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Peripheral tackykinin and excitatory amino acid receptors mediate hyperalgesia induced by *Phoneutria nigriventer* venom

Eliane Maria Zanchet, Yara Cury*

Laboratório de Fisiopatologia, Instituto Butantan, Avenida Vital Brazil, 1500, 05503-900, São Paulo, SP, Brazil

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Abstract

The generation of hyperalgesia by *Phoneutria nigriventer* venom was investigated in rats using the paw pressure test, through the intraplantar injection of the venom. Hyperalgesia was significantly inhibited by *N*-[2-(4-chlorophenyl) ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2*H*-2-benzazepine-2-carbothioamide (capsazepine), a vanilloid receptor antagonist, by the local administration of pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Pro (spiro-γ-lactam) Leu-Trp-NH_2 (GR82334) or of Phenyl-CO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (GR94800), inhibitors of tachykinin NK_1 and NK_2 receptors, respectively, or by the local injection of dizocilpine (MK 801), (±)-2-amino-5-phosphonopentanoic acid ((±)-AP-5), or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), antagonists of NMDA and non-NMDA excitatory amino acid receptors. The correlation between hyperalgesia and the inflammatory response induced by the venom was also investigated. The venom-induced edematogenic response was not modified by the pharmacological treatments. These results suggest that hyperalgesia induced by *P. nigriventer* venom is mediated by stimulation of capsaicin-sensitive neurons, with activation of peripheral tachykinin NK_1 and NK_2 receptors and of both the NMDA and AMPA receptors. Distinct mechanisms are involved in the development of hyperalgesia and edema induced by the venom.

Keywords: *Phoneutria nigriventer* venom; Hyperalgesia; Vanilloid receptor; Tachykinin receptor; Excitatory amino acid receptor

1. Introduction

The *Phoneutria nigriventer* spider represents the most important cause of arachnid envenomization in Brazil (Ministério da Saúde, 1998). The main local clinical manifestation of such envenomization is intense pain that may be followed by local swelling and erythema (Bucharetchi, 1992). The inflammatory response has usually been studied in rats and rabbits. The skin edema formation in rabbits is independent of the histamine and 5-hidroxytryptamine (5-HT, serotonin) components of the venom and is at least partially mediated by the tissue kallikrein–kinin system (Marangoni et al., 1993). In rats, the venom acts as a potent stimulant of capsaicin-sensitive sensory neurons (Costa et al., 1997), resulting in the local release of substance P and activation of tachykinin NK_1 receptors as well as activation of mast cells and release of biogenic amines (Palframan et al., 1996; Costa et al., 2001) that act on post-capillary venules eliciting plasma extravasation and edema formation (Palframan et al., 1996; Costa et al., 2001). Despite the clear involvement of neurogenic mechanisms in the inflammatory response induced by *P. nigriventer* venom, no information is available concerning the contribution of these mechanisms to the algogenic activity of this venom.

It was properly demonstrated that tachykinins and excitatory amino acids comprised the main neurotransmitters in pain transmission (reviewed in Yaksh, 1999); additionally to their central action, these neurotransmitters influence nociception in the periphery (Carlton et al., 1995, 1996, 1998; Heppelmann and Pawlak, 1997; Kessler et al., 1992; Davidson et al., 1997; Prioleau et al., 1996). Tachykinins are released from both central and peripheral endings of primary afferent capsaicin-sensitive neurons (Otsuka and Yoshioka, 1993; Holzer, 1988) and, when delivered from peripheral endings of primary sensory neurons, induce neurogenic inflammation (Holzer, 1998). This local response may contribute to the generation of pain. Fur-

* Corresponding author. Tel.: +55-11-3726-7222; fax: +55-11-3726-1505.
E-mail address: yarac@attglobal.net (Y. Cury).

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thermore, peripheral application of substance P sensitizes afferent neurons, causing painful states. The effect of substance P on these cells involves the activation of tachykinin NK_1 receptors, localized on peripheral terminals of unmyelinated axons (Carlton et al., 1996; Heppelmann and Pawlak, 1997). Concerning the peripheral algogenic effect of excitatory amino acids, experimental findings have demonstrated that glutamate, through an action on NMDA and non-NMDA receptors, acts as mediator of inflammatory or thermal hyperalgesia (Davidson et al., 1997; Jackson et al., 1995). The possibility of a peripheral action of glutamate on unmyelinated sensory axons was also supported by the demonstration that these sensory fibers immunostain for NMDA, AMPA or kainate receptors (Carlton et al., 1995; Coggeshall and Carlton, 1998). Several lines of evidence indicate that, in the case of C fibers, glutamate coexists with substance P (De Biasi and Rustioni, 1988). These neurotransmitters interact synergistically for the pain frame (Rusin et al., 1993; Liu et al., 1997; Dougherty et al., 1993; Dougherty and Willis, 1991; Ma and Woolf, 1995; Carlton et al., 1998); this synergism being important for the generation of central sensitization (Dougherty et al., 1993; Ma and Woolf, 1995). Recently, Carlton et al. (1998) showed a similar interaction among glutamate and substance P in the periphery, suggesting that primary afferent neurons may be under the same control as are the neurons in the dorsal horn.

In the light of these observations, the aim of the present study was to investigate: (a) the hyperalgesic response induced by *P. nigriventer* spider venom; (b) the contribution of capsaicin-sensitive neurons to this phenomenon; (c) the involvement of peripheral tachykinin NK_1 and NK_2 receptors and peripheral NMDA and AMPA glutamate receptors in the hyperalgesic manifestation and (d) the possible correlation between hyperalgesia and edematogenic response induced by the venom.

