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The effect of a tachykinin NK_1 receptor antagonist, SR140333, on oedema formation induced in rat skin by venom from the *Phoneutria nigriventer* spider

*R.T. Palframan, S.K.P. Costa, P. Wilsoncroft, *E. Antunes, *G. de Nucci & [1]S.D. Brain

Pharmacology Group, Division of Biomedical Sciences, King's College, Manresa Road, London SW3 6LX and *Department of Pharmacology, Faculty of Medical Sciences, UNICAMP, PO Box 61111, Campinas, São Paulo, Brazil

1 The possibility that tachykinin NK_1 receptors are involved in the plasma extravasation evoked by intradermal (i.d.) injection of *Phoneutria nigriventer* venom (PNV) in rat dorsal skin *in vivo* has been investigated.

2 Local oedema formation induced by the i.d. injection of test agents was measured by the extravascular accumulation of intravenously (i.v.) injected ^{125}I-labelled human serum albumin over a 30 min period.

3 The tachykinin NK_1 agonist, GR73632 (30 pmol per site), induced local oedema formation which was potentiated by co-injection with the neuropeptide vasodilator, calcitonin gene-related peptide (CGRP, 10 pmol per site). The non-peptide tachykinin NK_1 receptor antagonist, SR140333 (0.03-1 nmol per site co-injected, i.d.) significantly inhibited (0.3 nmol per site, $P<0.05$; 1 nmol per site, $P<0.001$) local oedema formation induced by GR73632 with CGRP but not that induced by histamine (10 nmol per site) with CGRP.

4 PNV (0.03-0.3 μg per site) injected i.d. induced dose-dependent local oedema formation. SR140333 (1 nmol per site, co-injected i.d.) inhibited oedema formation; with complete inhibition observed at doses of 0.03 μg ($P<0.05$) and 0.1 μg ($P<0.001$); and partial inhibition (50%) observed with the highest dose of PNV, 0.3 μg ($P<0.05$).

5 Local oedema formation induced by PNV was not affected by systemic pretreatment with the bradykinin B_2 receptor antagonist, Hoe 140 (80 nmol kg^{-1}, i.v.), which was used at a dose which significantly inhibited oedema formation by bradykinin (1 nmol per site).

6 Local oedema formation induced by PNV was significantly inhibited ($P<0.01$) by co-injection of the histamine H_1 receptor antagonist, mepyramine (2.5 nmol per site), together with the 5-hydroxytryptamine (5-HT) antagonist, methysergide (2.8 nmol per site).

7 In the presence of all three antagonists (mepyramine 2.5 nmol per site; methysergide, 2.8 nmol per site and SR140333 1 nmol per site), the plasma extravasation induced by PNV was further significantly inhibited ($P<0.001$, when compared with PNV injected i.d. alone; $P<0.05$ when compared with PNV co-injected with mepyramine and methysergide and $P<0.01$, when compared with PNV co-injected with SR140333).

8 These results suggest that oedema formation evoked by i.d. PNV in rat skin may be partially mediated via a mechanism involving tachykinin NK_1 receptors and that this effect is independent of histamine and 5-HT.

Keywords: *Phoneutria nigriventer* venom; oedema; skin; SR140333; tachykinins; NK_1 receptors

Introduction

The 'armed spider' *Phoneutria nigriventer*, commonly found in Brazil, has a venom which can have fatal effects in a variety of species, including man (Lucas, 1988). *Phoneutria nigriventer* venom (PNV) induces local oedema formation when injected intradermally (i.d.) in rat and rabbit skin. Oedema formation in both rat and rabbit skin is thought to be mediated by components other than the neurotoxic polypeptides which are responsible for the major toxic effects of the venom (Antunes *et al.*, 1992). PNV has been found to contain varying amounts of the inflammatory mediators histamine (0.06-1%) 5-hydroxytryptamine (5-HT, 0.03-0.25%) (Schenberg & Pereira-Lima, 1971). In rabbit skin, local oedema formation is independent of the histamine and 5-HT components, and is at least partly mediated by activation of the tissue kallikrein-kinin system (Marangoni *et al.*, 1993). In contrast, local oedema formation in rat skin is partially dependent upon the histamine and 5-HT present in the venom, as determined by the level of oedema observed following dialysis of the venom to remove the histamine and 5-HT. However, dialysing the whole venom, or pretreatment of the rats with histamine and 5-HT antagonists, does not abolish the local oedema formation induced by i.d. injection of PNV (Antunes *et al.*, 1992).

