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Contribution of C-fibers to leucocyte recruitment in bronchoalveolar lavage fluid and pleural cavity in the rat

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Abstract

The effect of neonatal capsaicin (8 methyl-*N*-vanillyl-6-nonenamide) treatment on the leucocyte infiltration into the airways and pleural cavity was investigated in rats actively sensitized with ovalbumin. The animals were neonatally injected with either capsaicin (50 mg/kg, s.c., 2nd day of life) or vehicle (10% ethanol and 10% Tween 80). At adult ages, the animals were actively sensitized with ovalbumin (200 μg, s.c.) and 14 days later they were intratracheally (or intrapleurally) challenged with ovalbumin. The substance P level in bronchoalveolar lavage fluid of the capsaicin group was reduced by $>90\%$ compared to control group (vehicle), confirming the efficacy of capsaicin treatment. In the capsaicin group, the number of neutrophils (but not of eosinophils and mononuclear cells) in bronchoalveolar lavage fluid of sensitized animals was significantly higher than the control group. Intrapleural injection of ovalbumin in sensitized rats caused a significant neutrophil influx at 6 h that was markedly increased in the capsaicin-pretreated animals compared to control group. The counts of eosinophils and mononuclear cells in the pleural exudates did not differ significantly between capsaicin and control groups. The increased levels of immunoglobulin (Ig)E, IgG_1 and IgG_{2a} anti-ovalbumin antibodies in serum of sensitized rats did not differ between capsaicin and control groups. In conclusion, the exacerbated pulmonary neutrophil recruitment caused by the capsaicin neonatal treatment is unrelated to increase in serum immunoglobulin antibodies, and suggests a protective role for C-fibers in attenuating the allergic neutrophil infiltration.

Keywords: Non-adrenergic non-cholinergic (NANC) fiber; Lung inflammation; Neutrophil; Ovalbumin; Pleurisy; Bronchoalveolar lavage fluid

1. Introduction

Capsaicin (8 methyl-*N*-vanillyl-6-nonenamide) is a pungent ingredient contained in a wide variety of red peppers of the genus *Capsicum* and excites a subset of primary sensory neurons with somata in dorsal root ganglion or trigeminal ganglion via the so-called vanilloid receptors (Szallasi and Blumberg, 1999). Capsaicin stimulates the peripheral terminals of vanilloid-sensitive neurons causing the release of neuropeptides such as substance P, neurokinin A, calcitonin gene-related peptide (CGRP) and somatostatin, thus initiating the cascade of neurogenic inflammation. When given to newborn rats, capsaicin degenerates the neurons located in dorsal root ganglion (Jancsó et al., 1977) and therefore has largely been used to identify capsaicin-sensitive neuronal pathways and to explore their contributions to evaluate sensory neuron mechanisms (Holzer, 1991).

Asthma is a multifactorial disease characterized by reversible episodes of bronchoconstriction and increase in airway hyperresponsiveness to various bronchoconstrictor stimuli such as allergens, chemical irritants, cold air, exercise and viral or bacterial infections (Barnes, 1989). This is associated with airway inflammation caused mainly by a prominent leucocyte infiltration into the airways and release of a complex mixture of inflammatory mediators (Kroegel et al., 1994). The mechanisms underlying the pathophysiology of this disease have been under intense investigation and include abnormalities of the cholinergic and inhibitory non-adrenergic non-cholinergic innervation (NANC) as well as dysfunction of the β-adrenoceptors on airway smooth muscle (Barnes, 1989). Additionally, capsaicin-sensitive sensory C-fibers found beneath and within the epithelium, around blood vessels and submucosal

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glands, and within the bronchial smooth muscle layer contain substance P and neurokinin A, which might be released in chronic inflammation (Lundberg et al., 1984; Van der Velden and Hulsmann, 1999). These neuropeptides are suggested to modulate bronchial smooth muscle tone and leucocyte migration in airways of different animal species (Maggi et al., 1995) and humans (Ben-Jebria et al., 1993; Carolan and Casale, 1993; Crimi et al., 1994; Fayon et al., 1994; Naline et al., 1996; Joos et al., 1996). Additionally, the level of substance P is increased in bronchoalveolar lavage fluid (Nieber et al., 1992) and sputum (Tomaki et al., 1995) of asthmatic subjects. Paradoxically, in rats treated neonatally with capsaicin, the neutrophil influx to airways is increased in response to certain non-allergic stimuli such as ozone (Sterner-Kock et al., 1996) and lipopolyssacharide (Long et al., 1996). Since studies of neonatal capsaicin treatment on allergic lung inflammation are generally lacking, this study was undertaken to examine the contribution of capsaicin-sensitive sensory C-fibers to the leucocyte infiltration in the airways and pleural cavity of rats actively sensitized with ovalbumin. Therefore, a pretreatment of neonate rats with capsaicin was carried out and used at adult ages (8–10 weeks).