2. Materials and methods

2.1. Venom

Lyophilized crude venom of *P. nigriventer* was supplied by Laboratório de Artrópodes, Instituto Butantan (São Paulo, Brazil), collected by electrical stimulation. Samples were kept at -20 °C until use.

2.2. Animals

Male Wistar rats, weighing between 170 and 180 g, were used. All procedures were in accordance with the guidelines for the ethical use of conscious animals in pain research published by the International Association for the Study of Pain (Zimmermann, 1983). The practices were approved by the Institutional Animal Care Committee at the Instituto Butantan (CEUAIB, Protocol Number 022/2000).

2.3. Evaluation of hyperalgesia

The rats were subcutaneously injected with either 0.1 ml of sterile 0.15 M NaCl solution—control group—or 0.1 ml of saline solution containing the appropriate concentrations of *P. nigriventer* venom into the plantar surface of one hind paw. The contralateral paw was not injected. The pain threshold was measured at different times after venom or saline injection using an Ugo-Basile pressure apparatus as described by Randall and Selitto (1957). Briefly, a force (in g) of increasing magnitude was applied to the paw and when the animals reacted by withdrawing the paw, the force needed to induce such response was recorded and represented the pain threshold. To reduce stress, rats were habituated to the apparatus 1 day before the experiments.

2.4. Evaluation of edema formation

Venom samples were dissolved in sterile 0.15 M NaCl solution and 0.1 ml of the final preparation was injected into the subplantar surface of one hind footpad. An equal volume of saline was injected into the control contralateral paw. The volume increase of paws (edema) was measured plethysmographically at several intervals following injection according to the method of Van Arman et al. (1996). The results were calculated as the difference between the values obtained in both paws and expressed as the percentage of the increased volume.

2.5. Drugs treatments

In order to assess the involvement of capsaicin-sensitive neurons in the algogenic effect, the rats were treated with the vanilloid receptor antagonist, *N*-[2-(4-chlorophenyl) ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2*H*-2-benzazepine-2-carbothioamide (capsazepine, 120 μmol kg^{-1}, i.v.), 15 min before venom injection. For i.v. treatment, unanesthetized animals received a single injection of the antagonist in the tail vein. To investigate the participation of peripheral tackykinin receptors, the tachykinin NK_1 receptor antagonist, pGlu-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Pro(spiro-γ-lactam)Leu-Trp-NH_2 (GR82334) or tachykinin NK_2 receptor antagonist, Phenyl-CO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2 (GR94800) (10 μmol/paw) was administered 15 min before the intraplantar venom injection. To examine the involvement of peripheral excitatory amino acid receptors, dizocilpine (MK 801,100 pmol/paw) or (±)-2-amino-5-phosphonopentanoic acid ((±)-AP-5, 40 pmol/paw), a noncompetitive and a competitive NMDA receptor antagonist, respectively, or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 100 nmol/paw), an AMPA receptor antagonist, was injected simultaneously with venom. None of the drugs used altered, per se, the pain threshold of the animals. The doses of the drugs used were based on earlier data (O'Shaughnessy and Connor, 1994; Guo et al., 1995; Jackson et al.,

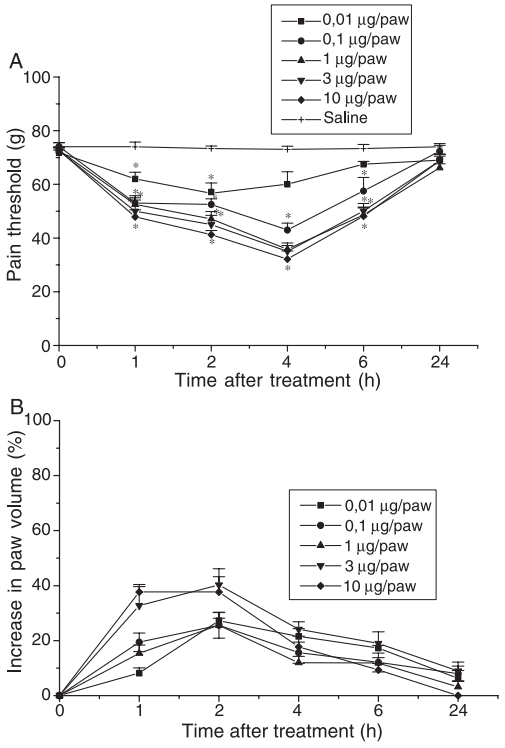

Fig. 1. Comparison of hyperalgesia effect and edema-inducing activity of *P. nigriventer* venom in rat hind paws. Decrease in pain threshold (A) and increase in paw volume (B) were determined before and at different times after the intraplantar injection of saline in the control group, or of crude venom, dose varying from 0.01 to 10 µg/paw. Sensitivity to pain was measured as the threshold response to pressure and expressed as g. Edema is taken as a percentage increase in volume in relation to the initial paw volume. Each point represents the mean ± S.E.M. for six animals. *Significantly different from mean values before venom injection ($P<0.05$).

1995; Costa et al., 2000). However, to assure the efficacy of the pharmacological interventions used, the agonists of tachykinin NK_1, H_2N-$(CH_2)_4$-CO-Phe-Phe-Pro-NmeLeu-Met-NH_2 (GR73632, 1 nmol/paw) and of NK_2, Lys-Asp-Ser-Phe-Val-Gly-R-γ-lactam-Leu-Met-NH_2 (GR64349, 100 nmol/paw) receptors, as well as glutamate (30 nmol/paw), or capsaicin (50 µg/paw, diluted in 1:1:8 of ethanol, DMSO and saline, respectively), injected by the i.pl. route, were used as positive controls.

