ELSEVIER

European Journal of Pharmacology 376 (1999) 293–300

ejp

www.elsevier.nl/locate/ejphar

Effects of tachykinin NK_1 or PAF receptor blockade on the lung injury induced by scorpion venom in rats

Ione M. Matos, Danielle G. Souza, Danielle G. Seabra, Lineu Freire-Maia, Mauro M. Teixeira *

Departamento de Farmacologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627 Pampulha, 31270-901 Belo Horizonte, MG, Brazil

Received 11 February 1999; received in revised form 11 May 1999; accepted 18 May 1999

Abstract

In cases of severe human scorpion envenoming, lung injury is a common finding and frequently the cause of death. In the rat, two distinct mechanisms account for oedema following the intravenous injection of the venom — acute left ventricular failure resulting from a massive release of catecholamines and an increase in pulmonary vascular permeability. In the present work, we investigated the effects of a tachykinin NK_1 receptor antagonist (CP96,345, the dihydrochloride salt of (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)methyl)-1-azabicycol[2.2.2]octan-3-amine) and its 2*R*–3*R* inactive enantiomer (CP96,344) on the acute lung injury induced by the i.v. injection of *Tityus serrulatus* venom in rats. Lung injury was assessed by evaluating the extravasation of Evans blue dye in the bronchoalveolar lavage fluid and in the lung of venom-treated and control animals. The effects of the platelet-activating factor (PAF) receptor antagonist WEB2170 (2-methyl-1-phenylimidazol[4,5*c*]pyridine) were evaluated for comparison. The i.v. injection of the venom induced the extravasation of Evans blue in the bronchoalveolar lavage fluid and into the left lung. Pretreament with the tachykinin NK_1 receptor antagonist CP96,345, but not CP96,344, inhibited Evans blue dye extravasation in the bronchoalveolar lavage fluid and in the lung by 96% and 86%, respectively. The PAF receptor antagonist WEB2170 inhibited the increase in vascular permeability in the bronchoalveolar lavage fluid by 60% and had no effect on the extravasation to the lung parenchyma of venom-injected animals. In addition to abrogating lung injury, pretreatment of rats with CP96,345, but not CP96,344 or WEB2170, decreased by 70% the mortality induced by the venom. This is the first study to show the relevance of the tachykinin NK_1 receptor in mediating lung injury and mortality in animals injected with the neurotoxic *T. serrulatus* venom. Blockade of the tachykinin NK_1 receptor may represent an important strategy in the treatment of patients with signs of severe envenoming and clearly deserves further studies. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Lung injury; *(Tityus serrulatus)*; Neurotoxin; Substance P; Tachykinin NK_1 receptor; PAF receptor; Oedema; CP96,345; WEB2170

1. Introduction

Human scorpion envenoming is a frequent and sometimes fatal occurrence in tropical and subtropical countries, specially amongst children (Freire-Maia et al., 1994). In Brazil, *Tityus serrulatus* is the most important scorpion species, once it causes most accidents and induces the most severe forms of poisoning. The clinical manifestations of severe envenoming include excruciating pain, unremitting nausea and vomiting, diarrhoea, cardiac arrhythmias and arterial hypertension followed by hypotension and shock. In the most severe cases, lung injury is a common finding and frequently the cause of death (Amaral et al., 1993).

In experimental animals, the i.v. injection of scorpion venom from several species has been shown to induce pulmonary oedema (Bertke and Atkins, 1964; Yaron and Braun, 1970; Freire-Maia et al., 1978). Two distinct mechanisms have been suggested to account for lung oedema in these animals — acute left ventricular failure resulting from a massive release of catecholamines (Freire-Maia et al., 1978) and an increase in pulmonary vascular permeability following the release of inflammatory mediators, such as platelet-activating factor (PAF), leukotrienes and prostaglandins (Freire-Maia and De Matos, 1993; De Matos et al., 1997). Since most of the active peptides obtained from *T. serrulatus* venom are neurotoxins, the mecha-

* Corresponding author. Tel.: +55-31-499-2723; fax: +55-31-499-2695; E-mail: mmtex@icb.ufmg.br

0014-2999/99/$ - see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(99)00382-9

nisms underlying the ability of scorpion venom to initiate the inflammatory cascade and subsequent increase in vascular permeability are not clear. We have recently demonstrated that the venom from *T. serrulatus* induced the contraction of the isolated guinea pig ileum by a mechanism partially dependent on the release of neuropeptide(s) which activate tachykinin NK_1 receptors (De Matos et al., 1999). Thus, it was possible that neurotoxins present in the venom could also induce the release of neuropeptides in the lung of venom-injected animals. The neuropeptides released could then be responsible for the initiation of the inflammatory cascade and ensuing lung oedema. In support of our hypothesis, it has been shown that neuropeptides which activate the tachykinin NK_1 receptor, such as substance P, are capable of inducing the release of inflammatory mediators from a range of cells (e.g., mast cells and macrophages) involved in the inflammatory process (Brain, 1997).

