British Journal of Pharmacology (2003) 140, 847–854
www.nature.com/bjp

Mechanisms underlying the inhibitory effects of tachykinin receptor antagonists on eosinophil recruitment in an allergic pleurisy model in mice

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1 The activation of tachykinin NK receptors by neuropeptides may induce the recruitment of eosinophils *in vivo*. The aim of the present study was to investigate the effects and underlying mechanism(s) of the action of tachykinin receptor antagonists on eosinophil recruitment in a model of allergic pleurisy in mice.

2 Pretreatment of immunized mice with capsaicin partially prevented the recruitment of eosinophils after antigen challenge, suggesting the potential contribution of sensory nerves for the recruitment of eosinophils

3 Local (10–50 nmol per pleural cavity) or systemic (100–300 nmol per animal) pretreatment with the tachykinin NK_1 receptor antagonist SR140333 prevented the recruitment of eosinophils induced by antigen challenge of immunized mice. Neither tachykinin NK_2 nor NK_3 receptor antagonists suppressed eosinophil recruitment.

4 Pretreatment with SR140333 failed to prevent the antigen-induced increase of interleukin-5 concentrations in the pleural cavity. Similarly, SR140333 failed to affect the bone marrow eosinophilia observed at 48 h after antigen challenge of immunized mice.

5 SR140333 induced a significant increase in the concentrations of antigen-induced eotaxin at 6 h after challenge.

6 Antigen challenge of immunized mice induced a significant increase of Leucotriene B_4 (LTB_4) concentrations at 6 h after challenge. Pretreatment with SR140333 prevented the antigen-induced increase of LTB_4 concentrations.

7 Our data suggest an important role for NK_1 receptor activation with consequent LTB_4 release and eosinophil recruitment in a model of allergic pleurisy in the mouse. Tachykinins appear to be released mainly from peripheral endings of capsaicin-sensitive sensory neurons and may act on mast cells to facilitate antigen-driven release of LTB_4.
British Journal of Pharmacology (2003) **140,** 847–854. doi:10.1038/sj.bjp.0705515

Keywords: Eosinophil recruitment; LTB_4; eotaxin; IL-5; bone marrow

Abbreviations: IL-5, interleukin-5; LTB_4, Leucotriene B_4; OVA, ovalbumin

Introduction

There is much evidence suggesting an important role for eosinophils in the pathophysiology of allergic diseases (Schroder *et al*., 1996; Giembycz & Lindsay, 1999). In these conditions, eosinophils may be a crucial source of cationic proteins, lipid mediators, oxygen-derived radicals, cytokines and chemokines that contribute to severity of disease (Cara *et al*., 2000). Thus, the understanding of the mechanisms underlying eosinophil recruitment *in vivo* may aid in the development of novel strategies for the treatment of allergic disorders. Leucotriene B_4 (LTB_4), the chemokine eotaxin and interleukin-5 (IL-5) are among the mediators of the inflammatory process known to play an important role in inducing eosinophil migration during allergic processes (Giembycz & Lindsay, 1999; Cara *et al*., 2000; Klein *et al*., 2000; 2001; 2002).

Tachykinins are a group of neuropeptides that include substance P, neurokinin A and neurokinin B, and are released from peripheral endings of capsaicin-sensitive sensory nerves. These fibres are stimulated by a large variety of agents (Geppetti *et al*., 1991; Geppetti, 1993) and are depleted by pretreatment with capsaicin (Jancso *et al*., 1977; Holzer, 1991). The biological actions of tachykinins are mediated by the tachykinins receptors NK_1, NK_2 and NK_3, and their release may cause, among other things, oedema formation (Walsh

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Advance online publication: 6 October 2003

et al., 1995), vasodilatation (Holzer, 1998), mast cell degranulation (Lau *et al*., 2001) and recruitment of inflammatory cells (Maggi, 1997; Saban *et al*., 1997; Frode-Saleh *et al*., 1999).

Several studies have investigated the ability of neuropeptides, especially substance P, to induce eosinophil chemotaxis *in vitro* and migration *in vivo* (Matsuda *et al*., 1989; Numao & Agrawal, 1992; Iwamoto *et al*., 1993; Smith *et al*., 1993; Baluk *et al*., 1995; Walsh *et al*., 1995; Saban *et al*., 1997; Dunzendorfer *et al*., 1998; Frode-Saleh *et al*., 1999). It is clear from these studies that neuropeptides may not only activate eosinophil recruitment directly, but also induce the release of mediators of the inflammatory process, especially LTB_4, which in turn induce the recruitment of eosinophils (Iwamoto *et al*., 1993; Walsh *et al*., 1995: Saban *et al*., 1997). Moreover, it appears that activation of mast cells, either *via* NK_1 receptor-dependent or -independent mechanisms, is a relevant mechanism for neuropeptide-induced eosinophil migration (Matsuda *et al*., 1989; Iwamoto *et al*., 1993; Walsh *et al*., 1995). The importance of endogenous neuropeptide release and action on tachykinin NK receptors for the recruitment of eosinophils in various models of inflammation has also been demonstrated. Thus, blockade of tachykinin receptors was associated with the inhibition of eosinophil influx after pulmonary administration of sephadex (Tramontana *et al*., 2002) or in models of allergic pulmonary inflammation (Schuiling *et al*., 1999a,b; Nénan *et al*., 2001). Few of the studies above have evaluated the comparative effect of drugs that inhibit each of the tachykinin NK receptors. Furthermore, we are not aware of studies investigating the effects of these drugs on the local release of mediators of inflammation known to participate in the cascade of events leading to eosinophil recruitment after antigen challenge of sensitized animals. Thus, the aim of the present study was to investigate the effects and underlying mechanism(s) of the action of tachykinin receptor antagonists on eosinophil recruitment in a model of allergic pleurisy in mice.

Methods

Animals

Female Balb/C mice (18–22 g) were used throughout these experiments. Animals were housed in a temperature-controlled room with free access to water and food. All experimental procedures have been subjected to evaluation and were approved by animal ethics committee of the Universidade Federal de Minas Gerais.