2.6. Drugs

Capsaicin, capsazepine, GR73632, GR64349, GR82334, GR94800, MK 801, (±)-AP-5, CNQX and L-glutamic acid were purchased from Sigma (USA).

2.7. Statistics

Statistical evaluation of the data was carried out by analysis of variance and sequential differences between means according to Tukey contrast analysis at $P<0.05$ (Gad and Weil, 1989).

3. Results

3.1. Hyperalgesia and edema induced by *P. nigriventer* venom

The intraplantar injection of *P. nigriventer* venom, doses varying from 0.01 to 10 µg/paw/100 µl into the rat hind paw, evoked a significant decrease in pain threshold (Fig. 1A). The peak of the hyperalgesic response for 0.01 µg occurred 2 h and, for the other doses, 4 h after venom injection. Thereafter, hyperalgesia decreased and disappeared within 24 h. Except with the dose of 0.01 µg/paw, the maximum responses induced by the distinct venom doses were not statistically different from each other. The 1 µg/paw administration was chosen for the subsequent studies since it reduced the pain threshold by 50%. No effect was found in the control/saline injected rats. The same doses of *P. nigriventer* venom induced a dose and time-dependent edema. The maximum increase in the hind-paw swelling occurred 2 h after venom injection, decreasing and disappearing within 24 h (Fig. 1B). The maximum edematogenic response observed for the 1 µg/paw dose was 26%.

3.2. Effects of pharmacological treatments on *P. nigriventer* venom-induced hyperalgesia and edema

3.2.1. Capsazepine effect

Pre-treatment of the animals with the vanilloid receptor antagonist, capsazepine, significantly reduced the hyperalgesic response induced by the venom (Fig. 2). On the contrary, the edematogenic reaction was not altered (Table 1).

3.2.2. Effects of tackykinin receptor antagonists

P. nigriventer venom-induced hyperalgesia was significantly inhibited by the intraplantar injection of both the

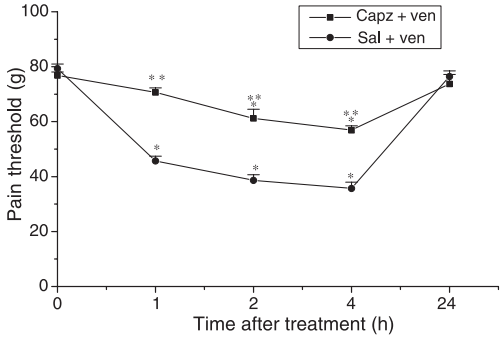

Fig. 2. Effect of capsazepine on hyperalgesia induced by *P. nigriventer* venom in rat hind paws. Capsazepine (Capz, 120 µmol kg^{-1}, i.v.) or saline (Sal, control group) was injected 15 min before venom (ven, 1 µg/paw). Decrease in pain threshold was determined in rat hind paw before and at different times after injection of the venom. Sensitivity to pain was measured as the threshold response to pressure and expressed as g. Each point represents the mean ± S.E.M. for six animals. *Significantly different from mean values before venom injection, **significantly different from mean values for Sal + ven group ($P<0.05$).

Table 1
Effect of vanilloid, tackykinin or excitatory amino acid receptor antagonists on edema induced by *P. nigriventer* venom

Treatment	Increase in paw volume (%)
Saline + venom	27 ± 2.4
Capsazepine + venom	27 ± 1.8
GR 82334 (i.pl.) + venom	33 ± 4.6
GR 82334 (i.v.) + venom	20 ± 2.7
GR 94800 (i.pl.) + venom	30 ± 3.6
GR 94800 (i.v.) + venom	20 ± 3.9
MK 801 + venom	20 ± 3.9
AP5 + venom	29 ± 2.8
CNQX + venom	26 ± 4.8

Capsazepine (120 μmol kg^{-1}, i.v.), GR82334 and GR94800 (10 μmol/paw or 0.02 mg kg^{-1}, i.v.) were injected 15 min before the venom. MK801 (100 pmol/paw), AP5 (40 pmol/paw) and CNQX (100 nmol/paw) were injected concomitantly with the venom. Data represent the values obtained 2 h after venom (1 μg/paw) injection. Edema is expressed as percentage increase in volume in relation to the initial volume of the paw. Each point represents the mean ± S.E.M. for six animals.

tachykinin NK_1 (GR 82334) or NK_2 (GR 94800) receptor antagonists; this inhibition being greater for the tachykinin NK_2 receptor antagonist (Fig. 3A). However, it must be stressed that the intraplantar injection of GR 82334 caused per se a significant edematogenic response as compared to GR 94800 (data not showed). Based on these observations, both tackykinin receptor antagonists were administered by the intravenous route at 0.02 mg kg^{-1}, 15 min before the intraplantar injection of the venom. For this experiment, unanesthetized animals received a single injection of the antagonists in the tail vein.

The tachykinin NK_1 receptor antagonist abolished the hyperalgesia until the second hour after the venom administration (Fig. 3B), whereas GR 94800 partially reduced this phenomenon (Fig. 3B). The edematogenic response was not altered by these treatments (Table 1).