In the present work, we investigated the effects of a tachykinin NK_1 receptor antagonist (CP96,345) and its inactive enantiomer (CP96,344) in the acute lung injury induced by the i.v. injection of *T. serrulatus* venom in rats. Initial experiments were conducted to confirm in rats that *T. serrulatus* venom induces the contraction of the distal ileum by a mechanism partially dependent on the local release of neuropeptides acting on the tachykinin NK_1 receptor. Lung injury was then assessed by evaluating the extravasation of Evans blue dye in the bronchoalveolar lavage fluid and in the lung of venom-treated and control animals. Because PAF receptor antagonists have been previously shown to inhibit scorpion venom-induced lung injury (Freire-Maia and De Matos, 1993; De Matos et al., 1997), we also evaluated the effects of the PAF receptor antagonist WEB2170 for comparison.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–230 g) were fed a normal laboratory chow (Nuvilab, Brazil), given water to drink ad libitum and kept in the Bioscience Unit of our Institution.

2.2. Induction of lung oedema

Rats were anaesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (40 mg/kg) and had cannulas inserted into the trachea and into the femoral artery and vein. The femoral vein access was used to administer i.v. solutions and the femoral artery access connected to a pressure transducer (Viggo-Spectramed, USA) and an amplifier (CWE, USA). The amplifier was connected to a computer and arterial pressure was recorded using the software WinDaq (USA). After the basal arterial pressure was recorded, both vagal nerves were cut in the cervical region and atropine (1 mg/kg, i.v.) was administered to prevent the apnea and the bradycardia, respectively, evoked by the venom (Freire-Maia et al., 1973, 1974). *T. serrulatus* venom was then injected i.v. (500 μg/kg) and the animals followed for periods of 60 min or until death occurred. In the latter, time of death was noted and bronchoalveolar lavage performed. In surviving animals (at 60 min), an overdose of sodium pentobarbital was given and bronchoalveolar lavage performed after the animals died. The bronchoalveolar lavage fluid was centrifuged (1500 rpm for 7 min) and the supernatant used for Evans blue determination and the pellet used to assess the number of infiltrating leukocytes. The lungs and heart were excised in block, the excess blood washed by inserting a cannula through the right ventriculum and perfusing with 20 ml of saline. The left and right lung were used for Evans blue and myeloperoxidase determinations, respectively (see below). The tachykinin NK_1 receptor antagonist, CP96,345 (2 mg/kg, i.v.), its inactive enantiomer, CP96,344 (2 mg/kg, i.v.), or the PAF receptor antagonist, WEB2170 (10 mg/kg, i.v.), were administered 10 min prior to the i.v. injection of venom.

2.3. Measurement of vascular permeability changes

Changes in vascular permeability to serum proteins were analyzed using an Evans blue technique (Saria and Lundberg, 1983). Briefly, Evans blue (20 mg/kg, 1 ml/kg) was injected into the femoral vein just prior to the administration of venom or vehicle (saline). Evans blue levels which were significantly higher in rats injected with scorpion venom than in control animals were assumed to represent increased vascular permeability. The left lung was excised and placed in 2 ml formamide and incubated without homogenization at 40°C for 24 h. Evans blue was quantified in a spectrophotometer (Spectronic 20, Baush and Lomb) by comparison with a standard curve at 620 nm wavelength. Evans blue levels in bronchoalveolar lavage fluid were determined after centrifugation.

2.4. Total and differential leukocyte count

The pellet containing cells from the bronchoalveolar lavage fluid was resuspended in 1 ml of phosphate buffered saline containing 3% bovine serum albumin and an aliquot diluted in Turk solution (1:20). Total leukocyte counts were then performed in a Neubauer chamber using an optical microscope (Standard 25, Zeiss, Germany). Differential cell counts were performed after cytocentrifugation (Cytospin 3, Shandon, USA) and staining with May-Grunwald-Giemsa. Analysis was carried out under an immersion objective (100×) and at least 300 cells were counted. Leucocyte types were defined using standard morphological criteria.

2.5. Tissue extraction and measurement of myeloperoxidase activity

The extent of neutrophil accumulation in the right lung tissue was measured by assaying myeloperoxidase activity

as previously described (Ivey et al., 1995). Briefly, the right lungs of animals that had received vehicle or *T. serrulatus* venom were removed and snap frozen in liquid nitrogen. Upon thawing, the tissue (1 g tissue/19 ml buffer) was homogenized in pH 4.7 buffer (0.1 M NaCl, 0.02 M $NaPO_4$, 0.015 M NaEDTA), centrifuged at $260 \times g$ for 10 min and the pellet underwent hypotonic lysis (15 ml of 0.2% NaCl solution followed 30 s later by addition of an equal volume of a solution containing NaCl 1.6% and glucose 5%). After a further centrifugation, the pellet was then resuspended in 0.05 M $NaPO_4$ buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) and re-homogenized. One milliliter aliquots of the suspension were transferred into 1.5-ml Eppendorf tubes followed by three freeze-thaw cycles using liquid nitrogen. These were then centrifuged for 15 min at $10{,}000 \times g$ and myeloperoxidase activity in the resuspended pellet was assayed by measuring the change in optical density (O.D.) at 450 nm using tetramethylbenzidine (1.6 mM) and H_2O_2 (0.5 mM). Results were expressed as changes in absorbance (O.D.) per milligram of lung tissue.