In this study we have investigated the possibility that, in rat skin, PNV mediates increased microvascular permeability via a tachykinin-dependent mechanism. The non-peptide tachykinin neurokinin-1 (NK_1) antagonist, SR140333 (Edmonds-Alt *et al.*, 1993) was used to investigate the involvement of NK_1 receptors in oedema formation induced by PNV.

Methods

Oedema formation in response to i.d. agents was measured by the accumulation of ^{125}I-labelled human serum albumin injected i.v. (Brain & Williams, 1985; Escott & Brain, 1993). Male Wistar rats (200-250 g) were anaesthetized with sodium

[1] Author for correspondence.

pentobarbitone (Sagatal, 40–50 mg kg^{-1}, i.p.) and maintenance doses given as required. The dorsal skin was shaved, and injection sites marked out to give a balanced site pattern. Each test agent was given in duplicate, one injection on each flank of the rat, according to a randomized injection protocol. Evan's blue dye (0.3 ml of 2.5% w/v solution made up in saline) and [^{125}I]-albumin (100 kB_q) were injected into the tail vein. Test agents (0.1 ml/site made up in Tyrode solution and kept on ice until use) were injected i.d.. Antagonists were injected as required by specific protocols. Following a 30 min accumulation period a blood sample was taken via cardiac puncture and the rat killed by cervical dislocation. The blood was centrifuged at 10,000 *g* for 4 min to obtain a plasma sample. The dorsal skin was removed from the rat, and the injection sites were punched out (16 mm diameter), the Evan's blue dye being used as a visual marker of plasma extravasation. The skin sites and plasma extravasation calculated by comparing the ^{125}I activity in the skin sites with that in 100 μl of plasma.

Materials

Mepyramine maleate, histamine, substance P, bradykinin and compound 48/80 were purchased from Sigma Chemical Company, Poole, U.K. Human α-calcitonin gene-related peptide (CGRP) was purchased from Bachem U.K., Saffron Walden, Essex. Hoe 140 (D-[Arg-Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin was purchased from Peninsula Laboratories, St Helens U.K. SR140333 ((S)1-{2-[3(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl) piperidine-3-yl]ethyl}-4-phenyl-1-azoniabicyclo [2.2.2]octone, chloride), was a gift from Dr Emonds-Alt, Sanofi Recherche, Montpellier, France. GR73632 (δAva[L-Pro^9,N-MeLeu^{10}]SP(7-11)), was a gift from Dr D. Beattie, Glaxo Group Research, Ware, U.K. Methysergide maleate was a gift from Sandoz Products Ltd, Eltham, Middx. ^{125}I-labelled human serum albumin was purchased from Amersham International, Amersham, U.K. Sodium pentobarbitone (Sagatal) was purchased from May & Baker, Dagenham, U.K. Lyophilized *Phoneutria nigriventer* venom (PNV), collected from spiders by electrical stimulation, was purchased from the Butantan Institute, Sao Paulo, Brazil. Modified Tyrode solution was made up as follows (mM): NaCl 137, KCl 2.7, $MgCl_2$ 0.5, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.6.

Statistical analysis

Results are shown as mean values for plasma extravasation ± s.e.mean. The one-way analysis of variance (ANOVA) test followed by Bonferroni's modified *t* test was performed to allow comparison of multiple injection sites.