3.2.3. Inhibition of hyperalgesia by excitatory amino acid receptor antagonists

The MK801 noncompetitive NMDA receptor antagonist partially inhibited, whereas the AP5 competitive NMDA antagonist abolished, hyperalgesia induced by *P. nigriventer* venom (Fig. 4A,B). The AMPA receptor antagonist, CNQX, reduced this response (Fig. 5). These same treatments did not cause significant changes in the edema induced by the venom (Table 1).

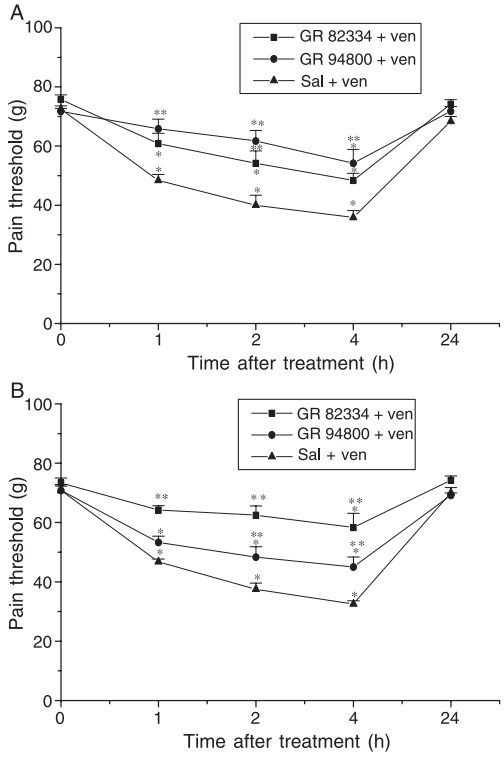

Fig. 3. Effect of GR82334 and GR94800 on hyperalgesia induced by *P. nigriventer* venom in rat hind paws. GR82334 or GR94800, 10 μmol/paw (A) or 0.02 mg/kg, i.v. (B) and saline (Sal, i.pl. or i.v., control group) were injected 15 min before venom (ven, 1 μg/paw). Decrease in pain threshold was determined in rat hind paw before and at different times after venom injection. Sensitivity to pain was measured as the threshold response to pressure and expressed as g. Each point represents the mean ± S.E.M. for six animals. *Significantly different from mean values before venom injection, **significantly different from mean values for Sal + ven group ($P < 0.05$).

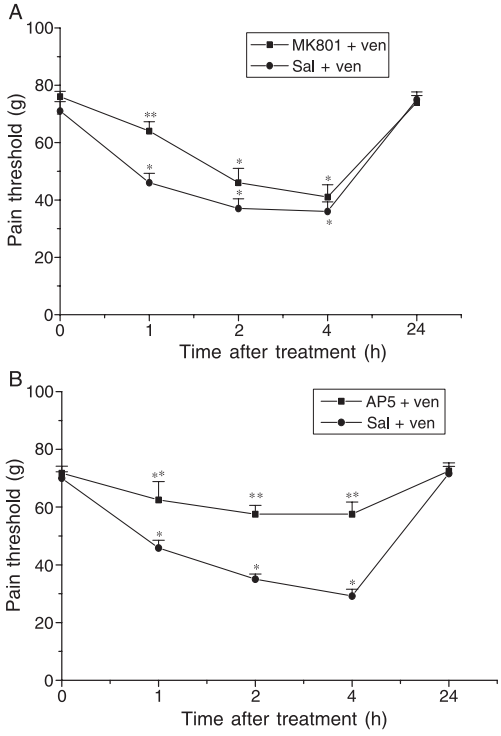

Fig. 4. Effect of MK801 (A) and AP5 (B) on hyperalgesia induced by *P. nigriventer* venom in rat hind paws. MK801 (100 pmol/paw), AP5 (40 pmol/paw) or saline (Sal, control group) were administered concomitantly with the venom (ven, 1 μg/paw). Decrease in pain threshold was determined in rat hind paw before and at different times after venom injection. Sensitivity to pain was measured as the threshold response to pressure and expressed as g. Each point represents the mean ± S.E.M. for six animals. *Significantly different from mean values before venom injection, **significantly different from mean values for Sal + ven group ($P < 0.05$).

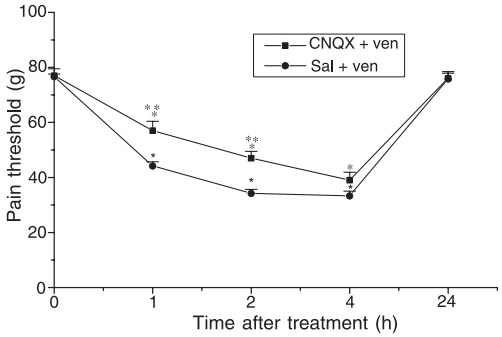

Fig. 5. Effect of CNQX on hyperalgesia induced by *P. nigriventer* venom in rat hind paws. CNQX (100 nmol/paw) or saline (Sal, control group) were injected simultaneously with the venom (ven, 1 μg/paw). Decrease in pain threshold was determined in rat hind paw before and at different times after the injection of the venom. Sensitivity to pain was measured as the threshold response to pressure and expressed as g. Each point represents the mean ± S.E.M. for six animals. *Significantly different from mean values before venom injection, **significantly different from mean values for Sal + ven group ($P < 0.05$).