Drugs and reagent

Bovine serum albumin (BSA), ovalbumin (OVA) and capsaicin were purchased from Sigma (St Louis, MO, U.S.A.). The NK_1 NK_2 and NK_3 receptor antagonists (SR140333, SR48968 and SR148201, respectively) were a kind gift of Sanofi Recherche. These antagonists were dissolved in DMSO and diluted further in phosphate-buffered saline (PBS, pH 7.4) just prior to use.

Sensitization

Animals were immunized with OVA adsorbed to aluminium hydroxide gel as previously described (Das *et al*., 1997). Briefly, mice were injected subcutaneously (s.c.) on days 1 and 8 with 0.2 ml of a solution containing 100 μg of OVA and 70 μg of aluminium hydroxide (Reheiss, Dublin, Ireland).

Leucocyte migration into the pleural cavity induced by antigen

At 8 days after the last immunization, antigen (OVA, 1.0 μg per pleural cavity) or vehicle was injected intrapleurally (i.pl.) in naïve or immunized mice. Animals were killed at 48 h after the i.pl. injection of the stimuli. The cells present in the cavity were harvested by 2 ml of PBS and total cell counts performed in a modified Neubauer chamber using Turk's stain. Differential cell counts were performed on cytospin preparations (Shandon III) stained with May Grunwald using standard morphologic criteria to identify cell types. The results are presented as the number of cells per cavity.

Tachykinin receptor antagonist pretreatment

To evaluate the role of endogenous tachykinins on the eosinophil recruitment induced by OVA in immunized animals, we prctreated mice with NK receptor antagonists at doses similar to those previously reported by others (Inoue *et al*., 1996; Daoui *et al*., 2001). In sensitized animals, the NK_1 receptor antagonist (SR140333, 10–50 nmol per pleural cavity), the NK_2 receptor antagonist (SR48968, 10–100 nmol per pleural cavity) or the NK_3 receptor antagonist (SR142801, 10–50 nmol per pleural cavity) was administered i.pl. 5 min prior to OVA challenge. In addition, experiments were carried out injecting the NK_1 receptor antagonist (SR140333, 100–300 nmol nmol per animal), the NK_2 receptor antagonist (SR48968, 100–300 nmol nmol per animal) or the NK_3 receptor antagonist (SR142801, 50–300 nmol nmol per animal) systemically (intravanously, i.v.) 15 min before antigen challenge. For the experiments that evaluated eosinophil release from the bone marrow, SR140333 (300 nmol per animal) was injected systemically 15 min prior to antigen challenge.

Capsaicin treatment

The protocol for capsaicin depletion was similar to that of Dickerson *et al*. (1998). Briefly, animals were immunized on days 1 and 8. On days 13, 14 and 15, each animal was anaesthetized i.p. with 0.2 ml of a solution containing xylazine (0.02 mg ml^{-1}), ketamine (50 mg ml^{-1}) and saline in a proportion of 1:0.5:3, respectively, and injected s.c. with capsaicin (50 μg per animal twice on day 13 and 300 μg per animal twice on days 14 and 15). Capsaicin was dissolved in absolute ethanol, stabilized with Tween 80 (50:50), and then further diluted in saline. The final concentration of ethanol and Tween 80 was 10%. The solution of capsaicin was made up daily just prior to the administration to the animals. On day 21, mice were challenged with antigen and leucocyte counts performed after a further 48 h. As a positive control for the depletion of capsaicin-sensitive sensory nerves, mice received an intraplantar injection of capsaicin (0.1 μg per paw; 20 μl) and the composite licking behaviour during the first 5 min assessed.

Collection of bone marrow cells

Bone marrow cells were isolated from the left femur. The femoral head and condyles were removed, and the displaceable cells were recovered by flushing the marrow cavity of the femur shaft with 1 ml PBS containing heparin (10 U ml^{-1}). Total cell counts were performed in a modified Neubauer chamber using Turk's stain. Differential cell counts were performed on cytospin preparations (Shandon III) stained with May Grunwald using standard morphologic criteria to identify cell types. The results are presented as the number of cells per left femur.

Measurement of eotaxin, LTB$_4$ and IL-5

At 6 h after challenge with OVA, animals were killed by cervical dislocation and the pleural cavity was washed with 1 ml of ice-cold PBS solution containing EDTA 10^{-3} M and 0.01% BSA and supernatants stored at -70°C until further analysis. The concentration of eotaxin protein in pleural effluents was measured by specific ELISA using commercially available antibodies (R&D Systems, Minneapolis, MN, U.S.A.), as reported elsewhere (Klein *et al*., 2000). Frozen supernatants were also assayed for LTB$_4$ and IL-5 levels. Concentrations of LTB$_4$ in each supernatant were assayed in duplicate by EIA according to the manufacturer's instructions (Cayman, Ann Arbor, MI, U.S.A.). IL-5 was determined using a specific IL-5 ELISA detection kit (Pharmingen), according to the instructions of the manufacturer.

Statistical analysis

All results are presented as the means ± s.e.m. Normalized data were analysed by one-way ANOVA, and differences between groups were assessed using Student – Newman – Keuls post-test. A P-value <0.05 was considered to be significant.

Results

Effects of sensory nerves depletion on eosinophil recruitment

In agreement with our previous studies, the i.pl. injection of antigen (OVA) in sensitized mice induced eosinophil recruitment after 48 h (Figure 1a). In contrast, OVA challenge of naïve animals did not induce significant recruitment at 48 h. In order to investigate the potential contribution to sensory nerves for the recruitment of eosinophils, sensitized animals were pretreated with capsaicin and challenged with OVA. As a positive control for the effectiveness of capsaicin in depleting sensory nerves, we evaluated a behavioural effect, the licking response, after the intraplantar injection of capsaicin (0.1 μg per paw). The pretreatment with capsaicin was inhibited by 54% of the licking response (data not shown, $P<0.001$). In a similar manner, the recruitment of eosinophils in capsaicin-pretreated animals was markedly inhibited in comparison to animals that were pretreated with vehicle (Figure 1b).