2. Materials and methods

2.1. Neonatal capsaicin treatment

All experiments were carried out in accordance with the guidelines of State University of Campinas (UNICAMP) for animal care. The experiments were carried out on both male and female Wistar rats bred in the animal house of Department of Pharmacology of Faculty of Medical Science, UNICAMP (Brazil). Litters of rats were injected subcutaneously (s.c.) on the second day of life with capsaicin (50 mg/kg) or the corresponding volume (100 μl) of capsaicin-vehicle (10% ethanol and 10% Tween 80, in 0.9% w/v NaCl solution), as previously described (Jancsó et al., 1977). The animals were then used at adult ages (8–10 weeks after vehicle or capsaicin pretreatment).

2.2. Sensitization procedure

At adult ages, both male and female rats (200–300 g) were actively sensitized against ovalbumin chicken egg (Grade III) according to a previous study (Vianna and Garcia-Leme, 1995), with slight modifications. Sensitization was performed by subcutaneous injection of ovalbumin (0.15 ml) solution containing 200 μg of ovalbumin and 8 mg $Al(OH)_3$ prepared in saline. Non-sensitized rats received only 8 mg $Al(OH)_3$. Fourteen days later, both sensitized and non-sensitized were employed for the different experimental protocols (see below).

2.3. Bronchoalveolar lavage fluid

Fourteen days after ovalbumin sensitization, both sensitized and non-sensitized (control and capsaicin-pretreated rats) rats were anaesthetized with chloral hydrate (300 mg/kg, i.p.). The trachea was exposed through a midline ventral incision of approximately 0.5 cm length in the neck. With the aid of a 26.5-gauge needle, 0.4 ml of 0.25% ovalbumin solution was injected into the airways.

Bronchoalveolar lavage was performed 6, 24 and 48 h after intratracheal injection of ovalbumin. Briefly, the animals were again anaesthetised with chloral hydrate (300 mg/kg, i.p.) and exsanguinated by cutting the abdominal aorta. The trachea was exposed and cannulated with a polyethylene tube (1 mm diameter) connected to a syringe. The lungs were washed by flushing with phosphate buffered saline (PBS) solution containing heparin (20 IU/ml) and 0.03% serum albumin. The PBS buffer was instilled through the tracheal cannula as one 10-ml aliquot followed by three 5-ml aliquots. The fluid recovered after each aliquot instillation was combined and centrifuged ($1000 \times g$ for 10 min at 20°C). The cell supernatant was discarded and the cell pellet was resuspended in 2 ml of PBS buffer. Total cell counts were done with an automated cell counter (CELL-DYN, 1600, USA). A cytocentrifuge (Revan, São Paulo, Brazil) was used to prepare slides that were stained with May–Grunwald–Giemsa. A minimum of 400 cells was counted and classified as neutrophils, eosinophils and mononuclear cells based on normal morphological criteria.

2.4. Pleurisy

Allergic pleurisy was induced by an intrathoracic injection of 12 μg/cavity (0.2 ml) of ovalbumin in both non-sensitized and ovalbumin-sensitized animals of the control and capsaicin groups (Lima et al., 1997). After 6 and 24 h, animals were anesthetized (300 mg/kg chloral hydrate, i.p.), and their thoracic cavity was opened and rinsed with 5 ml of saline containing heparin (20 U/ml). The pleural wash was collected and its volume measured with a plastic graduated syringe. The total and differential number of leucocytes was measured using conventional techniques.

2.5. Measurement of substance P in bronchoalveolar lavage fluid

The 3 ml C-18 reverse phase cartridge (RPC; Supelco, PA, USA) was activated by first passing 5 ml methanol, 10 ml Urea 8 M and then 10 ml UltraPure water through the cartridge. The samples of bronchoalveolar lavage fluid were diluted 1:4 with 4% acetic acid and passed slowly through the RPC after which RPC was washed with 10 ml acetic acid. Substance P was eluted by passing the methanol:water:acetic acid solution through the RPC 1 ml

at time. The samples were dried by lyophilization and reconstituted with a volume of enzyme immunoassay (EIA) buffer.