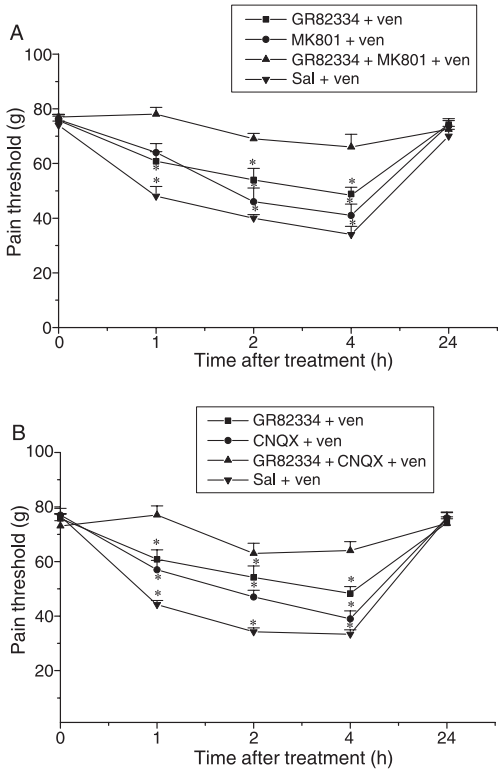

Fig. 6. Cooperative inhibitory action of tachykinin and glutamate receptor antagonists on hyperalgesia induced by *P. nigriventer* venom in rat hind paws. The animals were co-treated with GR82334 (A, B) and MK801 (A) or CNQX (B). GR82334 (10 μmol/paw) or saline (Sal, control group) were injected 15 min before venom (ven, 1 μg/paw). MK801 (100 pmol/paw), CNQX (100 nmol/paw) or saline were injected simultaneously with the venom. Decrease in pain threshold was determined in rat hind paw before and at different times after the injection of the venom. Sensitivity to pain was measured as the threshold response to pressure and expressed as g. Each point represents the mean ± S.E.M. for six animals. *Significantly different from mean values before venom injection ($P < 0.05$).

3.2.4. Cooperative action of tachykinin and glutamate receptor antagonists

In order to evaluate a possible cooperative action of tachykinin and glutamate receptor antagonists, the animals were treated with both tachykinin and glutamate receptor antagonists. The co-treatment with GR 82334 and MK801

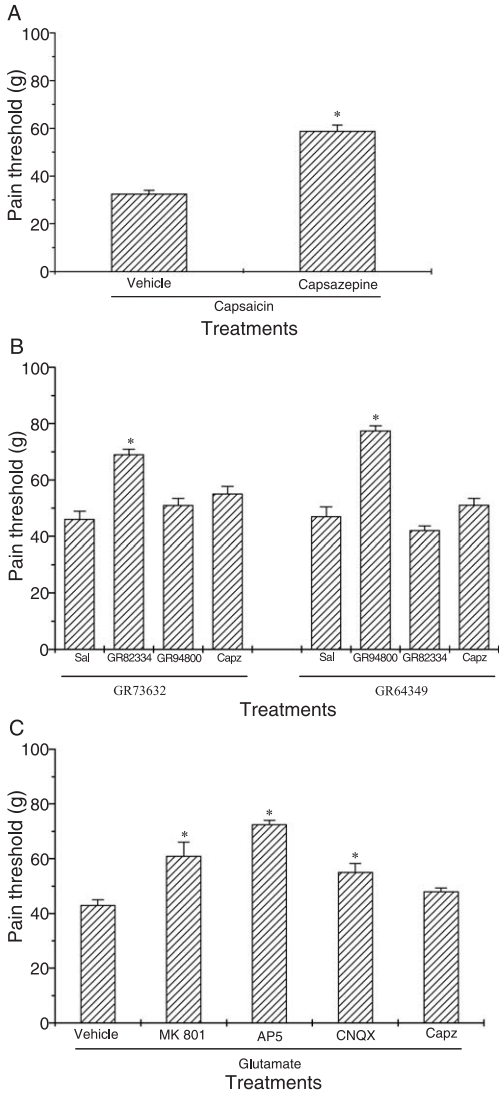

Fig. 7. Effect of vanilloid, tackykinin and excitatory amino acid receptor antagonists on hyperalgesia induced by capsaicin, tachykinin NK_1 or NK_2 receptors agonists and glutamate. Capsazepine (Capz, 120 μmol kg^{-1}, i.v.) was injected 15 min before the intraplantar injection of capsaicin (50 μg/paw) (A), GR73632 (10 nmol/paw) or GR64349 (10 nmol/paw) (B), or glutamate (30 nmol/paw) (C). GR82334 or GR94800 (10 μmol, i.pl.) was injected 15 min before GR73632 (10 nmol/paw) or GR64349 (10 nmol/paw), respectively (B). MK801 (100 pmol/paw), AP5 (40 pmol/paw) or CNQX (100 nmol/paw) was injected concomitantly with glutamate (30 nmol/paw) (C). Sensitivity to pain was measured as the threshold response to pressure and expressed as g. Data represent decrease in pain threshold 30 min after capsaicin injection (A), 2 h after injection of tachykinin NK_1 or NK_2 receptor agonists (B) or 1 h after glutamate injection (C). Each point represents the mean ± S.E.M. for six animals. *Significantly different from mean values for Caps (A), GR73632 or GR64349 (B) or GLU (C) group ($P < 0.05$).

abolished the nociceptive effect induced by *P. nigriventer* venom (Fig. 6A). The same effect was observed when the tachykinin receptor antagonist was co-administered with CNQX (Fig. 6B).

