-

min sensitised ileum, by further exposure to egg albumin. However, responses to venom (0.5 μg/ml) were significantly reduced compared to control responses [Fig. 1(d)].

Rat isolated vas deferens

Venom (0.5 μg/ml) had no contractile activity in the epididymal (unstimulated) segment of the vas deferens, although the α_1-adrenoceptor agonist phenylephrine (0.2 mM) produced contractions (2.0 ± 0.2 g, $n = 4$).

Venom (0.5 μg/ml, 9 ± 3%, $n = 3$) and the α_2-adrenoceptor agonist clonidine (10 nM, 45 ± 4%, $n = 7$) produced inhibition of stimulation-induced contractions in the prostatic segment of the vas deferens. Responses to clonidine (2 ± 1%, $n = 7$), but not venom (14 ± 1%, $n = 3$), were significantly inhibited by the α_2-adrenoceptor antagonist idazoxan (0.3 μM, $P < 0.05$, Student's paired t-test).

Rat isolated phrenic nerve–diaphragm

Venom (0.5 μg/ml) caused a time-dependent inhibition (maximum effect 24 ± 12%) of the electrically stimulated twitch response of the phrenic nerve–diaphragm preparation over a 1 hr duration. However, this was not significantly different from the effect of vehicle over the same time period (18 ± 8%, $n = 4$). In contrast, venom (10 μg/ml) caused time-dependent inhibition of the twitch response (100 ± 0% after 50 min) which was significantly different from vehicle (Fig. 2).

Rat isolated atria

Venom (10 μg/ml) produced positive inotropic (36 ± 5%) and chronotropic (9 ± 3%) responses in rat isolated atria ($n = 4$).

Rat isolated aortic rings

Venom (10 μg/ml) produced relaxation in endothelium-intact (70 ± 5%, $n = 9$) and endothelium-denuded (21 ± 3%, $n = 4$) aortic rings precontracted with phenylephrine (0.3 μM).

The nitric oxide synthase inhibitor NOLA (0.1 mM) significantly inhibited the relaxation to ACh (10 μM, $n = 5$, $P < 0.05$, Student's paired t-test) and venom (10 μg/ml, $n = 5$, $P < 0.05$, Student's unpaired t-test) in endothelium-intact aortic rings [Fig. 3(a)]. However, indomethacin (10 μM) had no significant effect on responses to ACh (10 μM) or venom (10 μg/ml) in endothelium-intact aortic rings [Fig. 3(b)].

Effect of venom on mean blood pressure in the anaesthetised rat

Venom (50 μg/kg, i.v.) caused an immediate drop in blood pressure followed by respiratory cessation and cardiovascular collapse [$n = 3$, Fig. 4(a)] in the anaesthetised rat. Respiration of the rat with room air (1.4 ml/100 g, 50 strokes/min, Ugo Basile 7025 rodent respirator) did not affect the venom response (data not shown, $n = 1$). Venom (5 μg/kg, i.v.) had little or no effect on blood pressure (data not shown, $n = 2$). Venom (10 μg/kg, i.v.) produced variable responses in the anaesthetised rat, causing either a slow, prolonged drop in blood pressure [$n = 3$, Fig. 4(b)], or a drop in blood pressure followed by temporary respiratory cessation and spontaneous respiratory and cardiovascular recovery [$n = 3$, Fig. 4(c)]. Responses to venom (10 μg/kg, i.v.) were not repeatable in the anaesthetised rat (data not shown).

