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Short communication

Phoneutria nigriventer spider venom induces oedema in rat skin by activation of capsaicin sensitive sensory nerves

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Abstract

Phoneeutria nigriventer venom induces oedema formation when injected in the rat dorsal skin and such oedema is, in part, dependent on the stimulation of tachykinin NK_1 receptors. This study investigated whether *Phoneutria nigriventer* venom acts directly on tachykinin NK_1 receptors, or indirectly to activate sensory neurones which in turn release a tachykinin NK_1 receptor agonist. The plasma extravasation induced by *Phoneutria nigriventer* venom (1–10 μg/site) in neonatally capsaicin (8-methyl *N*-vanillyl-6-nonenamide)-pretreated rats was substantially attenuated ($P < 0.05$) but the response to either the tachykinin NK_1 receptor agonist GR73632 ((δAva[L-Pro^9, *N*-MeLeu10] substance P-(7–11) 30 pmol/site) or bradykinin (0.3–3 nmol/site) was not affected. These results indicate that *Phoneutria nigriventer* venom stimulates sensory nerves indirectly. The lack of effect of capsaicin-pretreatment on the GR73632 and bradykinin responses indicated that the tachykinin NK_1 and bradykinin B_2 receptors remained functional. There was no evidence to suggest that *Phoneutria nigriventer* venom contains a tachykinin NK_1 receptor agonist. © 1997 Elsevier Science B.V.

Keywords: Sensory nerves; Capsaicin; *Phoneutria nigriventer* venom; Neurogenic oedema; Tachykinin NK_1 receptor agonist

1. Introduction

It is well established that acute electrical or chemical (e.g. capsaicin, 8-methyl *N*-vanillyl-6-nonenamide) stimulation of sensory nerve fibres leads to the release of neuropeptides (e.g. tachykinins such as substance P and also calcitonin gene-related peptide) and local inflammatory effects (Lembeck and Holzer, 1979; Escott and Brain, 1993). By comparison, it is well known that the levels of substance P are substantially and irreversibly reduced in primary sensory neurones when rats are treated with capsaicin at a neonatal stage (Gamse et al., 1980).

Venom from the spider *Phoneutria nigriventer* has a range of effects on both the central and peripheral nervous systems and on peripheral tissues (Entwistle et al., 1982; Diniz et al., 1990; Lopes-Martins et al., 1994; Costa et al., 1996). Indeed, *Phoneutria nigriventer* venom potently stimulates inflammatory oedema formation in rabbit and rat skin (Antunes et al., 1992, 1993; Marangoni et al., 1993; Palframan et al., 1996). Previous work has shown that the *Phoneutria nigriventer* venom-induced oedema in rats is mediated through tachykinin NK_1 receptor since the oedema is inhibited by the selective tachykinin NK_1 receptor antagonist 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), see Palframan et al., 1996). The present study was undertaken to determine whether *Phoneutria nigriventer* venom acts directly on tachykinin NK_1 receptors, or indirectly by activating sensory neurones which release a tachykinin NK_1 receptor agonist.

2. Materials and methods

2.1. Animals and capsaicin desensitisation

The experiments were carried out in Wistar rats of both sexes bred in the Departmental Animal House of the Faculty of Medical Science, UNICAMP (São Paulo, Brazil). Rats were pretreated subcutaneously (s.c.) on the second day of life with capsaicin (50 mg/kg) or the corresponding volume (100 μl) of capsaicin-vehicle (10% ethanol and 10% Tween 80, in 0.9% (w/v) NaCl solution)

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under ether anaesthesia (Jancsó et al., 1977). The rats were used 12–16 weeks after capsaicin pretreatment at which time they weighed 200–300 g. The animals were anaesthetised with sodium pentobarbitone (50 mg/kg, i.p.) for all of the oedema experiments.

2.2. Measurement of blood pressure

The mean arterial blood pressure was measured by an ultrasound tail-cuff method (Zatz, 1990) in two groups of conscious, randomised vehicle- and capsaicin-pretreated rats ($n = 11$).