2.6. Experiments with ileum strips

Rats were killed by cervical dislocation and exsanguinated. After laparotomy, the distal ileum was removed and 1-cm strips were suspended in 10 ml aerated Tyrode's solution (136.8 mM NaCl; 2.7 mM KCl; 1.4 mM $CaCl_2$; 12.0 mM $NaHCO_3$; 5.5 mM glucose; 1.0 mM $MgCl_2$; 0.4 mM NaH_2PO_4) at 37°C and attached to a tension transducer (Grass, USA) connected to a data acquisition device (CWE, USA). All strips were put under an initial tension of 1 g. After 30 min to allow stabilisation of the preparation, contractions were recorded in the presence of various substances or *T. serrulatus* venom. The following agents were used to induce contraction of the preparation: acetylcholine (5×10^{-7} M), histamine (10^{-6} M) and substance P (10^{-8} M). After the contraction reached a peak, the preparation was washed thrice and allowed to rest for 5 min between each addition. Then, scorpion venom was used at the concentration of 5 μg/ml and allowed to act for periods of up to 5 min. The use of this concentration of venom was based on preliminary results demonstrating optimal contraction of the rat ileum at 5 μg/ml. Atropine (1.5×10^{-7} M), the tachykinin NK_1 receptor antagonist CP96,345 (5.0×10^{-7} M) or atropine + CP96,345 were added to the Tyrode's solution prior to addition of acetylcholine, substance P, histamine or scorpion venom.

2.7. Scorpion venom and drugs

T. serrulatus scorpion venom was kindly provided by Instituto Butantan (São Paulo, Brazil). The following drugs were used: sodium pentobarbital (Cristália, São Paulo, Brazil), atropine sulphate and Evans blue (Sigma, USA). The tachykinin NK_1 receptor antagonist CP96,345 (the dihydrochloride salt of (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)methyl)-1-azabicycol[2.2.2]octan-3-amine), its inactive enantiomer CP96,344 (the 2*R*–3*R* enantiomer of CP96,345) were a kind gift of Pfizer (Groton, USA). The PAF receptor antagonist WEB2170 (2-methyl-1-phenylimidazol[4,5*c*]pyridine) was kindly provided by Institute Henry Beaufor-IPSEN (France).

2.8. Statistical analysis

Results are expressed as means ± S.E.M. Statistical analysis was carried out by using one-way analysis of variance (ANOVA) and P values were assigned using Student–Newman–Keuls test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effects of CP96,345 on the contraction of the rat ileum induced by T. serrulatus venom

We have previously shown that *T. serrulatus* venom induced the contraction of guinea pig ilea by a mechanism partially dependent on the release of neuropeptides, possibly substance P, which act on the tachykinin NK_1 receptor (De Matos et al., 1999). In order to confirm that the venom could also induce the release of neuropeptides in the rat, preliminary experiments assessed the ability of CP96,345 to inhibit the venom-induced contraction of the rat ileum. Addition of *T. serrulatus* venom induced a sustained contraction of the ileum (Basal, 1.0 g; venom, 3.26 ± 0.27 g; $n = 6$). Pretreament of the preparation with atropine (10^{-7} M) or CP96,345 (10^{-7} M) inhibited the venom-induced contraction by 37% and 50%, respectively (Fig. 1). At these concentrations, atropine and CP96,345 inhibited by over 90% the contractile responses after stimulation with acetylcholine and substance P, respectively (data not shown). Moreover, pretreatment with a combination of atropine and CP96,345 almost completely abolished the venom-induced responses (Fig. 1).

3.2. Effects of the i.v. injection of crude venom in rats

The i.v. injection of *T. serrulatus* scorpion venom (500 μg/kg) in rats submitted to bilateral vagotomy and pretreated with atropine induced a significant increase in mean arterial pressure which peaked approximately 1 min after the venom and returned to basal levels by 10 min (basal, 114 ± 5 mm Hg; 1 min, 188 mm Hg; 10 min, 112 ± 7 mm Hg; $n = 4$). In these animals, there was a significant increase in the extravasation of Evans blue in the bronchoalveolar lavage fluid (Fig. 2A) and in the left lung (Fig. 2B). The i.v. injection of venom had little effect

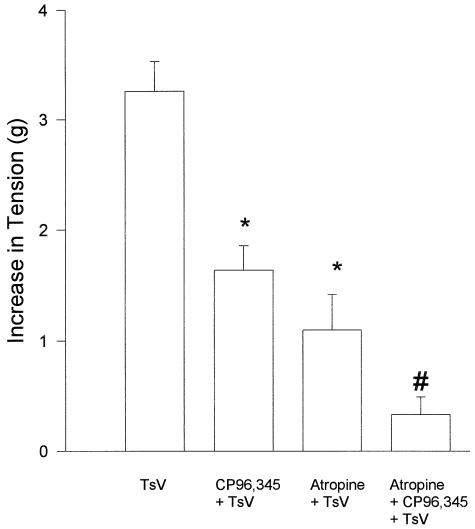

Fig. 1. Effects of the tachykinin NK_1 receptor antagonist, CP96,345, alone or in combination with atropine on the contractile responses induced by scorpion venom on the rat ileum. *T. serrulatus* venom (TsV) was used at the concentration of 5 μg/ml. CP96,345 (5×10^{-7} M) and/or atropine (1.5×10^{-7} M) were added 3 min prior to the addition of the venom. Results are the means ± S.E.M. for four animals in each group. Strips from rat ileum were immersed in 10 ml aerated Tyrode's solution at 37°C and put under an initial tension of 1.0 g. $^{*}P < 0.05$ in comparison with venom alone and $\#P < 0.05$ in comparison with venom-induced contractions in the presence of atropine or CP96,345 only.