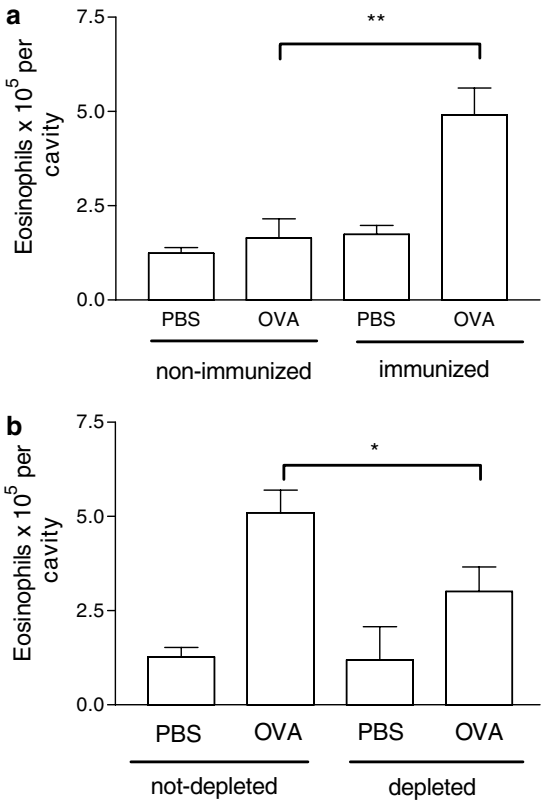

Figure 1 Effects of sensory nerve ending depletion with capsaicin on the recruitment of eosinophils induced by antigen challenge of sensitized mice. In (a), naïve or immunized mice were challenged with antigen (OVA, 1 μg per pleural cavity) or PBS (100 μl per cavity) and the number of infiltrating eosinophils assessed after 48 h. In (b), immunized animals were treated with capsaicin (50 μg per animal twice on day 13 and 300 μg per animal twice on days 14 and 15) or capsaicin vehicle (not depleted), challenged with antigen and the number of infiltrating eosinophils examined after 48 h. The results are expressed as means ± s.e.m. of 5–6 animals in each group. *$P<0.05$ and **$P<0.01$.

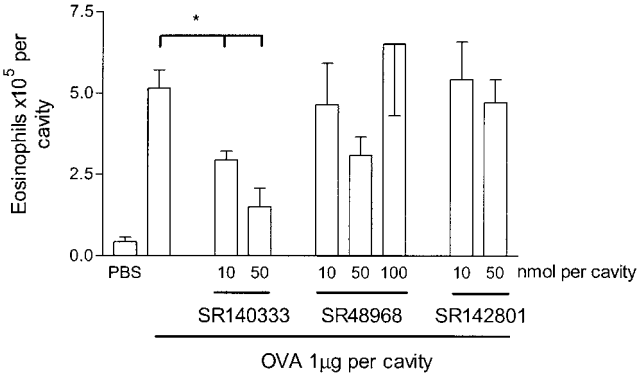

Figure 2 Effects of the local pretreatment with tachykinin NK$_1$ (SR140333), NK$_2$ (SR48968) or NK$_3$ (SR142801) receptor antagonists on the recruitment of eosinophils induced by antigen challenge of sensitized mice. Immunized mice were challenged with antigen (OVA, 1 μg per pleural cavity) or PBS (100 μl per cavity) and the number of infiltrating eosinophils assessed after 48 h. For the local pretreatment with tachykinin NK receptor antagonists, mice were injected i.pl. with SR140333, SR48968 or SR142801 (10–L100 nmol per pleural cavity) 5 min prior to the antigen challenge. Results are expressed as means ± s.e.m. of 5–6 animals in each group. *$P<0.05$.

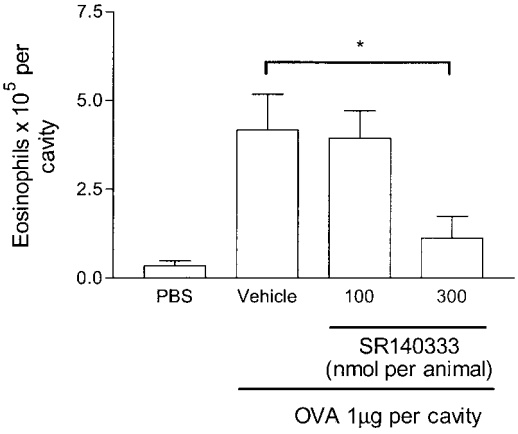

Figure 3 Effects of the systemic pretreatment with the tachykinin NK_1 receptor antagonist SR140333 on the recruitment of eosinophils induced by antigen challenge of sensitized mice. Immunized mice were challenged with antigen (OVA, 1 μg per pleural cavity) or PBS (100 μl per cavity) and the number of infiltrating eosinophils assessed after 48 h. SR140333 (100–300 nmol per animal) was injected i.v. 15 min before the antigen challenge. The results are expressed as means ± s.e.m. of 5–6 animals in each group. $^*P<0.05$.

Effects of the treatment with tachykinin receptor antagonists on eosinophil recruitment

In order to evaluate the potential contribution of tachykinin receptors for the recruitment of eosinophils, sensitized animals were pretreated i.pl. with a tachykinin NK_1 receptor antagonist (SR140333), a tachykinin NK_2 receptor antagonist (SR48968) or a tachykinin NK_3 receptor antagonist (SR142801) 5 min prior to OVA challenge. Only SR140333 suppressed the recruitment of eosinophils in a dose-dependent manner (Figure 2). Pretreatment with similar doses of SR48968 or SR142801 failed to affect significantly the recruitment of eosinophils at 48 h after antigen challenge (Figure 2).

Similarly, the systemic injection of SR140333 prevented the influx of eosinophils following antigen challenge of sensitized mice (Figure 3). Systemic treatment with SR48968 (50–300 nmol per animal) or SR142801 (50–300 nmol per animal) failed to affect the recruitment of eosinophils following antigen challenge of immunized animals (data not shown).

Effects of the treatment with tachykinin receptor antagonists on mediator release

The next series of experiments were designed to investigate whether pretreatment with SR140333 decreased the concentration of IL-5, eotaxin or LTB_4, substances reportedly known to mediate eosinophil differentiation and recruitment *in vivo* (Sanderson, 1992; Foster *et al*., 1996; Gonzalo *et al*., 1996; Kopf *et al*., 1996; Conroy & Williams, 2001; Klein *et al*., 2000; 2001).