The preparation of the 96-well plates preparation as well as distribution of reagents and samples were made as indicated in reagent preparation section. Briefly, 100 μl of EIA buffer to non-specific binding (NSB) wells and 50 μl of buffer to maximum binding (Bo) wells. Aliquots (50 μl) of either substance P (7.8–1000 pg/ml) or samples were added in duplicate to wells. Then, 50 μl of substance P acetylcholinesterase tracer were added to each well except the total activity (TA) and the blank (B) wells. Substance P antiserum (50 μl) was added to each well except TA, NSB and B wells. The plate was covered with a plastic film and incubated overnight at 4°C. The plate was empty and the wells washed five times with wash buffer. Two hundred microliters of Ellman's reagents was added to each well, and incubated in the dark at room temperature using a orbital shaker for 90–120 min. The plate was read at 412 nm (SpectraMAX-340, Molecular Devices, CA, USA).

2.6. Measurement of serum immunoglobulins

Two weeks after ovalbumin sensitization, the animals were anaesthetized and blood collected. The serum samples resulting from the different experimental groups were used to measure specific immunoglobulin (Ig)E, IgG_1 and IgG_{2a} anti-ovalbumin levels. Quantification of serum specific IgG_1 and IgG_{2a} anti-ovalbumin was made using indirect enzyme immunoassay. Microplates were coated overnight at 4°C with 100 μl of ovalbumin in borate buffer at pH 9.5 (10 μg/ml). Plates were washed three times with PBS supplemented with 0.1% Tween 20 at each time of assay. Plates were blocked with proteins from skimmed milk in PBS for 1 h at 37°C. One hundred microliters of serial 1/2 dilution of serum (beginning at 1/20) were then applied for 2 h. Peroxidase labelled monoclonal antibodies against rat IgG_1 or IgG_{2a} were then added and the plates were incubated for 1 h. Finally, 0.4 mg/ml orthophenyldiamine (diluted in citrate/phosphate buffer pH 5.5 and H_2O_2) was applied. The reaction was stopped with 50 μl of 1 M H_2SO_4, and optical densities were read at 492 nm with an automatic Titertek-Multiskan reader (Flow Laboratories, Finland). Quantification of serum IgE anti-ovalbumin antibodies were performed using capture enzyme immunoassay procedure. Microplates were coated with monoclonal antibody against rat IgE. Serial 1/2 dilutions of serum (beginning at 1/5) were applied for 1 h. Finally, peroxidase labelled ovalbumin was used, and the reaction was developed as described above.

2.7. Drugs

Capsaicin and ovalbumin chicken egg (Grade III) were obtained from Sigma (St. Louis, USA).

2.8. Statistical analysis

Data are presented as the mean values ± S.E.M. for n animals, and were analysed by analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons or Student's t-test where appropriate. A P value of less than 0.05 was considered to indicate significance.

3. Results

3.1. Number of leucocytes in the bronchoalveolar lavage fluid: basal cell population

Adult rats treated neonatally with capsaicin (or vehicle) had their airways washed with phosphate-buffered saline (PBS), and the basal leucocyte content in bronchoalveolar lavage fluid was evaluated. In both control and capsaicin groups, the leucocyte population in bronchoalveolar lavage fluid was constituted by approximately 95% mononuclear cells and 4.5% neutrophils ($n = 5$ each; Table 1). Eosinophils were very few (1.0–1.5%).

3.2. Number of total and differential leucocytes in bronchoalveolar lavage fluid in control and capsaicin groups: non-sensitized and ovalbumin-sensitized rats

In control rats (vehicle), the content of total leucocytes in bronchoalveolar lavage fluid in response to intratracheal injection of ovalbumin was significantly larger ($P < 0.05$) in sensitized compared to non-sensitized animals, except at

Table 1
Basal leucocyte population in BAL fluid and pleural exudate in adult rats treated neonatally with capsaicin or vehicle (control)
The results represent the mean ± S.E.M ($n = 5$ for each experimental group).