on the total number of leukocytes in the bronchoalveolar lavage fluid (saline, $2.7 \pm 0.8 \times 10^6$ leukocytes in the bronchoalveolar lavage fluid; venom, $3.0 \pm 1.0 \times 10^6$ leukocytes; $n = 4$). Most leukocytes present were mononuclear and there was no significant difference in the differential cell count in the bronchoalveolar lavage fluid of control and venom-treated animals (data not shown). The pulmonary levels of myeloperoxidase, an index of the number of infiltrating neutrophils, in venom-treated animals were similar to those of control animals (control, 0.14 ± 0.02 absorbance/mg tissue; venom, 0.10 ± 0.03; $n = 8$).

3.3. Effects of CP96,345 and WEB2170 on the extravasation of evans blue in the lung

Next, we examined whether the action of neuropeptides, such as substance P, on the tachykinin NK_1 receptor was important for the lung injury induced by *T. serrulatus* venom in the rat. Animals were pretreated with CP96,345 (2 mg/kg, i.v.) 10 min prior to the administration of the venom. This dose was chosen for its ability to inhibit effectively the hypotensive effects of tachykinin NK_1 receptor agonists in the rat (Floch et al., 1994) and substance P-induced oedema in the guinea pig (Sakamoto et al., 1993). Pretreatment with CP96,345 had no significant effect on the increase of mean arterial pressure 1 min after the administration of the venom (increase in blood pressure: venom alone, 74 ± 2 mm Hg; CP96,345 + venom, 64 ± 8 mm Hg; $n = 4$, $P > 0.05$). Nevertheless, CP96,345 significantly inhibited the increase in Evans blue dye extravasation in the bronchoalveolar lavage fluid (Fig. 3A) and in the left lung (Fig. 3B) of venom-injected animals. In contrast to the effects of CP96,345, its inactive enantiomer, CP96,344, had no significant effect on the levels of

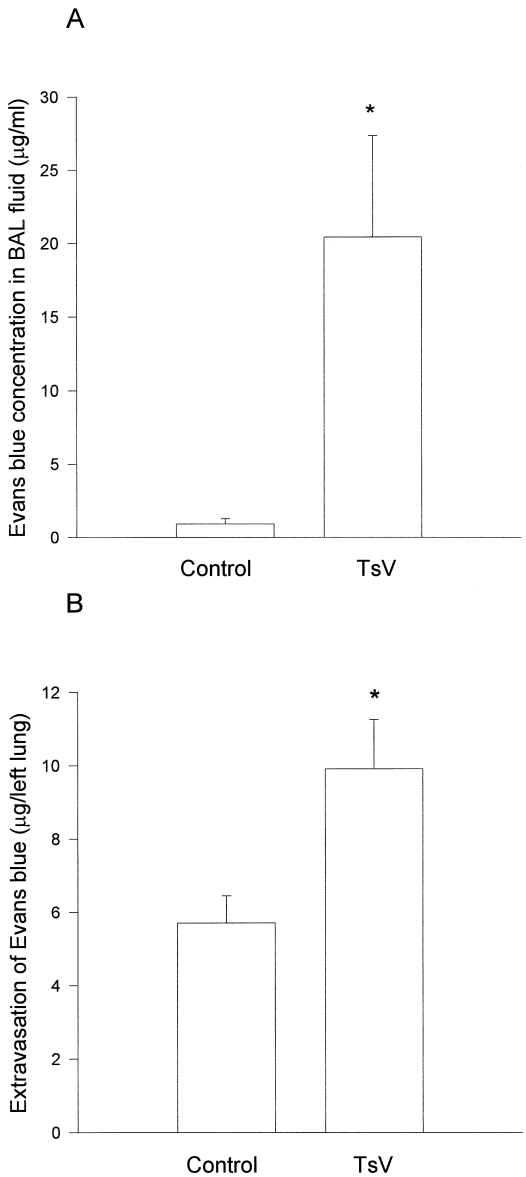

Fig. 2. Effects of the i.v. administration of *T. serrulatus* venom on the extravasation of Evans blue dye in the (A) bronchoalveolar lavage fluid or in the (B) left lung in the rat. *T. serrulatus* venom (TsV, 500 μg/kg) or saline (control) were injected i.v. and the animals followed up for periods of 60 min or until death occurred. At time of animal death or at 60 min, bronchoalveolar lavage was performed and the left lung excised, washed with excess saline and placed in 2 ml of formamide. Evans blue levels in the centrifuged bronchoalveolar lavage fluid and in formamide were quantified in a spectrophotometer by comparison with a standard curve at 620 nm wavelength. Results are the means ± S.E.M. for 5–6 animals in each group. $^{*}P < 0.05$ in comparison with saline-injected (control) animals.