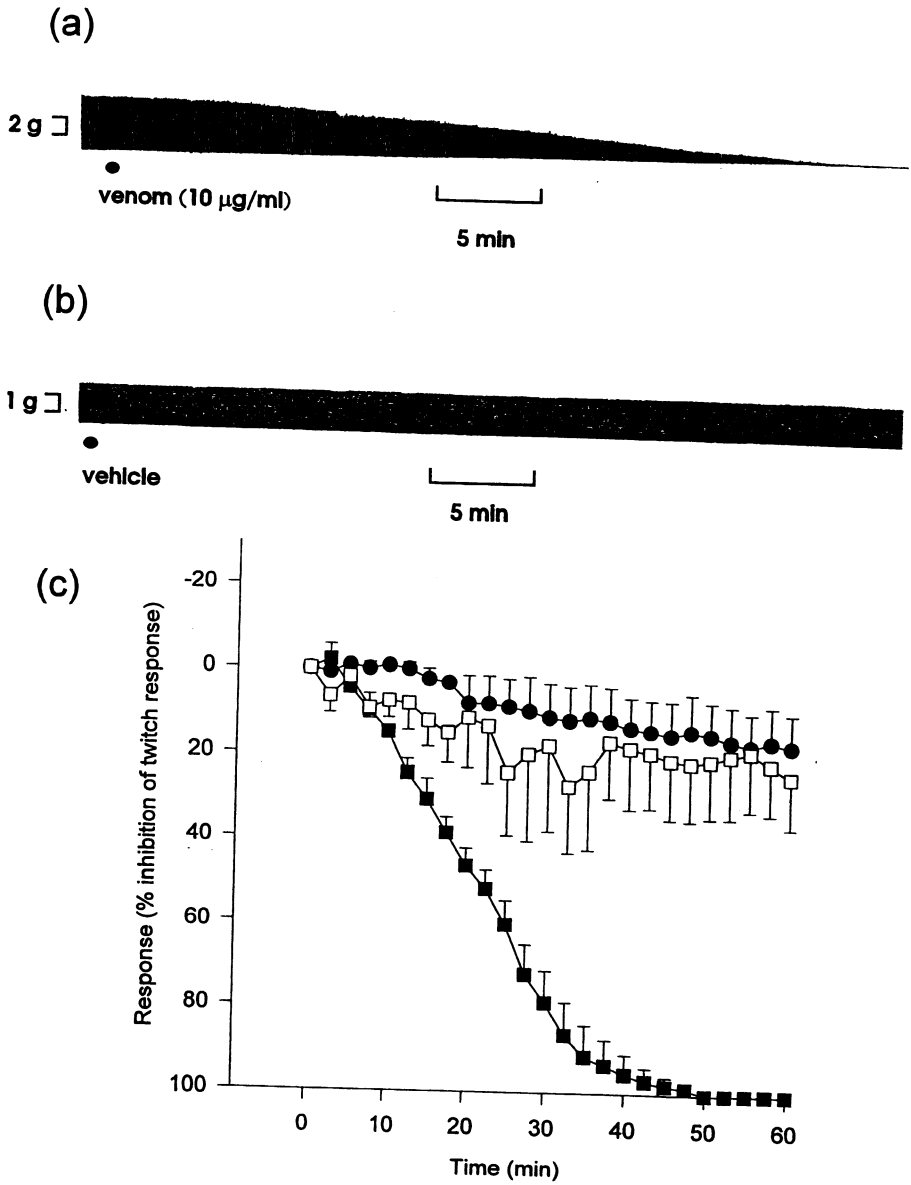

Fig. 2. Response of rat phrenic nerve–diaphragm to inland taipan venom. Trace showing response to (a) venom (10 μg/ml) and (b) vehicle. (c) Response of rat isolated phrenic nerve–diaphragm to vehicle (filled circle, $n = 4$) and inland taipan venom (0.5 μg/ml, open square, $n = 4$; 10 μg/ml, filled square, $n = 4$).

PLA_2 assay

Venom was assayed for PLA_2 activity according to its ability to hydrolyse chicken egg yolk phospholipids in an agarose gel. Venom (100 μg/ml) cleared the agarose gel, indicating the presence of PLA_2.