Results

Local oedema formation induced by the peptide NK_1 agonist, GR73632 (30 pmol per site) was potentiated by co-injection of the neuropeptide vasodilator CGRP (10 pmol per site), see Figure 1, as expected (see Brain & Williams, 1985). Oedema formation induced by co-injection of GR73632 with CGRP was reduced by co-injection together with the NK_1 receptor antagonist SR140333 (0.03–1 nmol per site). SR140333 (1 nmol per site) did not reduce oedema formation induced by histamine (10 nmol per site) co-injected with CGRP (10 nmol per site) as also shown in Figure 1.

Intradermal injection of PNV venom (0.03–0.3 μg per site) induced local oedema formation in a dose-related manner as previously described by Antunes *et al.* (1992). Oedema formation induced by PNV was significantly (0.03 and 0.3 μg per site, $P<0.05$; 0.1 μg per site, $P<0.001$) reduced at all doses when co-injected with SR140333, as shown in Figure 2. The bradykinin B_2 receptor antagonist, Hoe 140 (80 nmol kg^{-1}) significantly inhibited oedema formation induced by bradykinin in the presence and absence of CGRP, but had no effect on oedema formation induced by PNV as shown in Figure 3. In these experiments CGRP did not significantly potentiate the oedema induced by bradykinin. The reason for this is unknown. Mepyramine, a histamine H_1 receptor antagonist, and methysergide, a 5-HT receptor antagonist, were co-injected together with test agents, as shown in Figure 4. Local oedema induced by the mast cell amine releasing compound, compound 48/80 (500 ng per site), was abolished by co-injection with mepyramine (2.5 nmol per site) and methysergide (2.8 nmol per site). PNV (0.3 μg per site) injected i.d. induced local oedema formation which was significantly inhibited ($P<0.01$) by co-injection with mepyramine (2.5 nmol per site)

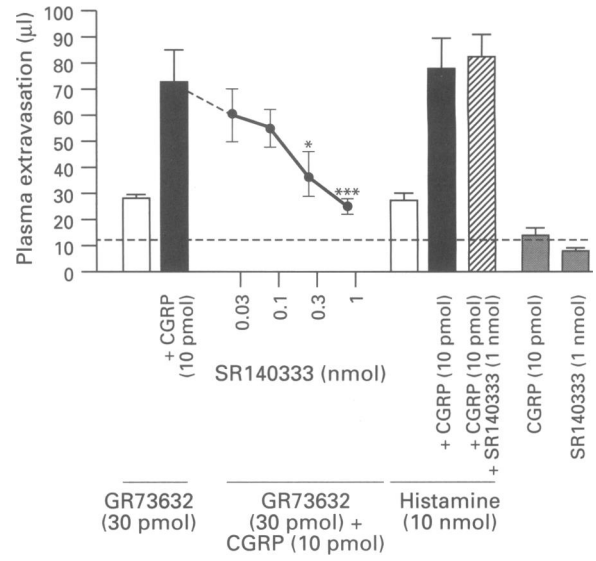

Figure 1 The effect of SR140333 on oedema induced by GR73632 and histamine. Oedema induced by GR73632 (30 nmol per site) and histamine (10 nmol per site) is shown by the open columns. GR73632 or histamine co-injected with CGRP (10 pmol $site^{-1}$) is shown by the solid columns. The effect of co-injection of the NK_1 antagonist SR140333 (0.03–1 nmol per site ●) together with GR73632 and CGRP (10 pmol per site) or with histamine and CGRP (10 pmol per site) is shown by the hatched column. CGRP (10 pmol per site) or SR140333 (1 nmol per site) injected alone is also shown. The effect of Tyrode solution injected alone is shown by the dotted line. ***$P<0.001$, *$P<0.05$, when compared with GR73632 co-injected with CGRP. Values are mean ± s.e.mean, $n=5–6$.