3.2.5. Pharmacological treatment effects on hyperalgesia induced by capsaicin, tachykinin receptor agonists or glutamate

The intraplantar injection of capsaicin caused hyperalgesia that peaked 30 min after injection, decreasing thereafter. This hyperalgesic response was inhibited by the capsazepine pretreatment (Fig. 7A). The injection of tachykinin NK_1 (GR73632) or NK_2 (GR64349) receptor agonists into the rat footpad induced a maximal hyperalgesia 2 h after the agonists' administration. This nociceptive effect was abolished by the respective tachykinin NK_1 or NK_2 receptor antagonist, but was not modified by the noncorresponding antagonist (Fig. 7B). Furthermore, capsazepine did not modify the nociceptive response induced by tachykinin receptor agonists (Fig. 7B).

The intraplantar injection of glutamate elicited a significant decrease in pain threshold, and the maximum hyperalgesic response was observed 1 h after administration. This phenomenon was inhibited by the NMDA or non-NMDA receptor antagonists (Fig. 7C). On the other hand, glutamate-induced hyperalgesia was not modified by capsazepine (Fig. 7C).

4. Discussion

The results presented herein demonstrate that the intraplantar injection of *P. nigriventer* spider venom causes significant local hyperalgesia and edema in the rat hind paw.

The contribution of the activity of capsaicin-sensitive sensory neurons and the role of neurotransmitters released from the peripheral endings of sensory neurons in *P. nigriventer* venom-induced hyperalgesia were analyzed using specific antagonists of vanilloid or of tackykinin and excitatory amino acid receptors. The results obtained support the evidence that the quantities of antagonists used were adequate to block the nociceptive effect of the venom, since these doses are active against hyperalgesia induced by the corresponding receptor agonists.

Activation of vanilloid receptors contributes to the evolution of hyperalgesia induced by *P. nigriventer* venom, since capsazepine, an antagonist of the vanilloid receptor (Caterina et al., 1997) reduced this phenomenon. These data suggest that the nociceptive effect of the venom involves sensory C-fiber-mediated mechanisms. Vanilloid receptors of type 1 (VR1) are expressed by sensory neurons and is sensitive to heat and protons at body temperature (Bevan and Geppetti, 1994; Caterina et al., 1997; Szallasi and Blumberg, 1999, for review). The mechanisms involved in the activation of capsaicin-sensitive receptors by *P. nigriventer* venom are still undetermined. It seems clear, however, that the eventual contribution of the solution acidity of the venom preparation to the activation of these receptors can be ruled out, since the pH of the solution injected into the hind paw of the animals was 6.2.

In addition, these results provide evidence that peripheral tachykinin receptors mediate the algogenic effect of *P. nigriventer* venom. This suggestion relies on the fact that the intraplantar injection of antagonists of tachykinin NK_1 or NK_2 receptors reduced the nociceptive effect of the venom. Based on the observation that the tachykinin NK_1 receptor antagonist itself caused an edematogenic response, the antagonists were also administered by the i.v. route, and inhibition of venom-induced hyperalgesia was also observed. However, a central action of the antagonists must also be considered for the drugs given i.v. The results indicate that activation of both peripheral tachykinin NK_1 and NK_2 receptors is an important mechanism concerning the *P. nigriventer* venom-induced hyperalgesia. The tachykinin receptors are expressed in many kinds of cells and tissues, including peripheral unmyelinated sensory afferents (Carlton et al., 1996). Moreover, the expression of these receptors is increased during inflammation (Hökfelt, 1991). The tachykinin NK_1 receptor exhibits a preference for substance P and the tachykinin NK_2 receptor for neurokinin A; however, these tackykinins are not highly selective for any given receptor (Regoli et al., 1994). The present results are in accordance with data showing that activation of peripheral tachykinin NK_1 receptors, e.g., following local injection of substance P in rat glabrous skin, results in hyperalgesia (Carlton et al., 1996). Based on these data and the observation that *P. nigriventer* venom does not contain a tachykinin receptor agonist (Costa et al., 1997), it can be suggested that venom-induced hyperalgesia results, at least partially, from stimulation of capsaicin-sensitive neurons and peripheral release of tackykinins.

The present results also suggest that peripheral excitatory amino acid receptors are involved in the algogenic effect of *P. nigriventer* venom, since the intraplantar injection of NMDA or AMPA receptor antagonists inhibited hyperalgesia. Several lines of evidence indicate that, peripherally, both NMDA and non-NMDA glutamate receptors contribute to nociceptive behavior during an inflammatory process (Prioleau et al., 1996; Davidson et al., 1997; Lawand et al., 1997; Davidson and Carlton, 1998). The present demonstration that the venom stimulates capsaicin-sensitive neurons together with data showing that, in these neurons, glutamate and substance P coexist, being released by the same stimulus (De Biasi and Rustioni, 1988; Carlton et al., 1998), indicates that *P. nigriventer* venom, by stimulation of capsaicin-sensitive neurons, evokes the peripheral liberation of tachykinins and glutamate. The concomitant release of tachykinins and glutamate could also increase the algogenic activity of the venom, corroborating the idea that, in primary afferent neurons, through an action on tachykinin NK_1 receptors, substance P enhances the algogenic activity of glutamate (Carlton et al., 1998). The possibility of a

cooperative action of tachykinin and glutamate receptors on venom-induced hyperalgesia was supported by the demonstration that co-treatment with tachykinin and glutamate receptor antagonists further reduced the nociceptive effect of the venom as compared to the inhibition observed with either antagonist alone.