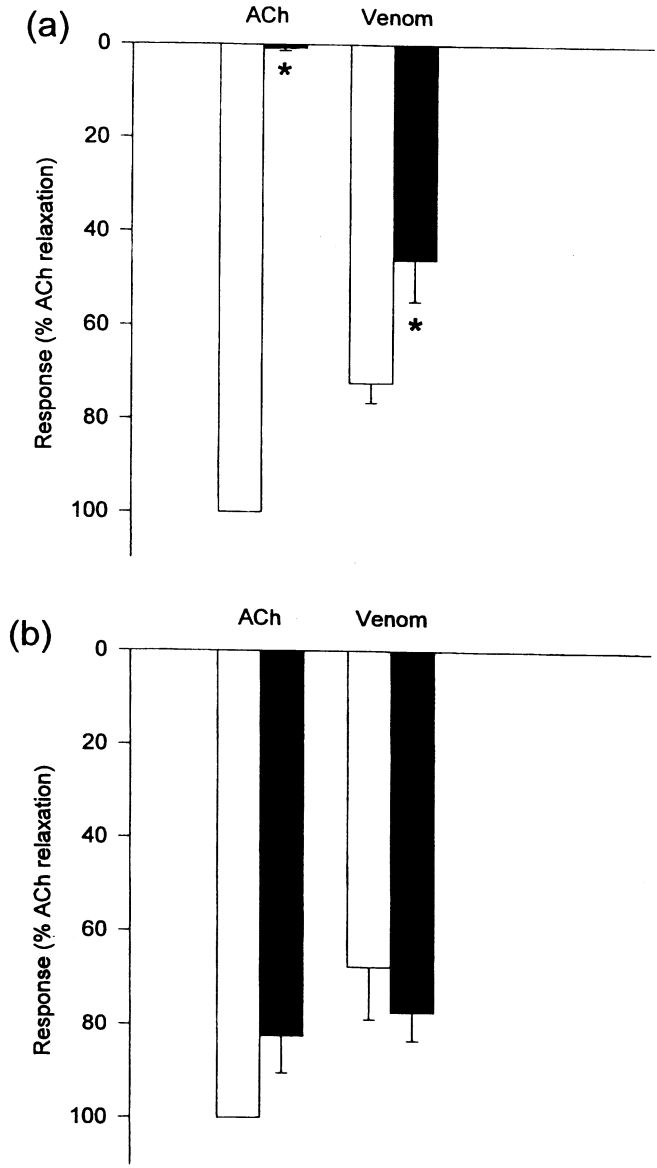

Fig. 3. Effect of (a) NOLA (0.1 mM) or (b) indomethacin (10 μM) on responses to venom (10 μg/ml, n = 4–5) or acetylcholine (ACh, 10 μM, n = 4–5) in rat endothelium-intact aortae. Open bars represent control values, filled bars represent treatment effect. *P < 0.05, significantly different from response in controls.

DISCUSSION

As the stability of the venom preparation was unknown, stability studies on contractile activity were carried out to ascertain optimal storage conditions. It was found that the venom could be stored over a wide range of temperatures for up to 72 hr without any significant loss of activity in the ileum. Despite this apparent stability, venom was