	BAL fluid ($\times 10^6$ cells/BAL)		Pleural exudate ($\times 10^6$ cells/cavity)	
	Control	Capsaicin	Control	Capsaicin
Total	2.1 ± 0.3	3.5 ± 1.3	17.6 ± 0.5	18.2 ± 1.0
Neutrophils	0.1 ± 0.05	0.15 ± 0.06	0	0
Eosinophils	0.02 ± 0.01	0.05 ± 0.05	3.8 ± 0.7	4.1 ± 0.4
Mononuclear cells	2.0 ± 0.3	3.3 ± 1.3	13.8 ± 0.4	14.1 ± 0.9

24 h where the difference did not reach statistical significance (Table 2). Similarly, in rats treated neonatally with capsaicin, the intratracheal injection of ovalbumin caused a higher cell influx in sensitized rats compared to non-sensitized animals, except at 24 h where the difference did not reach statistical significance (Table 2). However, no statistical differences in the total cell counts between control and capsaicin groups were observed (Table 2).

In the control group, the number of neutrophils in bronchoalveolar lavage fluid did not significantly differ between non-sensitized and sensitized animals, irrespective of the time evaluated (Fig. 1A). However, in the capsaicin group, the neutrophil number in the sensitized animals was significantly higher than the non-sensitized animals, as detected at 6, 24 and 48 h post-ovalbumin injection. In addition, the number of neutrophils in sensitized animals was significantly higher ($P < 0.05$) in the capsaicin group at 6 and 24 h, as compared to control group (Fig. 1A).

In the control group, a significant ($P < 0.05$) eosinophil recruitment in bronchoalveolar lavage fluid was observed at 24 and 48 h post-ovalbumin injection in sensitized rats, as compared to the non-sensitized animals (Fig. 1B). A similar result was observed in the capsaicin group when both non-sensitized and sensitized animals were compared. However, no statistical differences in sensitized animals where observed between control and capsaicin groups (Fig. 1B).

In the control group, the number of mononuclear cells in bronchoalveolar lavage fluid from non-sensitized and sensitized animals did not significantly differ, except at 6 h where a higher ($P < 0.05$) cell influx was observed in sensitized animals (Fig. 1C). In the capsaicin group, no differences between non-sensitized and sensitized rats were observed (Fig. 1C). When the mononuclear cell counts in sensitized rats in both control and capsaicin groups were compared, a significant reduction in the capsaicin group was observed at 6 h (Fig. 1C).

3.3. Pleurisy induced by ovalbumin

Adult rats treated neonatally with capsaicin (or vehicle) had their pleural cavities washed with PBS, and the basal leucocyte content present in pleural exudates was evaluated. In both capsaicin and control groups, leucocyte population was constituted by approximately 78% mononuclear cells and 22% eosinophils ($n = 5$; Table 1). Neutrophils were virtually absent in both groups of animals.

The pleural leucocyte number in both control and capsaicin groups was also examined in animals previously sensitized with ovalbumin in comparison with non-sensitized rats. In these animals, ovalbumin was injected into the pleural cavities and pleural exudates evaluated at 6 and 24 h post-ovalbumin injection. At 6 h, pleural exudates in