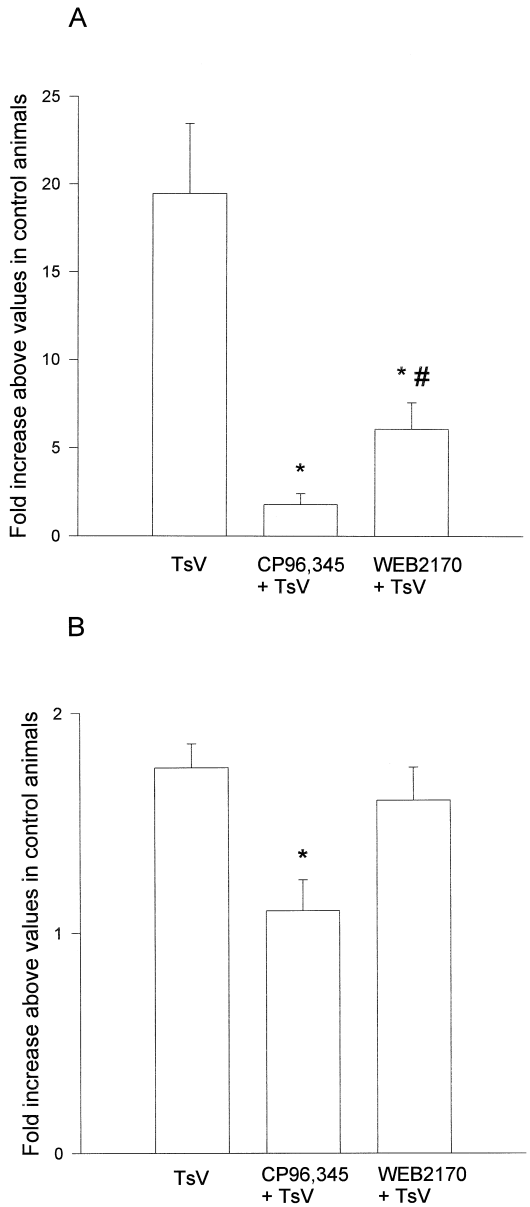

Fig. 3. Effects of the tachykinin NK_1 receptor antagonist, CP96,345, or the PAF receptor antagonist, WEB2170, on the extravasation of Evans blue dye in the (A) bronchoalveolar lavage fluid or in the (B) left lung induced by the injection of scorpion venom in the rat. *T. serrulatus* venom (TsV, 500 μg/kg) or saline (control) were injected i.v. and the animals followed up for periods of 60 min or until death occurred. CP96,345 (2 mg/kg) and WEB 2170 (10 mg/kg) were administered i.v. 10 min prior to the injection of the venom. At time of animal death or at 60 min, bronchoalveolar lavage was performed and the left lung excised, washed with excess saline and placed in 2 ml of formamide. Evans blue levels in the centrifuged bronchoalveolar lavage fluid and in formamide were quantified in a spectrophotometer by comparison with a standard curve at 620 nm wavelength. Results are shown as fold increase in the extravasation of Evans blue dye values in control animals and are the means ± S.E.M. for 7–9 animals in each group. $^*P < 0.01$ in comparison with TsV-injected animals and $\#P < 0.05$ when compared to animal injected with CP96345 and TSV.

Evans blue in the bronchoalveolar lavage fluid of venom-treated animals (venom, 18.2 ± 2.0 μg/ml of bronchoalveolar lavage fluid; CP96,344 + venom, 15.8 ± 7.6 μg/ml; $n = 4$).

Pretreatment of animals with WEB2170 partially inhibited the extravasation of Evans blue in the bronchoalveolar lavage fluid (Fig. 3A) but had little effect on the extravasation of Evans blue in the left lungs (Fig. 3B) of venom-treated animals.