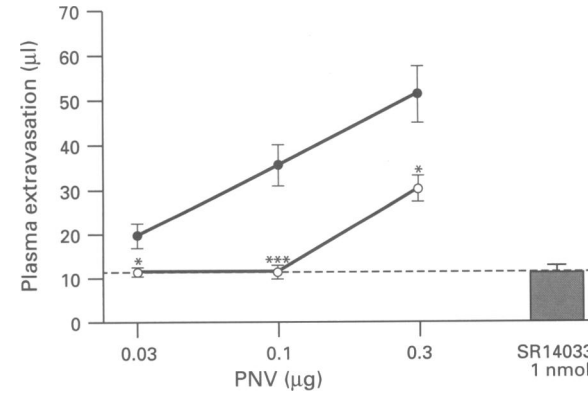

Figure 2 Local oedema formation induced by *Phoneutria nigriventer* venom (PNV). Venom (0.03–0.3 μg per site) was injected alone (●) or co-injected with the NK_1 antagonist SR140333 (1 nmol per site). SR140333 injected alone is shown by the stippled column. The dotted line represents Tyrode injected alone. *$P<0.05$, ***$P<0.001$, Bonferroni's modified *t* test, when compared to PNV injected alone. Values are means ± s.e.mean. $n=6$.

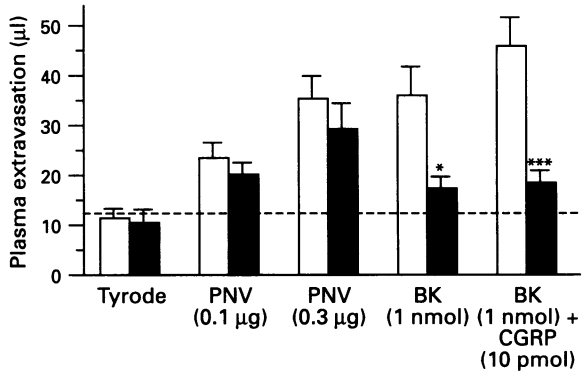

Figure 3 The effect of a bradykinin B_2 receptor antagonist on oedema induced by PNV in rat skin. Oedema induced by PNV (0.1–0.3 µg per site) and for bradykinin (1 nmol per site) ± CGRP (10 nmol per site) is shown for control rats (saline i.v.; open columns) and for rats which received Hoe 140 (80 nmol kg^{-1} i.v.; closed columns). The dotted line represents Tyrode solution injected alone. ***$P<0.001$, *$P<0.05$, when compared to comparative response in control rats. Values are mean ± s.e.mean, $n=6$.

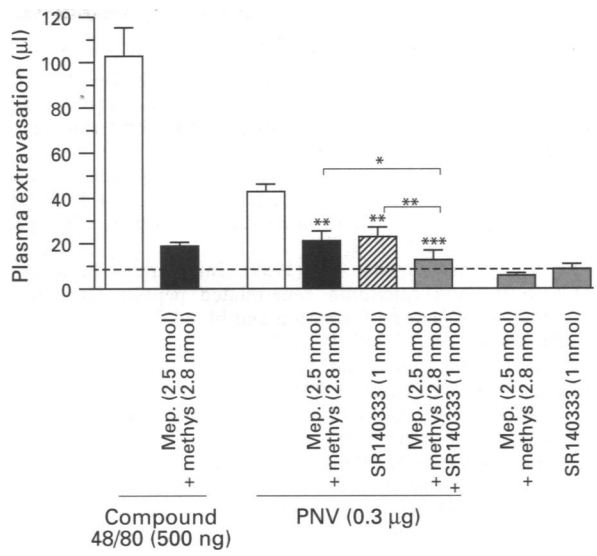

Figure 4 The effect of histamine and 5-HT antagonists in combination with the NK_1 receptor antagonist on oedema induced by PNV in rat skin. Oedema induced by compound 48/80 (500 ng per site), and by PNV (0.3 µg per site) is shown by open columns. Oedema induced by compound 48/80 and PNV co-injected with mepyramine (Mep 2.5 nmol per site) and methysergide (Methys, 2.8 nmol per site) is shown by the solid columns. PNV (0.3 µg per site) co-injected with only SR140333 (1 nmol per site) is shown by the hatched column. The effect of all three antagonists on the response to PNV (0.3 µg per site) is shown by the dark stippled columns. Mepyramine (2.5 nmol per site) and methysergide (2.8 nmol per site) injected alone, and SR140333 (1 nmol per site) injected alone are shown by the light stippled columns. The dotted line represents Tyrode solution injected alone. ***$P<0.001$, **$P<0.01$, *$P<0.05$, when compared to PNV injected alone, or as illustrated. Values are mean ± s.e.mean, $n=6$.