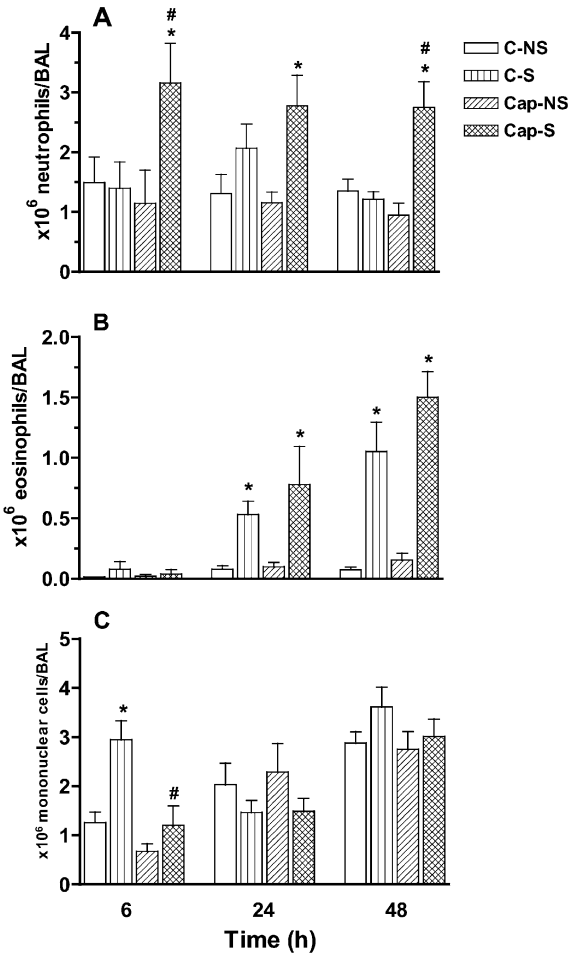

Fig. 1. Number of neutrophils (Panel A), eosinophils (Panel B) and mononuclear cells (Panel C) in bronchoalveolar lavage (BAL) fluid from rats neonatally treated with capsaicin or vehicle (control). At adult ages, ovalbumin-sensitized rats were intratracheally injected with ovalbumin (0.4 ml of 0.25% ovalbumin) and BAL fluid was evaluated at 6, 24 and 48 h post-ovalbumin injection. Open and vertical striped columns represent control animals (non-sensitized and sensitized, respectively). Hatched and cross-hatched columns represent capsaicin-pretreated rats (non-sensitized and sensitized, respectively). The number of animals used was: at 6 h, 9–10 rats in control and 5 in capsaicin-treated group; at 24 h, 12–18 rats in control and 12–14 in capsaicin-treated group; at 48 h, 20–23 rats in control and 16–18 in capsaicin-treated group. $^{*}P < 0.05$ compared to respective non-sensitized rats. $^{\#}P < 0.05$ compared to control, sensitized rats.

Table 2
Total leucocyte counts in BAL fluid from rats treated neonatally with capsaicin or vehicle
At adult ages, both non-sensitized and ovalbumin-sensitized rats were intratracheally injected with ovalbumin (0.4 ml of 0.25% ovalbumin solution). Bronchoalveolar lavage fluid was evaluated at 6, 24 and 48 h post-ovalbumin injection.

Time (h)	Control		Capsaicin	
	Non-sensitized	OVA-sensitized	Non-sensitized	OVA-sensitized
6	2.7 ± 0.6 (9)	4.4 ± 0.7[a] (10)	1.8 ± 0.8 (5)	4.4 ± 1.6[a] (5)
24	3.4 ± 0.6 (18)	4.1 ± 0.6 (12)	3.5 ± 0.6 (12)	5.1 ± 0.8 (14)
48	4.3 ± 0.3 (23)	5.9 ± 0.5[a] (20)	3.9 ± 0.5 (16)	7.3 ± 0.6[a] (18)

OVA, ovalbumin.
[a] $P < 0.05$ compared to respective non-sensitized groups. Results are mean values ± S.E.M. of data from (n) animals indicated in each group.

both control and capsaicin groups showed a marked ($P < 0.05$) increase in total leucocyte counts in sensitized animals in comparison with non-sensitized animals (not shown; $n = 5$ each). However, the number of total leucocytes in capsaicin group ($22.4 \pm 1.7 \times 10^6$ cells/cavity) was significantly higher than those of control group ($12.5 \pm 2.5 \times 10^6$ cells/cavity; $P < 0.05$). This increased leucocyte infiltration was mainly due to a large influx of neutrophil in capsaicin group as compared to vehicle group (Fig. 2). Regarding the counts of eosinophils and mononuclear cell, no statistical differences were observed between both groups of animals at 6 h (Fig. 2).