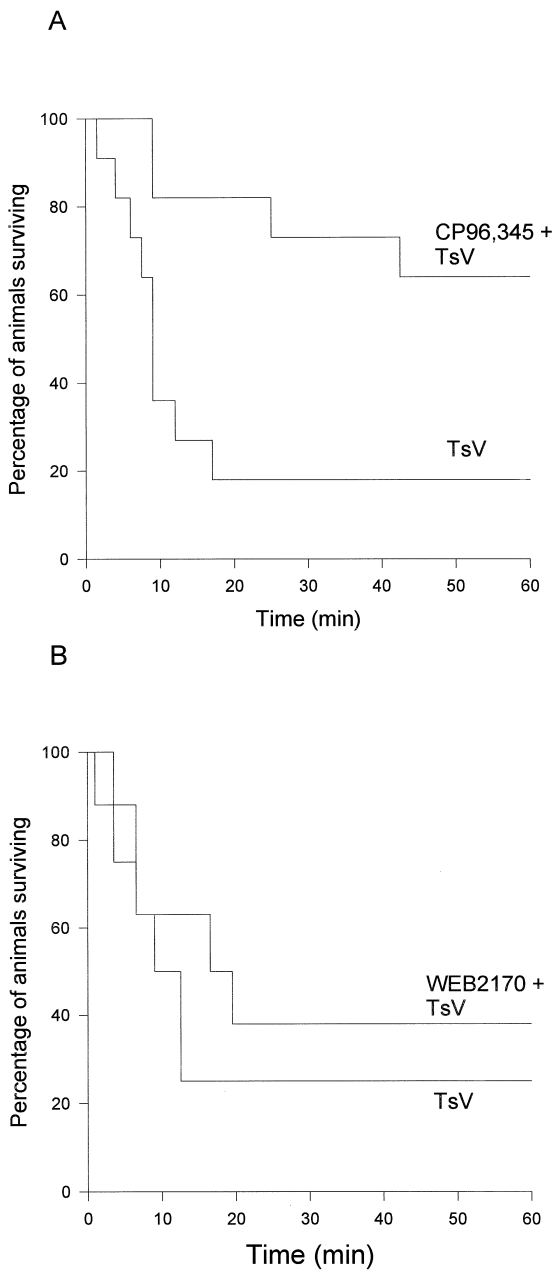

Fig. 4. Effects of the (A) tachykinin NK_1 receptor antagonist, CP96,345, or the (B) PAF receptor antagonist, WEB2170, on the percentage of surviving animals following the injection of scorpion venom in the rat. *T. serrulatus* venom (TsV, 500 μg/kg) was injected i.v. and the animals followed up for periods of 60 min or until death occurred. For the experiments depicted in panel A, 11 animals were used in each group and six animals in each group for the experiments depicted in panel B. $^*P < 0.05$ in comparison with TsV-injected animals.

3.4. Effects of cp96,345 and WEB2170 on mortality

As shown in Fig. 4, around 60–80% of rats died within 20 min of the i.v. administration of *T. serrulatus* venom. Pretreatment of animals with CP96,345 significantly decreased the mortality of the animals and over 60% were still alive 60 min following the injection of the venom (Fig. 4A). In contrast, pretreatment with CP96,344 (data not shown) or WEB2170 (Fig. 4B) had little effect on animal survival.

4. Discussion

The onset of pulmonary oedema in patients, especially children, stung by *T. serrulatus* is a sign of bad prognosis and is accompanied by significant lethality (Amaral et al., 1993). In experimental animals, one can model lung injury by injecting the venom intravenously. In the rat, two distinct mechanisms appear to account for the development of lung injury, as assessed by changes in lung water and histology — acute left ventricular failure due to systemic hypertension following a massive release of catecholamines (Freire-Maia et al., 1978) and an increase in vascular permeability following the release of inflammatory mediators, such as PAF (Freire-Maia and De Matos, 1993; De Matos et al., 1997). The mechanisms which trigger the release of such inflammatory mediators following the i.v. administration of the neurotoxic *T. serrulatus* venom are not known. One possibility is that the venom induces the release of neuropeptides and these mediators initiate the inflammatory cascade. There is evidence to suggest that *T. serrulatus* venom is capable of activating neuropeptide-containing sensory nerve endings (pain is a constant following envenoming) (Freire-Maia and Campos, 1989) and we have previously shown that the venom is capable of evoking the release of neuropeptides in the gut of guinea pigs (De Matos et al., 1999). The latter study demonstrates that the venom is capable of releasing functionally active neuropeptides in vitro that could also be active in different situations (i.e., venom-induced lung injury) in vivo. Moreover, there is much evidence demonstrating that neuropeptides, such as substance P, can induce the release of inflammatory mediators from several leukocytes, including mast cells and macrophages (Brain, 1997; Cooke et al., 1998; Ho et al., 1998), and other cell types, including epithelial cells (Koyama et al., 1998). Here, we have assessed the role of the tachykinin NK_1 receptor, of which substance P is the main ligand, in mediating lung injury following the i.v. injection of *T. serrulatus* venom in the rat.

Initial experiments were designed to confirm that the effects of *T. serrulatus* venom in the rat ileum were also partially dependent on the release of neuropeptides acting on the tachykinin NK_1 receptor. The venom contracted the ileum and this was partially blocked by pretreatment with atropine or the tachykinin NK_1 receptor antagonist, CP96,345. Combined pretreatment with both drugs virtually abolished the venom-induced contraction. These experiments are consistent with our previous findings in the guinea pig demonstrating that the effects of the venom or one of its purified toxins were partially dependent on the local release of neuropeptides, possibly substance P, acting on the tachykinin NK_1 receptor (De Matos et al., 1999).

We have previously shown that the i.v. injection of *T. serrulatus* venom in the rat induced a significant increase in water content (lung/body index) and in the amount of alveolar fluid as assessed by histology (Freire-Maia et al., 1978; De Matos et al., 1997). In the present study, the extravasation of Evans blue dye, a marker of vascular permeability changes, and the number of neutrophils in the lung were used as index of lung injury. The i.v. injection of *T. serrulatus* venom induced the extravasation of Evans blue in the bronchoalveolar lavage fluid and into the left lung of rats. In contrast, we failed to observe any increase in the number of neutrophils in the bronchoalveolar lavage fluid or in the right lung (as assessed by measuring myeloperoxidase levels). The latter results suggest that the recruitment of neutrophils does not appear to play a major role in the development of acute lung oedema following the i.v. injection of scorpion venom.

