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Short communication

The smooth muscle relaxant effects of venom from the inland taipan (*Oxyuranus microlepidotus*)

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Abstract

Venom (10 μg/ml) relaxed phenylephrine-precontracted aortae. This relaxation was unaffected by removal of the endothelium or a combination of N^G-nitro-L-arginine (L-NOARG; 0.1 mM), oxyhaemoglobin (10 μM) and indomethacin (10 μM). 4-BPB (0.78 mM), propranolol (1 μM), or a combination of apamin (0.1 μM), charybdotoxin (0.1 μM) and glibenclamide (10 μM) did not effect endothelium-independent relaxation, suggesting a lack of PLA_2 activity or an effect at β-adrenoceptors or K^+ channels. Venom (10 μg/ml) reversed Bay K 8644 (0.1 μM)-induced contraction indicating the venom may have an effect on L-type Ca^{2+} channels.

Despite the extreme lethality of *Oxyuranus microlepidotus* venom (Broad et al., 1979; Sutherland, 1984) little is known about its pharmacological activity. Among other effects, we have previously reported that the venom produces endothelium-independent relaxation of rat isolated aortae (Bell et al., 1997). The purpose of the present study was to further characterize this activity.

Inland taipans were collected from Goyders Lagoon in north eastern South Australia and venom prepared by Mr P. Mirtschin (Venom Supplies, South Australia). Freeze dried venom was dissolved in 0.1% bovine serum albumin.

Thoracic aortae were dissected from male Sprague Dawley rats killed by CO_2 asphyxiation and exsanguination. Where indicated, endothelial cells were removed

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by rubbing the intimal surface with a wooden rod. Rings (5 mm in length) were mounted between two stainless steel hooks under 10 g tension. Tissues were placed in organ baths containing Krebs solution (composition in mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.17, KH_2PO_4 1.18, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 11) and bubbled with carbogen (5% CO_2; 95% O_2) while maintained at 37°C. Contractions were recorded on a Grass Polygraph (model 79D) via a FT03 transducer. A paired Student's *t*-test was used to compare agonist responses before and after antagonist/inhibitors in the same tissue. As the venom response was not reproducible upon subsequent administration responses from different tissues were compared using an unpaired Student's *t*-test. Statistical significance is indicated by $P < 0.05$.

Aortic rings were precontracted to approximately 50% of tissue maximum, as determined by K^+ (124 mM) depolarizing solution, with phenylephrine (3–100 nM). Venom (10 μg/ml) caused relaxation in endothelium intact aortic rings (90% ± 5%, $n = 4$). This response was not significantly different in endothelium denuded aortae (100% ± 0%, $P > 0.05$, $n = 4$). In endothelium intact aortic rings, a combination of the nitric oxide (NO) synthase inhibitor, L-NOARG (0.1 mM), the NO scavenger, oxyhaemoglobin (10 μM) and the cyclo-oxygenase inhibitor indomethacin (10 μM) significantly inhibited the response to acetylcholine (10 μM; before 92% ± 5%; after 1% ± 0%; $P < 0.05$, $n = 3$) but had no significant effect on relaxation to venom (10 μg/ml; before 90% ± 5%; after 100% ± 0%; $P > 0.05$, $n = 3$). In endothelium denuded aortic rings, the β-adrenoceptor antagonist proproanolol (1 μM) significantly inhibited the response to isoprenaline (1 μM; before 93% ± 2%; after 0% ± 0%; $P < 0.05$, $n = 4$) but had no significant effect on the venom response (10 μg/ml; before 100% ± 0%; after 98% ± 2%; $P < 0.05$, $n = 4$). A combination of the medium and large conductance Ca^{2+}-activated K^+ channel antagonist, charybdotoxin (0.1 μM), the small conductance Ca^{2+}-activated K^+ channel antagonist, apamin (0.1 μM) and the ATP-sensitve K^+ antagonist, glibenclamide (10 μM) had no significant effect on the venom response (10 μg/ml; before 100% ± 0%; after 100% ± 0%; $P > 0.05$, $n = 4$) in endothelium denuded aortic rings. Incubation of the venom with the phospholipase A enyme inhibitor 4-bromophenacyl bromide (0.78 mM) had no significant effect on the venom response (10 μg/ml; before 100% ± 0%; after 91% ± 5%; $P > 0.05$, $n = 3$). Venom (10 μg/ml) caused relaxation (78% ± 11, $n = 4$) of endothelium denuded aortae precontracted with the calcium channel opener Bay K 8644 (0.1 μM) (Fig. 1).

The results of the present study indicate that *O. microlepidotus* venom contains a component that is capable of producing relaxation of vascular smooth muscle via a mechanism independent of the endothelial cell layer. In addition, this relaxation does not appear to be mediated by the activation of β-adrenoceptors, K^+ channels or be dependent on a phospholipase component in the venom. Preliminary evidence suggests that the venom may have an effect on L-type Ca^{2+} channels. Indeed, a blocker of high threshold cardiac calcium channels, taicatoxin, has been isolated from the venom of the closely related coastal taipan (*O. scutellatus*) (Brown et al., 1987; Possani et al., 1992). Isolation of the component

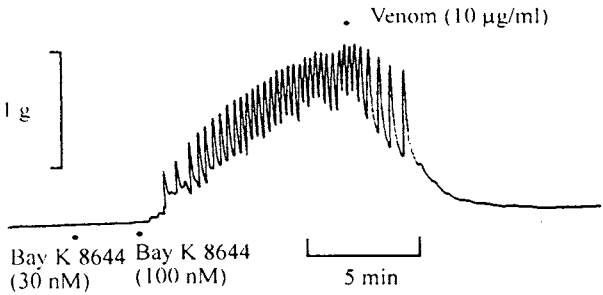

Fig. 1. Trace showing reversal of Bay K 8644 (100 nM) precontraction by venom (10 μg/ml) in an endothelium denuded aortic ring.

in *O. microlepidotus* venom responsible for this activity is required to further elucidate its mechanism of action.

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