There was no detectable IL-5 in the pleural cavity of sensitized and PBS-challenged mice, but the concentrations of this cytokine was elevated at 6 h after challenge with OVA (Figure 4a). This enhanced concentration of IL-5 at 6 h was mirrored by an increase in the number of mature eosinophils in the bone marrow of antigen-challenged mice (Figure 4b).

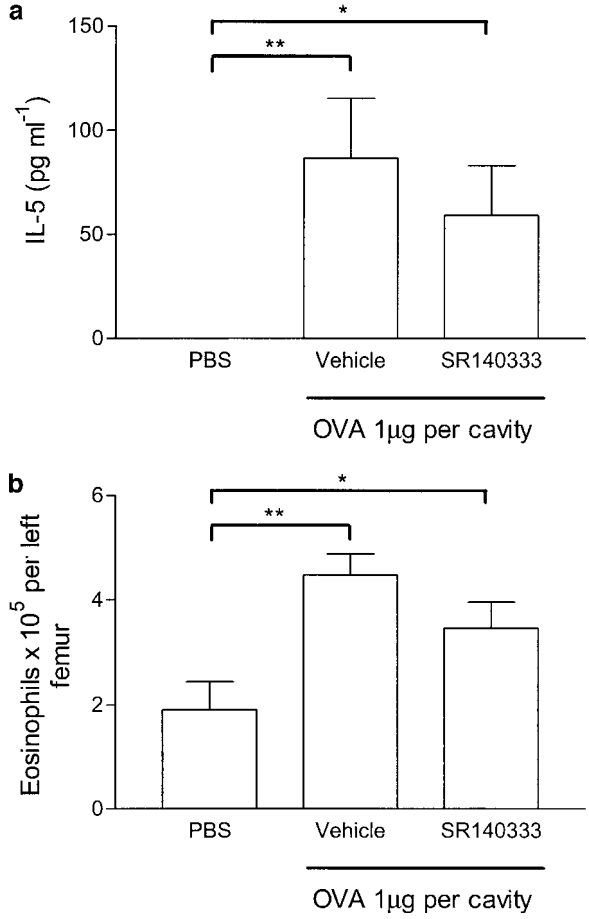

Figure 4 Effects of the pretreatment with the tachykinin NK_1 receptor antagonist SR140333 on the release of IL-5 into the pleural cavity (a) and the number of eosinophils in the bone marrow (b) after antigen challenge of sensitized mice. Animals were pretreated with SR140333 (50 nmol per pleural cavity) 5 min before the i.pl. injection of antigen and levels of the IL-5 on pleural wash assessed after 6 h. In other experiments, immunized mice were pretreated with SR140333 (300 nmol per animal) i.v. 15 min prior to the OVA challenge and the number of eosinophils in the bone marrow assessed after 48 h. Results are expressed as means ± s.e.m. of 5–6 animals in each group. $^{**}P<0.01$ and $^{***}P<0.001$.

Pretreatment with SR140333 had no significant effects on OVA-induced increase in IL-5 concentrations (Figure 4a). Furthermore, the drug failed to affect the number of mature eosinophils retrieved from the bone marrow of antigen-challenged sensitized mice (Figure 4b).

In agreement with our previous results (Klein *et al*., 2001), antigen challenge induced an increase in the amount of eotaxin immunoreactivity in the pleural cavity of sensitized mice (Figure 5). Pretreatment with SR140333 induced a significant increase in the concentrations of eotaxin detected in pleural wash supernatants 6 h after OVA challenge in comparison to animals pretreated with vehicle (Figure 5).

In the model of allergic pleurisy described herewith, LTB_4 is produced upon antigen challenge and seems to cooperate with eotaxin to facilitate the recruitment of eosinophils following antigen challenge (Klein *et al*., 2000; 2001). There was a significant increase in the concentrations of LTB_4 at 6 h after antigen challenge of sensitized mice (Figure 6). Pretreatment

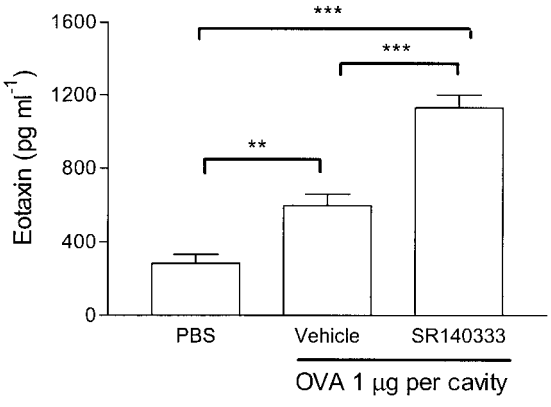

Figure 5 Effects of the local pretreatment with the tachykinin NK_1 receptor antagonist SRI40333 on the release of eotaxin into the pleural cavity after antigen challenge of sensitized mice. Immunized mice were challenged with antigen (OVA. 1 μg per pleural cavity) or PBS (100 μl per cavity) and the concentrations of the eotaxin on the pleural wash fluid assessed after 6 h. SR140333 (50 nmol per cavity) was injected i.pl. 5 min before the antigen challenge. Results are expressed as means $\pm$ s.e.m. of 5–6 animals in each group. $^{**}P<0.01$ and $^{***}P<0.001$.

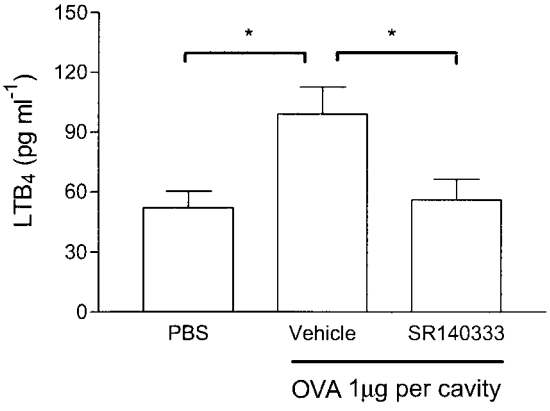

Figure 6 Effects of the local pretreatment with the tachykinin NK_1 receptor antagonist SR140333 on the release of LTB_4 into the pleural cavity after antigen challenge of sensitized mice. Immunized mice were challenged with antigen (OVA. 1 μg per pleural cavity) or PBS (100 μl per cavity) and concentrations of the LTB_4 on pleural wash fluid assessed after 6 h. SR140333 (50 nmol per cavity) was injected i.pl. 5 min before the antigen challenge. Results are expressed as means $\pm$ s.e.m. of 5–6 animals in each group. $^{*}P<0.05$.

with SR140333 prevented the increase in LTB_4 concentrations above the background levels found in sensitized PBS-challcnged mice (Figure 6).