It must be pointed out that *P. nigriventer* spider venom contains histamine and serotonin (Schenberg and Pereira-lima, 1971), which could interfere with sensory neuron activities and therefore contribute to the algogenic venom effect. The involvement of other inflammatory mediators, including biogenic amines, in the genesis of hyperalgesia by the venom is under investigation. Furthermore, in addition to biogenic amines, *P. nigriventer* venom contains neurotoxins with molecular weights ranging from 6000 to 9000 (Rezende et al., 1991), which might be affecting the activity of neurons. Regarding this, experimental studies have shown that the intraneural injection of *P. nigriventer* venom promotes repetitive firing of myelinated nerve fibers. This alteration is associated with transient swelling of axons at nodes of Ranvier, vacuole formation and modifications of the myelin sheath, due to delay in the inactivation of sodium current at the node of Ranvier (Cruz-Höfling et al., 1985; Love and Cruz-Höfling, 1986; Love et al., 1996).

The pharmacological modulation of edema was also investigated in the present study in order to further evaluate a possible correlation between this phenomenon and the hyperalgesic response. None of the treatments modified the edematogenic response induced by the venom. Costa et al. (2000, 2001) showed that plasma extravasation in rat skin and rat paw edema induced by *P. nigriventer* venom results from stimulation of capsaicin-sensitive neurons. The doses (10 or 100 μg, respectively) of venom employed in those studies and the administration of venom dialysed, in order to remove histamine, serotonin, and neurotoxin contents, might contribute to the discrepancies observed between results. Based on our results with the 1 μg/paw dose, it can be suggested that distinct mechanisms are involved in the development of hyperalgesia and edema induced by the spider venom.

In conclusion, the hyperalgesia induced by *P. nigriventer* venom in the rat footpad model is mediated, at least partially, by stimulation of capsaicin-sensitive neurons and activation of peripheral tachykinin NK_1 and NK_2 receptors and NMDA and AMPA receptors. To our knowledge, this is the first experimental study of the mechanisms involved in the algogenic property of *P. nigriventer* spider venom and it may contribute to the understanding of the pathophysiology and the control of pain observed in human envenomization.

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References

Bevan, S., Geppetti, P., 1994. Protons: small stimulants of capsaicin-sensitive sensory nerves. Trends Neurosci. 17, 509–512.

Bucharetchi, F., 1992. Acidentes por *Phoneutria*. In: Schvartsman, S. (Ed.), Plantas venenosas e animais peçonhentos. Sarvier, São Paulo, SP, pp. 196–201.

Carlton, S.M., Hargett, G.L., Coggeshall, R.E., 1995. Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin. Neurosci. Lett. 197, 25–28.

Carlton, S.M., Zhou, S., Coggeshall, R.E., 1996. Localization and activation of substance P receptors in unmyelinated axons of rat glabrous skin. Brain Res. 734, 103–108.

Carlton, S.M., Zhou, S., Coggeshall, R.E., 1998. Evidence for the interaction of glutamate and NK1 receptors in the periphery. Brain Res. 790, 160–169.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Coggeshall, R.E., Carlton, S.M., 1998. Ultrastructural analysis of NMDA, AMPA and kainate receptors on unmyelinated and myelinated axons in the periphery. J. Comp. Neurol. 391, 78–86.

Costa, S.K.P., Antunes, E., De Nucci, G., Brain, S.D., 1997. *Phoneutria nigriventer* spider venom induces edema in rat skin by activation of capsaicin sensitive sensory nerves. Eur. J. Pharmacol. 339, 223–226.

Costa, S.K.P., De Nucci, G., Antunes, E., Brain, S.D., 2000. Involvement of vanilloid receptors and purinoceptors in the *Phoneutria nigriventer* spider venom-induced plasma extravasation in rat skins. Eur. J. Pharmacol. 391, 305–315.

Costa, S.K.P., De Nucci, G., Esquisatto, L.C.M., Camargo, E., Gambero, A., Brain, S.D., Antunes, E., 2001. Comparative effect of *Phoneutria nigriventer* spider venom and capsaicin on the rat paw oedema. Life Sci. 69, 1573–1585.

Cruz-Höfling, M.A., Love, S., Brook, G.A., Duchen, L.M., 1985. Effects of *Phoneutria nigriventer* spider venom on mouse peripheral nerve. Q.J. Exp. Physiol. 70, 623–640.

Davidson, E.M., Carlton, S.M., 1998. Intraplantar injection of dextrorphan, ketamine or memantine attenuates formalin-induced behaviors. Brain Res. 785, 136–142.

Davidson, E.M., Coggeshall, R.E., Carlton, S.M., 1997. Peripheral NMDA and non-NMDA glutamate receptors contribute to nociceptive behaviors in the rat formalin test. NeuroReport 8, 941–946.

De Biasi, S., Rustioni, A., 1988. Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord. Proc. Natl. Acad. Sci. U.S.A. 85, 7820–7824.

Dougherty, P.M., Willis, W.D., 1991. Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined micro-iontophoretic application of *N*-methyl-D-aspartic acid and substance P. Pain 47, 85–93.

Dougherty, P.M., Palecek, P., Zorn, S., Willis, W.D., 1993. Combined application of excitatory amino acids and substance P produces long-lasting changes in responses of primate spinothalamic tract neurons. Brain Res. Rev. 18, 227–246.

Gad, S.C., Weil, C.S., 1989. Statistics for toxicologists. In: Hayer, A.W. (Ed.), Principles and Methods of Toxicology, 2nd ed. Raven Press, New York, NY, pp. 435–483.