2.3. Measurement of hind paw and rat dorsal skin oedema

After the induction of anaesthesia, the hind limbs and/or dorsal skin was shaved and a mixture of Evan's blue dye (25 mg/kg) with ^{125}I-albumin (50 kBq) was then injected via the tail vein. 5 min later, capsaicin-vehicle (100 μl) or capsaicin solution (5%) was applied by a pipette to the dorsal surface of the right or left hind paw of capsaicin- or vehicle-pretreated animals ($n = 7$). In separate experiments, the dorsal skin oedema formation in response to the intradermal (i.d.) injection of *Phoneutria nigriventer* venom (1–10 μg/site), the tachykinin NK_1 receptor agonist GR73632 (30 pmol/site) or bradykinin (0.3–1 nmol/site) was measured. The tachykinin NK_1 receptor antagonist SR140333 (1 nmol/site) was co-injected with *Phoneutria nigriventer* venom (1–10 μg/site) or GR73632 (30 pmol/site) into the dorsal skin of capsaicin- or vehicle-pretreated animals. 30 min later, a blood sample was obtained from all animals which were then killed by cervical dislocation. The treated skin area of both paws was removed and weighed. The oedema formation was expressed as plasma volume/100 mg of paw skin by comparison with the level of ^{125}I-albumin present in plasma. For the measurement of dorsal skin oedema formation, the injection sites were punched out and oedema formation was expressed as plasma volume/skin site (Palframan et al., 1996).

2.4. Venom and drugs

Phoneutria nigriventer venom was obtained from the Arthropods Section of the Instituto Butantan (São Paulo, Brazil). The venom (10 ml of a 2 mg/ml solution in 0.9% (w/v) NaCl) was dialysed (MW cutoff, 12,000–14,000) for up to 48 h at 4–6°C against 2 l of saline in order to remove histamine and 5-HT (Antunes et al., 1992). Capsaicin, bradykinin and dialysis tubing were purchased from Sigma Chemical Co. (St. Louis, MO). ^{125}I-Human serum albumin and sodium pentobarbitone were purchased from Amersham International (Amersham, UK) and Rhone Merieux (Dublin, Ireland), respectively. GR73632 (δAva[L-Pro^9, *N*-MeLeu10] substance P-(7–11) was a gift from Dr. D. Beattie, Glaxo Group Research (Ware, UK). 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). *Phoneutria nigriventer* venom and test agents were stored at −20°C and diluted with a modified Tyrode solution composition (in mM: NaCl, 137; KCl, 2.7; $MgCl_2$, 0.5; NaH_2PO_4, 0.4; $NaHCO_3$, 11.9 and glucose, 5.6) immediately prior to use.

2.5. Analysis of results and statistics

The results are presented as the mean values for plasma extravasation ± S.E.M. for n experiments. Results not shown here indicated that neonatal capsaicin pretreatment was equally effective in rats of both sexes. Thus, the data from both sexes were pooled to form a single group. The multiple injection sites and tail-cuff pressures were evaluated by analysis of variance (ANOVA) followed by Bonferroni's modified t-test. A p-value < 0.05 was considered to indicate significance.

3. Results

3.1. Effect of neonatal capsaicin pretreatment on blood pressure and on oedema formation induced by topical capsaicin

The mean arterial blood pressure in conscious, vehicle-pretreated rats (127 ± 3 mm Hg; $n = 5$) was not significantly different from that of conscious, capsaicin-pretreated rats (125 ± 9 mm Hg; $n = 6$).

Topical administration of 5% capsaicin solution onto the dorsal hind paw skin of vehicle-pretreated rats significantly increased ($P < 0.05$) the oedema formation compared to the response obtained following the administration of vehicle to the contralateral paw (10.5 ± 0.9 and 6.2 ± 0.3 μl, respectively; $n = 4$ each). In rats pretreated neonatally with capsaicin, the plasma extravasation induced by the topical administration of 5% capsaicin (6.0 ± 1 μl) was not different from that of the contralateral paw treated with vehicle (6.2 ± 0.2 μl) but was significantly reduced ($P < 0.05$; $n = 7$) when compared to the response to topical capsaicin seen in control rats pretreated neonatally with the vehicle.