We then examined the effects of a tachykinin NK_1 receptor antagonist on the venom-induced lung injury. Pretreament with the tachykinin NK_1 receptor antagonist CP96,345 effectively inhibited lung injury, but had no effect on the rapid increase in blood pressure in rats injected with *T. serrulatus* venom. These results show that an increase in arterial pressure induced by the scorpion venom in presence of the tachykinin NK_1 receptor antagonist is still accompanied by inhibition of lung injury. Thus, an effect of the antagonist on vascular permeability is a more likely explanation for the inhibitory effects observed. This hypothesis is consistent with the ability of substance P, an agonist of tachykinin NK_1 receptors, to induce an increase in pulmonary vascular permeability in animal models (e.g., Saria et al., 1983) and with studies demonstrating a role for tachykinin NK_1 receptor activation in mediating injury following a range of different stimuli (Sakamoto et al., 1993; Bhathia et al., 1998). Interestingly, Palframan et al. (1996) demonstrated that the oedema formation induced by the neurotoxic venom of *Phoneutria nigriventer* spider in rat skin was mediated by a mechanism partially involving tachykinin NK_1 receptors. However, this is the first study to demonstrate a role for the tachykinin NK_1 receptor in mediating lung injury following injection of neurotoxins in rats.

A few studies have demonstrated non-specific inhibitory activities of CP96,345 which are not related to the ability of the drug to antagonise the tachykinin NK_1 receptor (e.g., Nagahisa et al., 1992, Schmidt et al., 1992; Guard et al., 1993). However, these studies demonstrate that these non-specific activities (e.g., interaction with

calcium channels) of CP96,345, but not the binding to the tachykinin NK_1 receptor, are also mimicked by CP96,344. In contrast, CP96,344 failed to affect scorpion venom-induced lung injury or mortality in rats. In addition, the tachykinin NK_1 receptor antagonist RP67580 also inhibited the extravasation of Evans blue in our model (data not shown). Thus, although there may be limitations in the use of CP96,345, our studies do suggest an important role for tachykinin NK_1 receptor in mediating the lung oedema induced by scorpion venom.

We have previously reported that PAF receptor antagonists were effective at inhibiting an increase in water content and histological modifications in the lungs of rats injected with *T. serrulatus* venom (Freire-Maia and De Matos, 1993; De Matos et al., 1997). It was, thus, of interest to compare the effects of a PAF receptor antagonist with the effects of CP96,345. In agreement with our previous findings, the PAF receptor antagonist WEB2170 partially inhibited the increase in vascular permeability in the bronchoalveolar lavage fluid of venom-injected animals. In contrast, WEB2170 failed to alter significantly the extravasation of Evans blue into the left lung of venom-injected animals. The reasons underlying the lack of inhibition of extravasation of Evans blue into the lung parenchyma are not clear but the result is consistent with our previous studies demonstrating that other inflammatory mediators, in addition to PAF, appear to be involved in the development of lung injury (De Matos et al., 1997). Whether the activation of tachykinin NK_1 receptors underlies the release of inflammatory mediators, such as PAF, is currently under investigation in our laboratory. Nevertheless, our results clearly demonstrate that blockade of the tachykinin NK_1 receptor is a most effective means of protecting the animals from the lung injury following i.v. administration of *T. serrulatus* venom.

In addition to abrogating lung injury, pretreatment of rats with CP96,345 effectively decreased the mortality induced by the venom. In contrast, the PAF receptor antagonist failed to alter mortality and only partially inhibited the lung injury. It is tempting to speculate that inhibition of mortality is linked to inhibition of lung injury. However, we have not measured injury to other vascular beds which could also play an important role in determining the outcome following the i.v. administration of *T. serrulatus* venom. Clearly, further studies are needed to determine the cause of death in our animal model. Nevertheless, our results strongly suggest that, whatever the mechanism(s) underlying the cause of death, the activation of tachykinin NK_1 receptors appears to be an essential part in the process.

This is the first study to show the relevance of the tachykinin NK_1 receptor in mediating lung injury and mortality in animals injected with the neurotoxic *T. serrulatus* venom. Inasmuch as lung injury is an important cause of death following envenoming in humans (Freire-Maia and Campos, 1989; Amaral et al., 1993), blockade of the tachykinin NK_1 receptor may represent an important strategy in the treatment of patients with signs of severe envenoming and clearly deserves further studies.

References

Amaral, C.F.S., Resende, N.A., Freire-Maia, L., 1993. Acute pulmonary edema after *Tityus serrulatus* scorpion sting in children. Am. J. Cardiol. 71, 242–245.

Bertke, E.M., Atkins, J.H., 1964. Effects of *Centruroides sculpuratus* venom upon rat tissue: a histopathologic study. Toxicon 2, 205–209.

Bhathia, M., Saluja, A.K., Hofbauer, B., Frossard, J.-L., Lee, H.S., Castagliuolo, I., Wang, C.-C., Gerard, N., Pothoulakis, C., Steer, M.L., 1998. Role of substance P and the neurokinin 1 receptor in acute pancreatitis and pancreatitis-associated lung injury. Proc. Natl. Acad. Sci. 95, 4760–4765.

Brain, S.D., 1997. Sensory neuropeptides: their role in inflammation and wound healing. Immunopharmacology 37 (2–3), 133–152.