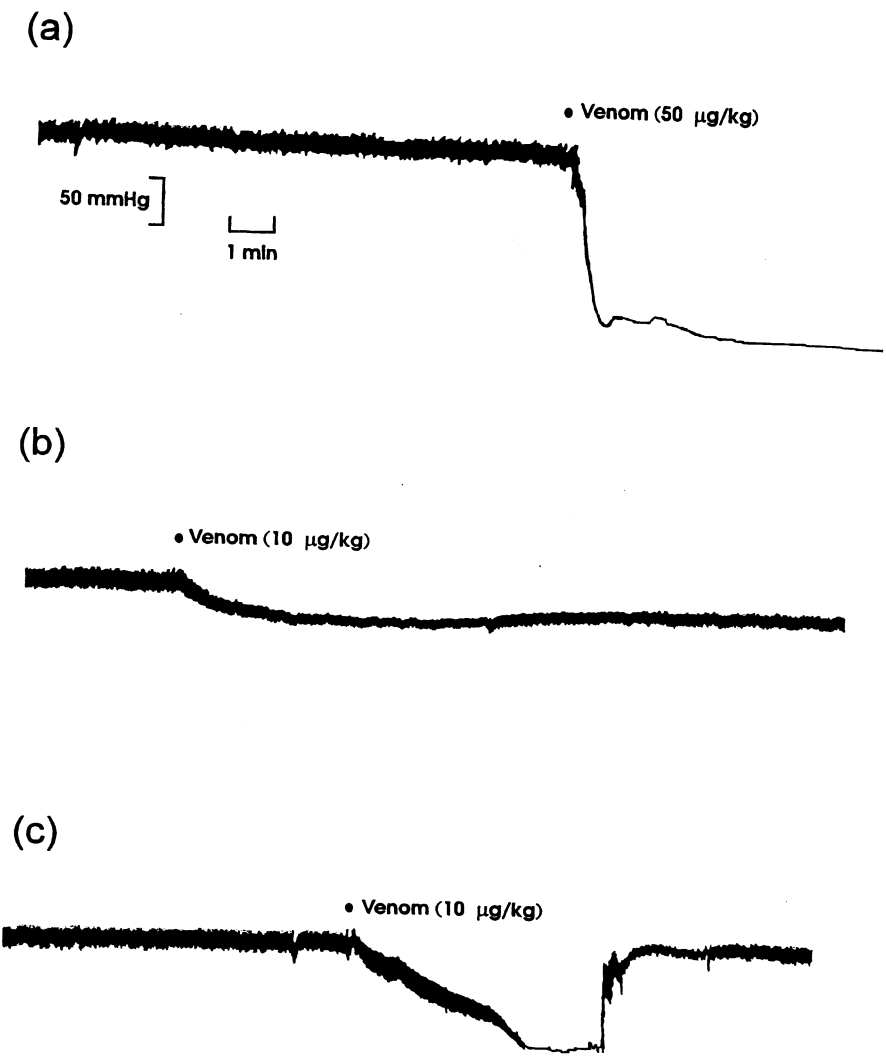

Fig. 4. Trace illustrating the varied effect of inland taipan venom administered i.v. to anaesthetised rats: (a) 50 µg/kg, (b) 10 µg/kg, (c) 10 µg/kg (alternative response).

always stored at −20°C and used within 72 hr of preparation as snake venoms are known to possess a wide variety of enzymes and proteins which may be considerably less stable. Interestingly, the contractile response to the venom in the ileum was subject to rapid desensitisation. Upon second administration the response was significantly reduced in magnitude. This desensitisation was also noted in rat aortae and anaesthetised rats.

Previous studies have shown that cobra (*Naja*) venoms cause the release of ACh from nerve terminals (Osman *et al.*, 1976). However, in the present study, atropine had no significant effect on the contractile responses to venom in ileum, indicating a lack of activity, direct or indirect, at muscarinic receptors.

As previously mentioned, PLA_2 has been identified in inland taipan venom (Tan and Ponnudurai, 1990). As PLA_2 hydrolyses membrane phospholipids, it is possible that the

venom may activate the eicosanoid pathway to cause the contractile response seen in ileum. In the present study, the phospholipase inhibitor 4-BPB abolished the venom response, suggesting that the activity seen in the ileum is due primarily to the presence of PLA_2 and, therefore, activation of the eicosanoid pathway. In addition, the cyclooxygenase inhibitor indomethacin significantly inhibited the response to venom in ileum. This finding indicates that the contractile response to the venom is partially mediated by the synthesis of cyclooxygenase metabolites (e.g. prostaglandins, thromboxane).

These findings are consistent with the nature of the contractile response in ileum. It has been previously shown that responses to PLA_2, isolated from the venom of *Vipera ammodytes*, were significantly reduced upon second administration in ileum (Sket and Gubensek, 1976), and responses to PLA_2 in the guinea-pig lung parenchyma are not repeatable owing to rapid homologous desensitisation (Ohara *et al.*, 1995). These findings may also explain the stability of the venom at temperatures up to 37°C, as PLA_2 is known to be heat stable (Iwanaga and Suzuki, 1978).

Many snake venoms contain, or cause the release of, histamine and 5-HT, either directly or by causing the degranulation of mast cells. There is evidence that venom PLA_2 may cause such a release of inflammatory mediators (Rosenberg, 1978; Nagai *et al.*, 1991; Lloret and Moreno, 1993). To investigate this, guinea-pigs were sensitised to chicken egg albumin and, on the day of experimentation, mast cells in segments of isolated ileum were degranulated with successive administrations of egg albumin. Under these conditions the contractile response to venom was significantly inhibited, indicating that the response to the venom may be partly due to the release of inflammatory mediators from mast cells. To investigate this further, the histamine receptor antagonist mepyramine and 5-HT receptor desensitisation were used. Interestingly, the venom was not significantly inhibited by either. This suggests that the contractile activity of the venom may be due to inflammatory mediators other than 5-HT and histamine, such as platelet activating factor or leukotrienes which are also released during an anaphylactic response.