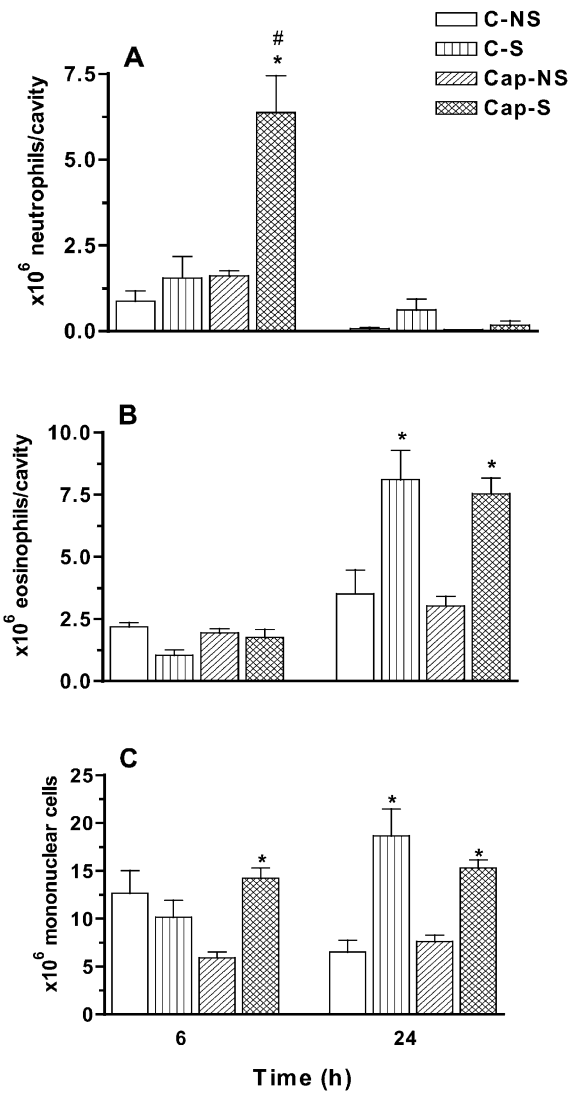

Fig. 2. Number of neutrophils (Panel A), eosinophils (Panel B) and mononuclear cells (Panel C) in pleural exudates of rats neonatally treated with capsaicin or vehicle (control). At adult ages, sensitized rats were intrapleurally injected with ovalbumin (12 μg/cavity; $n = 5–7$) and pleural exudates was evaluated at 6 and 24 h post-ovalbumin injection. Open and vertical striped columns represent control animals (non-sensitized and sensitized, respectively). Hatched and cross-hatched columns represent capsaicin-pretreated rats (non-sensitized and sensitized, respectively). $^{*}P < 0.05$ compared to respective non-sensitized rats. $^{\#}P < 0.05$ compared to control, sensitized rats.

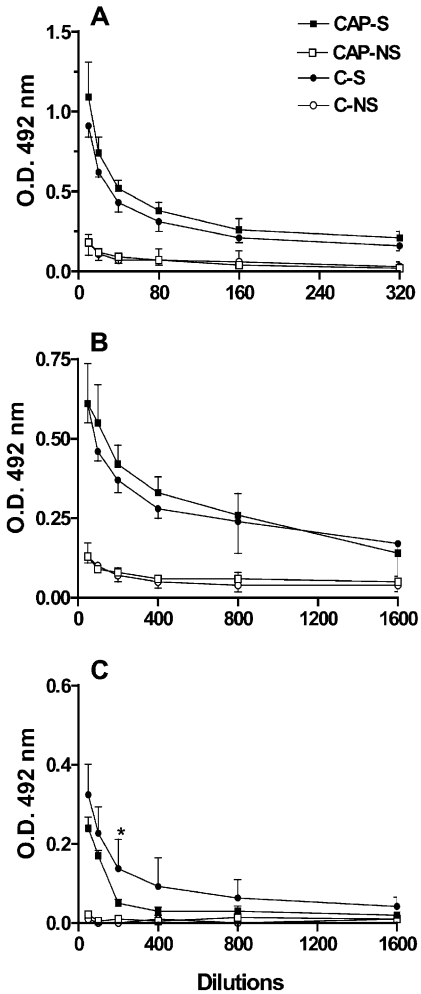

Fig. 3. Levels of immunoglobulin (Ig)E (dilution 1:10 to 1:320; Panel A), IgG_1 (dilution 1:50 to 1:1600; Panel B) and IgG_{2a} (dilution 1:25 to 1:1600; Panel C) anti-ovalbumin antibodies in serum of both capsaicin and control rats ($n = 5$ each). $^{*}P < 0.05$ compared to Cap-S animals. C-NS, control and non-sensitized rats; C-S, control and ovalbumin-sensitized rats; Cap-NS, capsaicin-pretreated rats and non-sensitized; Cap-S, capsaicin-pretreated rats and ovalbumin-sensitized.