and methysergide (2.8 nmol per site), and by co-injection with SR140333 (1 nmol per site). Oedema induced by PNV (0.3 µg per site) in the presence of all three antagonists mepyramine (2.5 nmol per site), methysergide (2.8 nmol per site) and SR140333 (1 nmol per site), was significantly inhibited when compared to oedema formation induced by PNV co-injected with mepyramine and methysergide ($P<0.05$), or when compared to PNV co-injected with SR140333 ($P<0.01$).

Discussion

The results of this study suggest that, in rat skin, local oedema formation induced by i.d. injection of PNV is partially dependent on a tachykinin NK_1 receptor-mediated mechanism. This was demonstrated by use of the non-peptide NK_1 receptor antagonist, SR140333, which has been shown to act as a potent NK_1 receptor antagonist *in vitro* and *in vivo* in several species (Emonds-Alt *et al.*, 1993). Our results confirm the selectivity of SR140333 at NK_1 receptors and demonstrate its usefulness when injected intradermally in rat skin.

It has been previously demonstrated that PNV contains histamine (0.06–1%) and 5-HT (0.03–0.25%; Schenberg & Pereira-Lima, 1971) both of which are potent oedema-inducing agents in rat skin (see Brain & Williams, 1989; Newbold & Brain, 1993). A combination of the histamine H_1 receptor antagonist, mepyramine and the 5-HT_2 receptor antagonist, methysergide, significantly inhibited oedema formation induced by PNV, suggesting that sufficient histamine and 5-HT were present in our samples, or released from skin mast cells, to promote oedema formation. Interestingly, SR140333, in the presence of mepyramine and methysergide, further inhibited oedema formation. This suggests that the mechanism which activates NK_1 receptors is independent of histamine H_1 and 5-HT receptor mechanisms.

PNV is extremely painful when injected subcutaneously in a variety of species (Schenberg & Pereira-Lima, 1971). It is therefore possible that the venom acts in a similar manner to capsaicin, simultaneously stimulating the afferent and efferent functions of sensory nerves. Although PNV contains neurotoxins with molecular weights in the range of 6,000–9,000 (Rezende *et al.*, 1991), our previous study demonstrated that the venom was still able to induce oedema formation after dialysis to remove components with molecular weights less than 12,000–14,000 (Antunes *et al.*, 1992). Therefore it is unlikely that neurotoxins are responsible for the NK_1 receptor-mediated oedema formation observed in the present study.

The mechanism by which PNV mediates NK_1 receptor-dependent plasma extravasation is not clear and will be the subject of further study. It is possible that PNV contains components which act as NK_1 agonists in rat skin. PNV may also contain components which directly or indirectly stimulate the release of vasoactive neuropeptides from sensory nerve terminals. The possible involvement of kinin generated by PNV, as observed in rabbit skin (Marangoni *et al.*, 1993), has been investigated in this study through the use of Hoe 140. It has previously been shown in certain systems that some of the acute inflammatory effects mediated by bradykinin are secondary to the activation of the efferent function of sensory nerves (see Walker *et al.*, 1995, for review). However in the case of PNV it would appear that B_2 receptors are not involved in releasing NK_1 agonists.

One aim of our studies on PNV is to suggest therapeutic approaches which can be used to support conventional polyclonal antibody therapy, especially in the early stages after injection of the venom when the predominant symptoms are pain and increased nasal/bronchial secretions (Schenberg & Pereira Lima, 1971). Based on the present results, we conclude that NK_1 receptor antagonists may be worth pursuing as a novel therapeutic approach in the clinic.

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