3.2. Effect of neonatal pretreatment with capsaicin on plasma protein extravasation induced by the i.d. injection of agents

Fig. 1 shows that the tachykinin NK_1 receptor agonist GR73632 (30 pmol/site, i.d.) caused a similar significant plasma protein exudation in the skin of vehicle- (65 ± 7 μl; $n = 6$) and capsaicin-pretreated (57 ± 4 μl; $n = 6$) rats. This response was markedly reduced by the co-injection of the tachykinin NK_1 receptor antagonist SR140333 (1 nmol/site) in all rats.

Phoneutria nigriventer venom induced significant dose-dependent oedema formation in the skin of neonatal

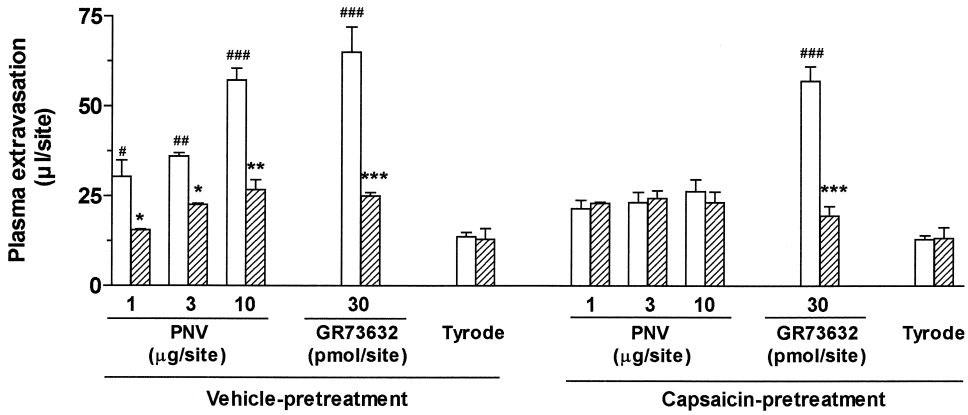

Fig. 1. Effect of neonatal capsaicin-pretreatment on *Phoneutria nigriventer* venom (PNV)-induced oedema formation. The response to venom (1–10 µg/site) and the tachykinin NK_1 receptor agonist GR73632 (30 pmol/site) in the absence (open bars) and in the presence (hatched bars) of the tachykinin NK_1 receptor antagonist SR140333 (1 nmol/site) is shown in both vehicle- and capsaicin-pretreated rats. The effect of Tyrode solution injected alone is also shown for both groups. The results are expressed as the mean ± S.E.M. for each group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$, compared to venom in the absence of SR140333. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, compared to the Tyrode group of vehicle- or capsaicin-pretreated rats.

vehicle-pretreated rats (Fig. 1). As expected, this response was reduced by the concomitant administration of SR140333 (1 nmol/site) (Fig. 1; see Palframan et al., 1996). In contrast, the oedema induced by *Phoneutria nigriventer* venom (1–10 µg/site) in neonatal capsaicin-pretreated rats skin was markedly reduced such that significant oedema was not observed (Fig. 1). The intradermal injection of bradykinin induced dose-dependent plasma protein exudation in both vehicle- and capsaicin-pretreated rats (Fig. 2).

4. Discussion

Various symptoms, including pain and cardiovascular disturbances, are observed when animals and humans are bitten by the armed spider *Phoneutria nigriventer* a species common in Brazil (Lucas, 1988). In addition to polypeptides of different molecular weights, the venom also contains varying amounts of histamine (0.06%) and 5-hydroxytryptamine (5-HT 0.03–0.24%; Schenberg and Pereira-Lima, 1971). We have previously shown that the oedema formation induced by undialysed *Phoneutria nigriventer* venom in rat skin can be reduced, but not abolished, by treatment with histamine and 5-hydroxytryptamine antagonists (Antunes et al., 1992). We confirm here our recent finding (Palframan et al., 1996) that the residual oedema seen with dialysed venom involves a tachykinin NK_1 receptor-mediated mechanism.