At 24 h post-ovalbumin injection, pleural exudates in both vehicle and capsaicin groups showed a marked ($P < 0.05$) increase in total leucocyte counts in sensitized animals in comparison with non-sensitized animals (not shown). In contrast to 6 h, no statistical differences on the total leucocyte number was observed between capsaicin and control groups (24.7 ± 1.4 and $28.0 \pm 3.5 \times 10^6$ cells/cavity, respectively; $n = 5–7$). At this time, the cell infiltration in both groups of animals was mainly due to eosinophils and mononuclear cells, but no statistical differences were observed (Fig. 2).

3.4. Substance P levels in bronchoalveolar lavage fluid

The levels of substance P in bronchoalveolar lavage fluid in control and capsaicin groups were evaluated at 24 h after intratracheal injection of ovalbumin. Our results showed a marked ($P < 0.01$) reduction of substance P

levels in the capsaicin group (0.042 ± 0.013 and 0.033 ± 0.015 ng/ml in non-sensitized and sensitized rats, respectively) compared to control group (7.3 ± 0.1 and 7.7 ± 0.2 ng/ml in non-sensitized and sensitized rats, respectively; $n = 5$).

3.5. Quantification of IgE, IgG_1 and IgG_{2a} anti-ovalbumin specific antibodies

Fig. 3 shows the levels of IgE (dilutions 1:10 to 1:320), IgG_1 (dilutions 1:50 to 1:1600) and IgG_{2a} (dilutions 1:25 to 1:1600) anti-ovalbumin antibodies in serum of both capsaicin- and vehicle-pretreated rats. Significant concentrations of IgE, IgG_1 and IgG_{2a} anti-ovalbumin were observed in serum from sensitized animals (vehicle and capsaicin groups) whereas serum non-sensitized animals did not show significant amount of these antibodies, as expected. In addition, no statistical differences were observed between the vehicle and capsaicin groups regarding the IgE (Fig. 3A) and IgG_1 levels (Fig. 3B). The IgG_{2a} serum levels in the capsaicin group were slightly (but significantly at dilution 1:200) smaller as compared to the vehicle group (Fig. 3C).

4. Discussion

This study shows that degeneration of capsaicin-sensitive primary afferent C-fibers by neonatal treatment of rats with capsaicin lead to an exacerbated neutrophil recruitment into the bronchoalveolar lavage fluid and pleural exudates in animals actively sensitized and challenged with ovalbumin, suggesting a protective role for C-fibers in attenuating allergic neutrophil infiltration into airways and pleural cavity of the rat. The levels of substance P in bronchoalveolar lavage fluid of the capsaicin group were reduced by $> 90\%$, indicating that neonatal treatment of rats with capsaicin was efficient to promote the neuropeptide depletion.

The mechanisms that influence neutrophil migration across endothelium towards sites of inflammation in tissues are complexes and involve the release of multiple mediators depending on the inflammatory state (Rossi and Hellewell, 1994). These mediators include those derived from microbe (*N*-formyl-methionyl-leucyl-phenilalanine and endotoxin), tissue fluid (complement fragment 5a) or cells (leukotriene B_4, platelet-activating factor, interleukin-8, interleukin-1 and tumor necrosis factor-α). Moreover, the pulmonary inflammation and the neutrophil functions seem to be influenced by the NANC innervation via different mechanisms such as cytokine release, T-cell activity and expression of certain adhesion molecules (Nakagawa et al., 1995). In our study, the intratracheal (or intrapleural) injection of ovalbumin into the non-sensitized rats evoked a higher neutrophil infiltration as compared to the basal neutrophil population, an effect observed in both control and capsaicin groups. This has generally been observed in studies with similar experimental protocols using ovalbumin-sensitized animals (Ferreira et al., 1998), and seems to be due to ovalbumin, a large protein that is able to evoke per se increases in vascular permeability and neutrophil migration. However, the intratracheal (or intrapleural) injection of ovalbumin in previously sensitized rats (control or capsaicin groups) caused a significant leucocyte infiltration compared to non-sensitized rats. Although the pattern of migration for neutrophils, eosinophils and mononuclear cells presented some differences regarding the model used (bronchoalveolar lavage or pleurisy), the initial neutrophil recruitment (at 6 and/or 24 h) was generally followed by a late eosinophil influx (at 24 and/or 48 h) whereas the mononuclear cell influx was not greatly affected by the ovalbumin challenge. This pattern of leucocyte migration corroborates previous studies carried out in rats exposed to ovalbumin (Vianna and Garcia-Leme, 1995; Lima et al., 1997; Ferreira et al., 1998). However, our results revealed that neutrophil counts in bronchoalveolar lavage fluid (6 and 48 h) and pleural exudates (6 h) of ovalbumin-sensitized animals were significantly larger in the capsaicin group compared to control group. The number of mononuclear cells was not enhanced by the capsaicin pretreatment in any time evaluated whereas a slight (but not significant) increase in the eosinophil number was observed at 24 and 48 h. A previous study showed that neonatal capsaicin treatment increases the lung neutrophil influx in rats submitted to ozone inhalation, suggesting that lung C-fibers protect lungs from the damaging effects of inhaled ozone (Sterner-Kock et al., 1996). The intratracheal injection of lipopolysaccharide in capsaicin-pretreated rats results in higher levels of tumor necrosis factor (TNF) in both bronchoalveolar lavage fluid and alveolar macrophages collected from naive capsaicin-treated rats, suggesting that macrophage-derived TNF contributes to the greater inflammatory responses of capsaicin-treated rats (Long et al., 1996). In fact, TNF induces neutrophil transendothelial and transepithelial migration, and has been shown to increase the expression of intercellular adhesion molecule-1 (ICAM-1) and endothelial leucocyte adhesion molecule (ELAM-1) on endothelial cells as well as the expression of the CD11/CD18 on the surface of neutrophils (Strieter and Kunkel, 1994). Thus, our findings that capsaicin pretreatment promoted an enhancement of neutrophils suggest that TNF may contributes to the increased neutrophil accumulation.