Hamilton *et al.* (1980) showed that inland taipan venom (0.5 μg/ml) caused an increase in the number of coated omega figures, a loss of synaptic vesicles and an increase in the number of neurofilaments in nerve terminals of the rat phrenic nerve–diaphragm. The authors hypothesised that the venom contained a potent PLA_2 neurotoxin which inhibits the release of ACh from presynaptic nerve terminals. These actions are very similar to presynaptic PLA_2 neurotoxins such as β-bungarotoxin from *Bungarus multicinctus*, crotoxin from *Crotalus durissus*, notexin from *Notechis scutatus scutatus* and taipoxin from *O. s. scuttelatus* (Chang *et al.*, 1977; Chang and Su, 1982). Whilst the effects of these neurotoxins on nerve–muscle preparations display species dependence, generally a triphasic response is observed, consisting of an initial depression of transmitter release, followed by a facilitation and finally a total block (Harvey, 1990).

Therefore, the effects of the venom were investigated in the rat isolated phrenic nerve–diaphragm preparation. Venom (10 μg/ml) caused abolition of stimulation-induced contractions after 50 min, without exhibiting the initial depression and subsequent facilitation described above. However, it has been shown that in mammalian nerve–muscle preparations the early depression may be independent of PLA_2, and the facilitation and the blocking phase dependent on PLA_2 (Chang and Su, 1982). Moreover, notexin does not display the second phase of facilitation even though PLA_2 is present (Harris, 1991). The activity observed in the present study is most likely to be

due to paradoxin, a potent presynaptic neurotoxin previously identified in the venom of *O. microlepidotus* (Fohlman, 1979).

The rat isolated vas deferens was used to investigate the possibility of adrenergic activity of the venom. Venom displayed no contractile activity in epididymal segments of vas deferens, indicating an apparent lack of α_1-adrenoceptor activity. However, venom caused inhibition of electrically induced contractions in stimulated prostatic segments of vas deferens, which was not significantly inhibited by the α_2-adrenoceptor antagonist idazoxan. Therefore, the venom may be producing its effects by a different mechanism. Indeed, it has been demonstrated that crotoxin causes inhibition of the response to field stimulation in guinea-pig vas deferens, possibly due to the release of prostaglandins (Anadon and Martinez-Larranaga, 1985).

Inland taipan envenomation is known to cause tachycardia (Mirtschin *et al.*, 1984). Therefore, it was of interest to investigate the direct action of the venom on isolated atria. Venom caused positive chronotropic and inotropic effects. These findings are interesting in light of the results of the experiments conducted in the anaesthetised rat. In the latter preparation venom (10 μg/kg, i.v.) caused a slow-onset, prolonged depression of blood pressure which was sometimes associated with temporary respiratory cessation. Accompanying this response was a slow-onset, prolonged fall in heart rate. Preliminary studies found that artificially respiring the animal had no effect on the hypotension or cardiovascular collapse seen at high doses (i.e. 50 μg/kg, i.v.). The mechanism behind the hypotension produced by venom in the anaesthetised rat was examined on an isolated vascular preparation. Venom caused relaxation in rat endothelium-intact and endothelium-denuded aortic rings. However, the relaxation seen in the endothelium-denuded aortae was notably less than the relaxation seen in endothelium-intact aortae. The response in endothelium-intact aortae was significantly inhibited by the nitric oxide synthase inhibitor NOLA but not by indomethacin. These findings suggest that the venom causes the release of nitric oxide as well having a direct action on the smooth muscle which does not involve the release of prostaglandins. Indeed, PLA_2 isolated from the venom of *Vipera russelli* produces endothelium-independent relaxation which is partially mediated by lipoxygenase products and cyclic GMP (Huang and Lee, 1985).

To confirm the presence of PLA_2 in the venom, a functional PLA_2 radial diffusion assay was conducted. PLA_2 was detected in the venom by its ability to hydrolyse phospholipids in an agarose gel. Controversy exists as to whether or not the enzymatic activity of PLA_2 in venoms is directly proportional to venom toxicity (Fatehi *et al.*, 1994). It has been found that the enzymatic activity in the five variants of β-bungarotoxin does not correlate with toxicity (Kondo *et al.*, 1982a,b). If this is correct, a quantitation of the PLA_2 enzymatic activity obtained from the assay would have no bearing on the toxicity of the venom. Therefore, no conclusions will be drawn from the assay apart from the apparent presence of PLA_2.

In conclusion, inland taipan venom liberates mediators of the eicosanoid pathway to cause contraction in the guinea-pig isolated ileum, and produces hypotension in the anaesthetised rat, relaxation of rat isolated aortic rings and neuromuscular blockade.

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