The present findings that the oedema formation seen following the i.d. injection of *Phoneutria nigriventer* venom was substantially attenuated in capsaicin-pretreated rats and that a tachykinin NK_1 receptor antagonist did not further reduce the venom-induced oedema, suggest that sensory nerve-mediated mechanisms are involved. The similar extent of oedema formation in response to the tachykinin NK_1 agonist GR73632 in both capsaicin- and

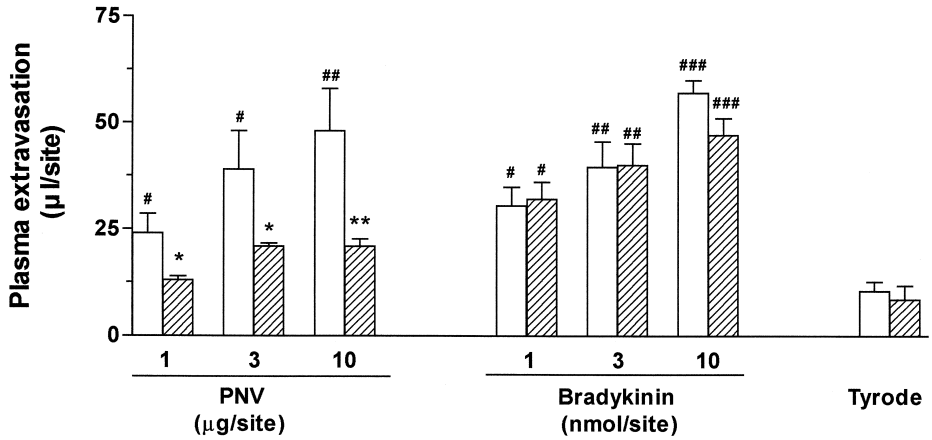

Fig. 2. Effect of neonatal capsaicin-pretreatment on oedema formation induced by bradykinin and *Phoneutria nigriventer* venom (PNV). The results (vehicle-pretreated rats, open bars; capsaicin-pretreated rats, hatched bars) are expressed as the mean ± S.E.M. of 7 rats. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to the vehicle-pretreated group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, compared to the Tyrode group of vehicle- or capsaicin-pretreated rats.

vehicle-pretreated rats indicates that the tachykinin NK_1 receptor remains functional after neonatal capsaicin pretreatment. The protocol used for the neonatal capsaicin pretreatment procedure has been reported to cause the destruction of a large percentage of peripheral, unmyelinated fibres in rats (Jancsó et al., 1977). We have demonstrated here that the procedure was successful since topical capsaicin caused minimal oedema formation in these rats when compared with those pretreated neonatally with vehicle. Moreover, it is unlikely that the reduction in *Phoneutria nigriventer* venom-induced oedema in capsaicin-pretreated rats was caused by disturbances in the cardiovascular system, since the basal mean arterial blood pressure was similar in vehicle- and capsaicin-pretreated rats, thus confirming the observations of others (Bachelard et al., 1992).

Bradykinin causes intense burning pain, probably due to the direct stimulation of nociceptors (Dray and Perkins, 1988) and produces oedema by increasing permeability in the microcirculation via bradykinin B_2 receptors (see Regoli and Barabe (1980), for review). We have shown elsewhere that the bradykinin B_2 receptor antagonist HOE 140 (D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin) inhibits *Phoneutria nigriventer* venom-induced local oedema formation in rabbit skin (Marangoni et al., 1993). Since bradykinin can release substance P from capsaicin-sensitive sensory neurones (Hakanson et al., 1987; Gepetti et al., 1988), we investigated whether the destruction of sensory neurones by neonatal capsaicin pretreatment could modulate the bradykinin response. The oedema formation induced by the i.d. injection of bradykinin in capsaicin-pretreated rats was not statistically different from that in vehicle-pretreated rats. This is in keeping with our previous finding that the B_2 receptor antagonist HOE 140 had no effect on *Phoneutria nigriventer* venom-induced oedema formation in rat skin (Palframan et al., 1996).

In conclusion, the present results indicate that *Phoneutria nigriventer* venom stimulates sensory nerves to release a tachykinin NK_1 receptor agonist which is responsible for the subsequent neurogenic oedema formation. This study provides no evidence to suggest that *Phoneutria nigriventer* venom contains an agent which stimulates the tachykinin NK_1 receptors directly.

Acknowledgements

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