Guo, J.-Z., Yoshioka, K., Zhao, F.-Y., Hosoki, R., Maehara, T., Yanagisawa, M., Hagan, R.M., Otsuka, M., 1995. Pharmacological characterization of GR82334, a tachykinin NK_1 receptor antagonist, in the isolated spinal cord of the neonatal rat. Eur. J. Pharmacol. 281, 49–54.

Heppelmann, B., Pawlak, M., 1997. Sensitization of articular afferents in normal and inflamed knee joints by substance P in the rat. Neurosci. Lett. 223, 97–100.

Hökfelt, T., 1991. Neuropeptides in perspective: the last ten years. Neuron 7, 867–879.

Holzer, P., 1988. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24, 739–768.

Holzer, P., 1998. Neurogenic vasodilatation and plasma leakage in the skin. Gen. Pharmacol. 30, 5–11.

Jackson, D.L., Graff, C.B., Richardson, J.D., Hargreaves, K.M., 1995. Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. Eur. J. Pharmacol. 284, 321–325.

Kessler, W., Kirchhoff, C., Reeh, P.W., Handwerker, H.O., 1992. Excitation of cutaneous afferent nerve endings in vitro by a combinations of inflammatory mediators and conditioning effect of substance P. Exp. Brain Res. 91, 467–476.

Lawand, N.B., Willis, W.D., Westlund, K.N., 1997. Excitatory amino acid receptor involvement in peripheral nociceptive transmission in rats. Eur. J. Pharmacol. 324, 169–177.

Liu, H., Mantyh, P.W., Basbaum, A.L., 1997. NMDA-receptor regulation of substance P release from primary afferent nociceptores. Nature 386, 721–724.

Love, S., Cruz-Höfling, M.A., 1986. Acute swelling of nodes of Ranvier caused by venoms which slow inactivation of sodium channels. Acta Neuropathol. 70, 1–9.

Love, S., Cruz-Höfling, M.A., Duchen, L.M., 1996. Morphological abnormalities in myelinated nerve fibres caused by *Leiurus*, *Centruroidis* and *Phoneutria* venoms and their prevention by tetrodotoxin. Q.J. Exp. Physiol. 71, 115–122.

Ma, Q.P., Woolf, C.J., 1995. Involvement of neurokinin receptors in the induction but not the maintenance of mechanical allodynia in rat flexor motoneurons. J. Physiol. 486, 769–777.

Marangoni, R.A., Antunes, E., Brain, S.D., De Nucci, G., 1993. Activation by *Phoneutria nigriventer* (armed spider) venom of tissue kallikrein–kininogen–kinin system in rabbit skin in vivo. Br. J. Pharmacol. 109, 539–543.

Ministério Da Saúde, 1998. Manual de diagnóstico e tratamento de acidentes por animais peçonhentos. Fundação de Saúde: Brasília, Ed. Gerência Técnica de Editoriações da Coordenação de Comunicação, Educação e documentação-COMED/ASPLAN/FNS-Setor de autarquia Sul, Quadra 4, Bloco N, sala 514, Brasília, DF.

O'Shaughnessy, C.T., Connor, H.E., 1994. Investigation of the role of tachykinin NK_1, NK_2 receptors and CGRP receptors in neurogenic plasma protein extravasation in dura mater. Eur. J. Pharmacol. 263, 193–198.

Otsuka, M., Yoshioka, S., 1993. Neurotransmitter functions of mammalian tachykinins. Physiol. Rev. 73, 229–308.

Palframan, R.T., Costa, S.K.P., Wilsocroft, P., Antunes, E., De Nucci, G., Brain, S.D., 1996. The effect of a tachykinin NK1 receptor antagonist, SR140333, on edema formation induced in rat skin by venom from the *Phoneutria nigriventer* spider. Br. J. Pharmacol. 18, 295–298.

Prioleau, C.E., Coggeshall, R.E., Carlton, S.M., 1996. Peripheral NMDA receptors play a role in capsaicin hyperalgesia. Soc. Neurosci. 22, 1370.

Randall, L.O., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. 111, 209–219.

Regoli, D., Boudon, A., Fauchere, J.L., 1994. Receptors and antagonists for substance P and related peptides. Pharmacol. Rev. 46, 551–599.

Rezende Jr., L., Cordeiro, M.N., Oliveira, E.B., Diniz, C.R. 1991. Isolation of neurotoxic peptides from the venom of the armed spider *Phoneutria nigriventer*. Toxicon 29, 1225–1233.

Rusin, K.L., Bleakman, D., Chard, P.S., Randic, M., Miller, R.J., 1993. Tachykinins potentiate *N*-methyl-D-aspartate responses in acutely isolated neurons from the dorsal horn. J. Neurochem. 60, 952–960.

Schenberg, S., Pereira-lima, F.A., 1971. *Phoneutria nigriventer* venom, pharmacology and biochemistry of its components. In: Bucherl, W., Buckley, E.E. (Eds.), Venomous Animals and Their Venoms. Academic Press, New York, NY, pp. 279–285.

Szallasi, A., Blumberg, P.M., 1999. Vanilloid (capsaicin) receptors and mechanisms. Pharmacol. Rev. 51, 159–212.

Van Arman, C.G., Begany, A.J., Miller, L.M., Pless, H.H., 1996. Some details of the inflammation caused by yeast and carrageenin. J. Pharmacol. Exp. Ther. 150, 328–333.

Yaksh, T.L., 1999. Central pharmacology of nociceptive transmission. In: Wall, P.D., Melzack, R. (Eds.), Textbook of Pain. Churchill Livingstone, London, pp. 253–308.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.