Discussion

Substance P, neurokinin A and neurokinin B are neuropeptides collectively known as tachykinins. These neuropeptides may be released antidromically from capsaicin-sensitive sensory neurons and produce a wide range of physiological and pathological events, including activation of the secretion of submucosal glands (Phillips *et al*., 2003), smooth muscle constriction (Murai *et al*., 1993), increase in vascular permeability (Holzer, 1998) and recruitment of leucocytes (Dunzendorfer *et al*., 1998). The ability of neuropeptides to induce the cardinal signs of inflammation is collectively referred to as neurogenic inflammation (Harrison & Geppetti, 2001). The biological actions of tachykinins are mediated by three tachykinin receptors, NK_1, NK_2 and NK_3 that exhibit preferences for substance P, neurokinin A and neurokinin B, respectively (Severini *et al*., 2002). In the present work, we have investigated the effects of tachykinin receptor antagonists on the recruitment of eosinophils and release of eosinophil-active mediators in a model of allergic pleurisy in mice.

As sensory nerves are a major source of tachykinins (Holzer, 1991; Quartara & Maggi, 1998), experiments were designed to investigate the role of sensory nerves as a source of neuropeptides in our model. To this end, sensitized animals were treated systemically with doses of capsaicin sufficient to cause neuropeptide depletion from C-fibres (Holzer, 1991; Dickerson *et al*., 1998). This protocol was chosen (instead of neonatal treatment) to avoid any possible effects of capsaicin treatment during the sensitization phase of the allergic pleurisy. As antigen challenge was given 13 days after the last sensitization, and not 8 days as in our previous studies (Klein *et al*., 2000; 2001; 2002), initial experiments confirmed that the recruitment of eosinophils was antigen and sensitization specific. The effectiveness of capsaicin depletion was confirmed by its ability to suppress the neuropetide-dependent behavioural response to intraplantar injection of capsaicin. In these animals, depleting of sensory nerves also effectively inhibited the eosinophil recruitment in response to antigen challenge of sensitized mice. Altogether, these results demonstrate the relevance of sensory nerve-derived neuropeptides for the recruitment of eosinophils in an allergic pleurisy model in mice.

The next series of experiments examined the participation of each of the tachykinin receptors in the process of eosinophil recruitment in the allergic pleurisy model. Our results clearly show that local or systemic administration of an NK_1 receptor antagonist prevented the recruitment of eosinophils induced by allergen challenge of sensitized mice. This is in agreement with other studies demonstrating a role of tachykinin NK_1 receptor for eosinophil recruitment in allergen-induced airway inflammation in conscious, unrestrained guinea-pigs (Schuiling *et al*., 1999a, b). In contrast, other studies in anaesthetized guinea-pigs or rabbits (Kudlacz *et al*., 1996; Costello *et al*., 1998; D'Agostino *et al*., 2002) failed to find any effects of NK_1 receptor antagonists on allergen-induced eosinophil recruitment. Our results also showed that neither NK_2 nor NK_3 receptor antagonists affected significantly the recruitment of eosinophils. This is consistent with studies showing lack of effect of NK_2 receptor antagonists on allergen-induced eosinophil recruitment (Kudlacz *et al*., 1996; D'Agostino *et al*., 2002). However, at least one study (Nénan *et al*., 2001) has shown that blockade of NK_3 receptors was associated with the inhibition of eosinophil infiltration in mice. Thus, it is clear from the discussion above that there is much controversy with regard to the effects of tachykinin NK receptor antagonists on the recruitment of eosinophils in response to allergen challenge. This is further complicated when one considers stimuli other than allergen challenge, including IL-5 and sephadex (eg Kraneveld *et al*., 1997; Tramontana *et al*., 2002). To our knowledge, there is no single

explanation to accommodate all these discrepant results, but differences in species, immunization and challenge procedures, and the use of anaesthetics may account for some of the differences. Moreover, the ability of tachykinin NK receptor activation to modulate the production of mediators of the inflammatory process differentially (see below) may also underlie the different ability of these drugs to function in animal models of allergen-induced eosinophil infiltration.

We have previously shown a role for both LTB_4 and cotaxin in mediating eosinophil recruitment in the model of allergic pleurisy used in the present study (Klein *et al*., 2000; 2001). Indeed, our previous work has demonstrated that PAF-induced eotaxin cooperates with stem cell factor-induced LTB_4 to induce eosinophil recruitment following allergen challenge of sensitized mice (Klein *et al*., 2000; 2001; 2002). Overall, the latter results are in good agreement with other studies demonstrating a role for eotaxin and LTB_4 in mediating eosinophil recruitment in several models of allergic inflammation (Teixeira & Hellewell, 1994; Teixeira *et al*., 1997; Turner *et al*., 1996; Humbles *et al*., 1997, Rothenberg, 1999). Furthermore, a recent study has shown a cooperation between eotaxin and substance P in inducing eosinophil cytotoxicity, which was at least in part due to tyrosine kinases pathyway(s) (El-Shazly & Ishikawa, 1999). IL-5 is another mediator of the inflammatory process known to play a most relevant role in allergen-induced eosinophil recruitment (Foster *et al*., 1996; Kopf *et al*., 1996). Indeed, IL-5 not only is essential for eosinophil differentiation and release from the bone marrow, but it also primes eosinophils, facilitating their migration *in vivo* (Mould *et al*., 1997). In our model, anti-IL-5 treatment totally abrogated eosinophil influx (data not shown). Next, we investigated the possible involvement in these mediators for the inhibitory effects of the tachykinin NK_1 receptor antagonist on eosinophil recruitment in the allergic pleurisy model.