Cooke, H.J., Fox, P., Alferes, L., Fox, C.C., Wolfe, S.A. Jr, 1998. Presence of NK1 receptors on mucosal-like mast cell line, RBL-2H3 cells. Can. J. Physiol. Pharmacol. 76, 188–193.

De Matos, I.M., Rocha, O.A., Leite, R., Freire-Maia, L., 1997. Lung oedema induced by *Tityus serrulatus* scorpion venom in the rat. Comp. Biochem. Physiol. 118C, 143–148.

De Matos, I.M., Teixeira, M.M., Leite, R., Freire-Maia, L., 1999. Pharmacological evidence that neuropeptides mediate part of the actions of scorpion venom on the guinea pig ileum. Eur. J. Pharmacol. 268, 231–236.

Floch, A., Fardin, V., Cavero, I., 1994. Characterization of NK_1 and NK2 tachykinin receptors in guinea-pig and rat bronchopulmonary and vascular systems. Br. J. Pharmacol. 111, 759–768.

Freire-Maia, L., Campos, J.A., 1989. Pathophysology and treatment of scorpion poisoning. In: C.L. Ownby, G.V. Odell (Eds.), Natural Toxins. Pergamon, Oxford, pp. 139–159.

Freire-Maia, L., De Matos, I.M., 1993. Heparin or a PAF antagonist (BN52021) prevents the acute pulmonary edema induced by *Tityus serrulatus* scorpion venom in the rat. Toxicon 31, 1207–1210.

Freire-Maia, L., Azevedo, A.D., Costa Val, V.P., 1973. Respiratory arrhythmias produced by purified scorpion toxin. Toxicon 11, 255–257.

Freire-Maia, L., Pinto, G.I., Franco, I., 1974. Mechanism of the cardiovascular effects produced by purified scorpion toxin in the rat. J. Pharmacol. Exp. Ther. 188, 207–213.

Freire-Maia, L., Almeida, H.O., Cunha-Melo, J.R., Azevedo, A.D., Barroso, J., 1978. Mechanism of the pulmonary edema induced by intravenous injection of scorpion toxin in the rat. Agents Actions 8, 113–118.

Freire-Maia, L., Campos, J.A., AmaraL, C.F.S., 1994. Approaches to the treatment of scorpion envenoming. Toxicon 12, 1009–1014.

Guard, S., Boyle, S.J., Tang, K.W., Watling, K.J., McKnight, A.T., Woodruff, G.N., 1993. The interaction of the NK1 receptor antagonist CP-96,345 with L-type calcium channels and its functional consequences. Br. J. Pharmacol. 110, 385–391.

Ho, W.Z., Stavropoulos, G., Lai, J.P., Hu, B.F., Magafa, V., Anagnostides, S., Douglas, S.D., 1998. Substance P C-terminal octapeptide analogues augment tumor necrosis factor-alpha release by human blood monocytes and macrophages. J. Neuroimmunol. 82, 126–132.

Ivey, C.L., Williams, F.M., Collins, P.D., Jose, P.J., Williams, T.J., 1995. Neutrophil chemoattractants generated in two phases during reperfusion of ischemic myocardium in the rabbit: evidence for a role for C5a and interleukin-8. J. Clin. Invest. 95, 2720–2728.

Koyama, S., Sato, E., Nomura, H., Kubo, K., Nagai, S., Izumi, T., 1998. Acetylcholine and substance P stimulate bronchial epithelial cells to release eosinophil chemotactic activity. J. Appl. Physiol. 84, 1528–1534.

Nagahisa, A., Asai, R., Kanai, Y., Murase, A., Tsuchiya-Nakagaki, M., Nakagaki, T., Shieh, T.C., taniguchi, K., 1992. Non-specific activity of (±)-CP-96,345 in models of pain and inflammation. Br. J. Pharmacol. 107, 273–275.

Palframan, R.T., Costa, S.K.P., Wilsoncroft, P., Antunes, E., de Nucci, G., Brain, S.D., 1996. The effect of a tachykinin NK_1 receptor antagonist, SR140333, on oedema formation induced in rat skin by venom from the *Phoneutria nigriventer* spider. Br. J. Pharmacol. 118, 295–298.

Sakamoto, T., Barnes, P.J., Fan Chung, K., 1993. Effect of CP96,345, a non-peptide NK_1 receptor antagonist, against substance P-, bradykinin- and allergen-induced airway microvascular leakage and bronchoconstriction in the guinea pig. Eur. J. Pharmacol. 231, 31–38.

Saria, A., Lundberg, J.M., 1983. Evans blue fluorescence: quantitative and morphological evaluation of vascular permeability in animal tissue. J. Neurosci. Meth. 8, 41–49.

Saria, A., Lundberg, J.M., Skofitsch, G., Lembeck, F., 1983. Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. Naunyn-Schmiedeberg's Arch. Pharmacol. 324, 212–218.

Schmidt, A.W., McLean, S., Heym, J., 1992. The substance P receptor antagonist CP-96,345 interacts with Ca^{2+} channels. Eur. J. Pharmacol. 219, 491–492.

Yaron, R., Braun, K., 1970. Cardiovascular effects of scorpion venom, morphological changes in the myocardium. Toxicon 8, 41–46.