It is well known that the antigen-specific immunoglobulin of IgE isotypes play a key role in allergic diseases in man and in certain allergy models in experimental animals. Thus, sensitization of experimental animals with antigens such as ovalbumin (Vianna and Garcia-Leme, 1995; Feder et al., 1997; Barton et al., 1991; Kung et al., 1994) stimulates an allergic inflammation mediated mainly by IgE antibodies, a phenomenon suggested to be also

influenced by sensory nerves (Nilsson et al., 1991). Our results showed a marked increase in IgE serum levels in ovalbumin-sensitized rats, indicating that sensitization with ovalbumin was efficient. However, no differences in the ovalbumin-specific serum IgE profile were observed between control and capsaicin groups, excluding the possibility that increased neutrophil counts in the capsaicin group reflect mechanisms involving cell accumulation via anaphylactic IgE-mediated responses. Interestingly, the neonatal treatment of rats with capsaicin slightly increases the serum IgE levels when animals are sensitized by daily subcutaneous injections of ovalbumin for 2 consecutive weeks but, on the other hand, this same treatment decreases the serum IgE levels when animals are sensitized by aerosol (Nilsson et al., 1991). The reasons for these discrepancies depending on the immunization model used are unclear; nevertheless, our results strongly suggest that IgE levels do not play a major role on neutrophil accumulation observed in rats with capsaicin-sensitive primary afferent fiber degeneration. The immunoglobulins of IgG isotypes also participate of the host defense system and, in fact, high amounts of this immunoglobulin class are detected in allergic states (Reynolds, 1988). In our study, marked levels of IgG_1 and IgG_{2a} were found in the serum from sensitized rats compared to non-sensitized animals. In agreement with Nilsson et al. (1991), no significant differences between control and capsaicin groups were observed (except for IgG_{2a} at dilution 1:200), suggesting that enhanced neutrophil infiltration in the latter group is also unrelated to high serum levels of this class of immunoglobulins. Regarding the decrease of IgG_{2a} levels in the capsacin group at dilution 1:200, we have no satisfactory explanation, but it seems to be an irrelevant aspect considering that such reduction was slight and appeared at just one dilution.

In summary, the findings that neonatal capsaicin treatment causes an exacerbated neutrophil recruitment into the bronchoalveolar lavage fluid and pleural exudates in response to ovalbumin in rats suggest that capsaicin-sensitive primary afferent C-fibers play a role in attenuating allergic neutrophil infiltration into lungs and pleural cavity. Future studies addressed to investigate certain neutrophil functions such as locomotion, adherence and adhesion molecule expression as well as production of selective neutrophil chemoattractant mediators (interleukin-8) in capsaicin-pretreated rats are necessary to elucidate the exact role of these neurons on the modulation of neutrophil infiltration into inflamed tissues.

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