Pretreatment with SR140333 had no significant effects on the concentrations of IL-5 in pleural wash supernatants. Likewise, the pretreatment with SR140333 did not modify the number of eosinophils in bone marrow. The drug also failed to diminish the enhanced eotaxin production that followed allergen challenge of sensitized mice. On the contrary, there was actually an increase in the concentrations of eotaxin. The rcason(s) for the latter findings are not known at present, but the results suggest that the release of neuropeptides and action on tachykinin NK_1 receptors play a negative modulatory role on the production of eotaxin following allergen challenge. Further studies in other experimental systems will be necessary to confirm whether this is also true in other conditions and to determine the cellular target of the actions of tachykinin NK_1 receptor agonists. Overall, these data argue that an effect on IL-5 and eotaxin release are not likely to be important mechanisms of the inhibitory action of the tachykinin NK_1 receptor antagonist in our system.

In contrast to its lack of effect on allergen-induced increases in eotaxin and IL-5 concentrations, pretreatment with SR140333 markedly suppressed the elevation of the concentration of LTB_4 in pleural cavity wash supernatants 6 h after allergen challenge. The latter results suggest that in the present model allergen-associated neuropeptides are not acting directly on eosinophils to induce migration, but facilitate and/or induce the release of an intermediate mediator of inflammation, LTB_4, which in turn induces eosinophil recruitment. This is in agreement with the shown ability of substance P to induce the migration of granulocytes (both eosinophils and neutrophils) *via* release of LTB_4 in several models of inflammation (Iwamoto *et al*., 1993; Saban *et al*., 1997; Okabe *et al*., 2001). The cell(s) that express tachykinin NK_1 receptors and are, thus, the target for the actions of neuropeptides released have not been investigated here. However, mast cells are a possible candidate, as several studies have demonstrated that mast cell degranulation induced by antigen or other stimuli is facilitated *via* tachykinin NK receptor activation (Krumins & Broomfield, 1992; 1993; Lilly *et al*., 1995; Hua *et al*., 1996). As in our system mast cells are a likely source of LTB_4 after antigen challenge of sensitized mice, neuropeptides may act on these cells to facilitate the release of LTB_4 and, consequently, enhance eosinophil migration into the pleural cavity. Interestingly, another mast cell-active mediator, stem cell factor, may facilitate the actions of substance P on murine mast cells *in vitro* (Karimi *et al*., 2000). As stem cell factor is released after allergen challenge and participates in the cascade of events leading to eosinophil recruitment (Klein *et al*., 2000), stem cell factor and substance P may also cooperate to facilitate eosinophil influx in our model.

In conclusion, our data suggest an important role for NK_1 receptor activation with consequent LTB_4 release and eosinophil recruitment in a model of allergic pleurisy in the mouse. Tachykinins appear to be released mainly from peripheral endings of capsaicin-sensitive sensory neurons and may act on mast cells to facilitate antigen-driven release of LTB_4.

We are grateful to Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq/Brasil) for financial support.

References

BALUK, P., BERTRAND, C., GEPPETTI, P., McDONALD, D.M. & NADEL, J.A. (1995). NK_1 receptors mediate leukocyte adhesion in neurogenic inflammation in rat trachea. *Am. J. Physiol.,* **268,** L263–L269.

CARA, D.C., NEGRAO-CORREA, D. & TEIXEIRA, M.M. (2000). Mechanisms underlying eosinophil trafficking and their relevance *in vivo*. *Histol. Histopathol.,* **15,** 899–920.

CONROY, D.M. & WILLIAMS, T.J. (2001). Eotaxin and the attraction of eosinophils to the asthmatic lung. *Respir. Res.,* **2,** 150–156.

COSTELLO, R.W., FRYER, A.D., BELMONTE, K.E. & JACOBY, D.B. (1998). Effects of tachykinin NK_1 receptor antagonists on vagal hyperreactivity and neuronal M2 muscarinic receptor function in antigen challenged guinea-pigs. *Br. J. Pharmacol.,* **124,** 267–276.

D'AGOSTINO, B., ADVENIER, C., DE PALMA, R., GALLELLI, L., MARROCCO, G., ABBATE, G.F. & ROSSI, F. (2002). The involvement of sensory neuropeptides in airway hyper-responsiveness in rabbits sensitized and challenged to Paricteria judaica. *Clin. Exp. Allergy,* **32,** 472–479.

DAOUI, S., AHNAOU, A., NALINE, E., EMONDS-ALT, X., LAGENTE, V. & ADVENIER, C. (2001). Tachykinin NK(3) receptor agonists induced microvascular leakage hypersensitivity in the guinea-pig airways. *Eur. J. Pharmacol.,* **433,** 199–207.

DAS, A.M., FLOWER, R.J., HELLEWELL, P.G., TEIXEIRA, M.M. & PERRETTI, M. (1997). A novel murine model of allergic inflammation to study effect of dexamethasone on eosinophil recruitment. *Br. J. Pharmacol.,* **121,** 97–104.

DICKERSON, C., UNDEM, B., BULLOCK, B. & WINCHURCH, R. (1998). Neuropeptides regulation of proinflammatory cytokine responses. *J. Leukocyte Biol.,* **63,** 602–605.

DUNZENDORFER, S., MEIERHOFER, C. & WIEDERMANN, C.J. (1998). Signaling in neuropeptide-induced migration of human cosinophils. *J. Leukocyte Biol.,* **64,** 828–834.

EL-SHAZLY, A. & ISHIKAWA, S.P. (1999). Novel co-operation between eotaxin and subtance-P in inducing eosinophil-derived neurotoxin release. *Mediators Inflamm.,* **8,** 177–179.

FOSTER, P.S., HOGAN, S.P., RAMSAY, A.J., MATTHAEI, K.I. & YOUNG, I.G. (1996). Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.,* **183,** 195–201.

FRODE-SALEH, T.S., CALIXTO, J.B. & MEDEIROS, Y.S. (1999). Analysis of the inflammatory response induced by substance P in the mouse pleural cavity. *Peptides,* **20,** 259–265.

GEPPETTI, P. (1993). Sensory neuropeptide release by bradykinin: mechanisms and pathophysiological implications. *Regul. Peptides,* **47,** 1–23.

GEPPETTI, P., DEL BIANCO, E., PATACCHINI, R., SANTICIOLI, P., MAGGI, C.A. & TRAMONTANA, M. (1991). Low pH-induced release of calcitonin gene-related peptide from capsaicin-sensitive sensory nerves: mechanism of action and biological response. *Neuroscience,* **41,** 295–301.

GIEMBYCZ, M.A. & LINDSAY, M.A. (1999). Pharmacology of the eosinophil. *Pharmacol. Rev.,* **51,** 213–340.

GONZALO, J.A., JIA, G.Q., AGUIRRE, V., FRIEND, D., COYLE, A.J., JENKINS, N.A., LIN, G.S., KATZ, H., LICHTMAN, A., COPELAND, N., KOPF, M. & GUTIERREZ-RAMOS, J.C. (1996). Mouse Eotaxin expression parallels eosinophil accumulation during lung allergic inflammation but it is not restricted to a Th2-type response. *Immunity,* **4,** 1–14.

HARRISON, S. & GEPPETTI, P. (2001). Substance P. *Int. J. Biochem. Cell Biol.,* **33,** 555–576.

HOLZER, P. (1991). Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol. Rev.,* **43,** 143–201.

HOLZER, P. (1998). Neurogenic vasodilatation and plasma leakage in the skin. *Gen. Pharmac.,* **30,** 5–11.

HUA, X.Y., BACK, S.M. & TAM, E.K. (1996). Substance P enhances electrical field stimulation-induced mast cell degranulation in rat trachea. *Am. J. Physiol.,* **270,** L985–L991.

HUMBLES, A.A., CONROY, D.M., MARLEAU, S., RANKIN, S.M., PALFRAMAN, R.T., PROUDFOOT, A.E., WELLS, T.N., LI, D., JEFFERY, P.K., GRIFFITHS-JOHNSON, D.A., WILLIAMS, T.J. & JOSE, P.J. (1997). Kinetics of eotaxin generation and its relationship to eosinophil accumulation in allergic airways disease: analysis in a guinea pig model *in vivo*. *J. Exp. Med.,* **186,** 601–612.

INOUE, H., NAGATA, N. & KOSHIHARA, Y. (1996). Involvement of tachykinin receptors in oedema formation and plasma extravasation induced by substance P, neurokinin A, and neurokinin B in mouse ear. *Inflamm. Res.,* **45,** 316–323.

IWAMOTO, I., TOMOE, S., TOMIOKA, H. & YOSHIDA, S. (1993). Leukotriene B_4 mediates substance P-induced granulocyte infiltrating into mouse skin. *J. Immunol.,* **151,** 2116–2123.

JANCSO, G., KIRALY, E. & JANCSO-GABOR, A. (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. *Nature,* **270,** 741–743.

KARIMI, K., REDEGELD, F.A., BLOM, R. & NIJKAMP, P.P. (2000). Stem cell factor and interleukin-4 increase responsiveness of mast cells to substance P. *Exp. Hematol.,* **28,** 626–634.

KLEIN, A., PINHO, V., ALESSANDRI, A.L., SHIMIZU, T., ISHII, S. & TEIXEIRA, M.M. (2002). Platelet-activating factor drives eotaxin production in an allergic pleurisy in mice. *Br. J. Pharmacol.,* **135,** 1213–1218.

KLEIN, A., TALVANI, A., CARA, D.C., GOMES, K.L., LUKACS, N.W. & TEIXEIRA, M.M. (2000). Stem cell factor plays a major role in the recruitment of eosinophils in allergic pleurisy in mice *via* the production of leukotriene B_4. *J. Immunol.,* **164,** 4271–4276.

KLEIN, A., TALVANI, A., SILVA, P.M., MARTINS, M.A., WELLS, T.N., PROUDFOOT, A., LUCKACS, N.W. & TEIXEIRA, M.M. (2001). Stem cell factor-induced leukotriene B4 production cooperates with cotaxin to mediate the recruitment of eosinophils during allergic pleurisy in mice. *J. Immunol.,* **167,** 524–531.

KOPF, M., BROMBACHER, F., HODGKIN, P.O., RAMSAY, A.J., MILBOURNE, E.A., DAÍ, W.J., OVINGTON, K.S., BEHM, C.A., KOHLER, G., YOUNG, I.G. & MATTHAEI, K.I. (1996). IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity,* **4,** 15–24.

KRANEVELD, A.D., NIJKAMP, P.P. & VAN OOSTERHOUT, A.J. (1997). Role for neurokinin-2 receptor in interleukin-5-induced airway hyperresponsiveness but not eosinophilia in guinea pigs. *Am. J. Respir. Crit. Care Med.,* **156,** 367–374.

KRUMINS, S.A. & BROOMFIELD, C.A. (1992). Evidence of NK_1 and NK_2 tachykinin receptors and their involvement in histamine release in a murine mast cell line. *Neuropeptides,* **21,** 65–72.

KRUMINS, S.A. & BROOMFIELD, C.A. (1993). C-terminal substance P fragments elicit histamine release from a murine mast cell line. *Neuropeptides,* **24,** 5–10.

KUDLACZ, E.M., KNIPPENBERG, R.W., LOGAN, D.E. & BURKHOLDER, T.P. (1996). Effect of MDL 105,212, a nonpeptide NK-l/NK-2 receptor antagonist in an allergic guinea pig model. *J. Pharmacol. Exp. Ther.,* **279,** 732–739.

LAU, A.H.Y., CHOW, S.S.M. & NG, Y.S. (2001). Immunologically induced histamine release from rat peritoneal mast cells is enhanced by low levels of substance P. *Eur. J. Pharmacol.,* **414,** 295–303.

LILLY, C.M., HALL, A.E., RODGER, I.W., KOBZIK, L., HALEY, K.J. & DRAZEN, J.M. (1995). Substance P-induced histamine release in tracheally perfused guinea pig lungs. *J. Appl. Physiol.,* **78,** 1234–1241.

MAGGI, C.A. (1997). The effects of tachykinin on inflammatory and immune cells. *Regul. Peptides,* **70,** 75–90.

MATSUDA, H., KAWAKITA, K., KISO, Y., NAKANO, T. & KITAMURA, Y. (1989). Substance P induces granulocyte infiltrating through dcgranulation of mast cells. *J. Immunol.,* **142,** 927–931.

MOULD, A.W., MATTHAEI, K.I., YOUNG, I.G. & FOSTER, P.S. (1997). Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J. Cin. Invest.,* **99,** 1064–1071.

MURAI, M., MAEDA, Y., HAGIWARA, D., MIYAKE, H., IKARI, N., MATSUO, M. & FUJII, T. (1993). Effects of an NK_1 receptor antagonist, FK888, on constriction and plasma extravasation induced in guinea pig airway by neurokinins and capsaicin. *Eur. J. Pharmacol.,* **236,** 7–13.

NÇNAN, S., GERMAIN, N., LAGENTE, V., EMONDS-ALT, X., ADVENIER, C. & BOICHOT, E. (2001). Inhibition of inflammatory cell recruitment by the tachykinin NK(3)-receptor antagonist, SR 142801, in a murine model of asthma. *Eur. J. Pharmacol.,* **421,** 201–205.

NUMAO, T. & AGRAWAL, D.K. (1992). Neuropeptides modulate human eosinophil chemotaxis. *J. Immunol.,* **149,** 3309–3315.

OKABE, T., HIDE, M., KORO, O., NIMI, N. & YAMAMOTO, S. (2001). The release of leukotriene B4 from human skin in response to substance P: evidence for the functional heterogeneity of human skin mast cells among individuals. *Clin. Exp. Immunol.,* **124,** 150–156.

PHILLIPS, J.E., HEY, J.A. & CORBOZ, M.R. (2003). Tachykinin NK(3) and NK(1) receptor activation elicits secretion from porcine airway submucosal glands. *Br. J. Pharmacol.,* **138,** 254–260.

QUARTARA, L. & MAGGI, C.A. (1998). The tachykinin NK, receptor Part II: distribution and pathophysiological roles. *Neuropeptides,* **32,** 1–49.

ROTHENBERG, M.E. (1999). Eotaxin. An essential mediator of eosinophil trafficking into mucosal tissues. *Am. J. Respir. Cell Mol. Biol.,* **21,** 291–295.

SABAN, M.R., SABAN, R., BJORLING, D. & HAAK-FRENDSCHO, M. (1997). Involvement of leukotrienes, TNF-α, and LFA-l/CAM-1 interaction in substance P-induced granulocyte infiltration. *J. Leukocyte Biol.,* **61,** 445–451.

SANDERSON, C.J. (1992). Interleukin-5, eosinophils, and disease. *Blood,* **97,** 3101–3109.

SCHRODER, J.M., NOSO, N., STICHERLING, M. & CHRISTOPHERS, E. (1996). Role of eosinophil-chemotactic C–C chemokines in cutaneous inflammation. *J. Leukocyte Biol.,* **59,** 1–5.

SCHUILING, M., ZUIDHOF, A.B., ZAAGSMA, J. & MEURS, H. (1999a). Involvement of tachykinin NK_1 receptor in the development of allergen-induced airway hyperreactivity and airway inflammatory in conscious, unrestrained guinea-pig. *Am. J. Respir. Crit. Care Med.,* **159,** 423–430.

SCHUILING, M., ZUIDHOF, A.B., ZAAGSMA, J. & MEURS, H. (1999b). Role of tachykinin NK_1 and NK_2 receptors in allergen-induced early and late asthmatic reactions, airway hyperresponsiveness, and airway inflammation in conscious, unrestrained guinea pigs. *Clin. Exp. Allergy,* **29,** 48–52.

SEVERINI, C., IMPROTA, G., FALCONIERI-ERSPAMER, G., SALVADORI, S. & ERSPAMER, V. (2002). The tachykinin peptide family. *Phannacol. Rev.,* **54,** 285–322.

SMITH, C.H., BARKER, J.N., MORRIS, R.W., MACDONALD, D.M. & LEE, T.H. (1993). Neuropeptides induce rapid expression of endothelial cell adhesion molecules and elicit granulocytic infiltration in human skin. *J. Immunol.,* **151,** 3274–3282.

TEIXEIRA, M.M. & HELLEWELL, P.G. (1994). Effect of a 5-lipoxygenase inhibitor, ZM 230487, on cutaneous allergic inflammation in the guinea-pig. *Br. J. Pharmacol.,* **111,** 1205–1211.

TEIXEIRA, M.M., WELLS, T.N., LUKACS, N.W., PROUDFOOT, A.E., KUNKEL, S.L., WILLIAMS, T.J. & HELLEWELL, P.G. (1997). Chemokine-induced eosinophil recruitment. Evidence of a role for endogenous eotaxin in an *in vivo* allergy model in mouse skin. *J. Clin. Invest.,* **100,** 1657–1666.

TRAMONTANA, M., SANTICIOLI, P., GIULIANI, S., CATALIOTO, R.M., LECCI, A., CARINI, F. & MAGGI, C.A. (2002). Role of tachykinins in scphadex-induced airway hyperreactivity and inflammation in guinea pigs. *Eur. J. Pharmacol.,* **439,** 149–158.

TURNER, C.R., BRESLOW, R., CONKLYN, M.J., ANDRESEN, C.J., PATTERSON, O.K., LOPEZ-ANAYA, A., OWENS, B., LEE, P., WATSON, J.W. & SHOWELL, H.J. (1996). *In vitro* and *in vivo* effects of leukotriene B_4 antagonism in a primate model of asthma. *J. Clin. Invest.,* **97,** 381–387.

WALSH, D.T., WEG, V.B., WILLIAMS, T.J. & NOURSHARGH, S. (1995). Substance P-induced inflammatory responses in guinea-pig skin: the effect of specific NK_1 receptor antagonists and the role of endogenous mediators. *Br. J. Pharmacol.,* **114,** 1343–1350.

(Received August 12, 2003
Accepted August 26, 2003)