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Inactivation of capsaicin-sensitive nerves reduces pulmonary remodeling in guinea pigs with chronic allergic pulmonary inflammation

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Abstract

Pulmonary remodeling is an important feature of asthma physiopathology that can contribute to irreversible changes in lung function. Although neurokinins influence lung inflammation, their exact role in the extracellular matrix (ECM) remodeling remains to be determined. Our objective was to investigate whether inactivation of capsaicin-sensitive nerves modulates pulmonary ECM remodeling in animals with chronic lung inflammation. After 14 days of capsaicin (50 mg/kg, *sc*) or vehicle administration, male Hartley guinea pigs weighing 250-300 g were submitted to seven inhalations of increasing doses of ovalbumin (1, 2.5, and 5 mg/mL) or saline for 4 weeks. Seventy-two hours after the seventh inhalation, animals were anesthetized and mechanically ventilated and the lung mechanics and collagen and elastic fiber content in the airways, vessels and lung parenchyma were evaluated. Ovalbumin-exposed animals presented increasing collagen and elastic fiber content, respectively, in the airways (9.2 ± 0.9; 13.8 ± 1.2), vessels (19.8 ± 0.8; 13.4 ± 0.5) and lung parenchyma (18.8 ± 1.1; 25.31 ± 1.1) compared to control ($P < 0.05$). Capsaicin treatment reduced collagen and elastic fibers, respectively, in airways (1.7 ± 1.1; 7.9 ± 1.5), vessels (2.8 ± 1.1; 4.4 ± 1.1) and lung tissue (12.46 ± 1.0; 15.05 ± 1.5) of ovalbumin-exposed animals ($P < 0.05$). These findings were positively correlated with lung mechanical responses to antigenic challenge ($P < 0.05$). In conclusion, inactivation of capsaicin-sensitive nerve fibers reduces pulmonary remodeling, particularly collagen and elastic fibers, which contributes to the attenuation of pulmonary functional parameters.

Key words: Collagen; Elastic fibers; Capsaicin; Chronic lung inflammation

Introduction

Chronic lung inflammation is a fundamental feature of several respiratory diseases such as asthma. Several mediators modulate this chronic inflammatory process, including the release of neurokinins stored in sensitive afferent nerve terminals (1) and in inflammatory cells present in airways and lung tissue (2,3). It has been established that neurokinins, such as substance P and neurokinin A, can induce smooth muscle contraction (3), facilitation of cholinergic neurotransmission, submucosal gland secretion (4), vasodilation (4), increases in vascular permeability (5), chemoattraction of eosinophils (5) and neutrophils (4), vascular adhesion of neutrophils, and stimulation of mast cells, B and T lymphocytes and macrophages (4). Approximately 20 to 35% of asthmatics were shown to have their exacerbations modulated by stress responses, and primary afferent sensitive C fiber stimulation and non-adrenergic, non-cholinergic responses were found to be the main pathways involved (6).

Capsaicin, the main pungent ingredient of red chili pepper, binds to specific vanilloid (capsaicin) receptors on non-myelinated C fiber primary sensitive sensory nerve terminals. High doses of capsaicin treatment were initially

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associated with acute release of neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide. However, after 10 days of this treatment there was an inactivation of capsaicin-sensitive nerves associated with an irreversible degeneration of these nerve terminals, which led to a greater than 90% reduction in substance P and neurokinin A in the lungs. These are the main neuropeptides involved in pulmonary responses (2,5,7-9). For these reasons, capsaicin has been used as a research tool to evaluate the importance of neurogenic inflammation in pulmonary diseases.

The persistence of chronic inflammatory processes contributes to pulmonary remodeling that is related to the progressive nature of airflow limitation, vascular pulmonary hypertension and reduction of lung tissue compliance (10,11). A myriad of histological changes, such as epithelial damage, hypertrophy and hyperplasia of goblet cells, airway smooth muscle, submucosal glands, and fibroblasts/myofibroblasts are typical characteristics of remodeling (10). Several vascular alterations can also be observed in patients with fatal asthma, such as blood vessel dilation and congestion, an increase in the total number of vessels, and an increase in the vascular area of the airways (12-14). All of these qualitative and quantitative changes that occur in lung blood vessels can contribute to the thickening of the airway wall, which in turn may lead to critical narrowing of the bronchial lumen when bronchial smooth muscle contraction occurs. This amplifies the airway inflammation and remodeling observed in asthma (10,11). In addition, it has been shown that inflammation and remodeling occur both in distal airways and in lung parenchyma tissue in animal models of chronic pulmonary inflammation and in patients who die from fatal asthma (15-17).

Another important aspect of pulmonary remodeling is related to an intense deposition of extracellular matrix (ECM) molecules, including collagen types I, II, and V, fibronectin, tenascin and proteoglycans, lumican, biglycan, versican, and decorin in the different lung compartments (10,11,18). The irreversibility of these remodeling alterations may contribute negatively to the severity of clinical symptoms of asthmatic patients. Until now, there was no specific therapeutic intervention that might target this process.

Although the importance of neurogenic inflammation in chronic pulmonary inflammation and airway responsiveness has been suggested by previous data (19,20), few studies have focused on pulmonary remodeling. De Swert et al. (21) demonstrated that tachykinin NK1 receptors are involved in goblet cells hyperplasia but not in allergic airway inflammation in a mouse model of allergic asthma. In addition, other studies have observed that neuropeptides are involved in fibroblast proliferation (22) and chemotaxis (23,24). To the best of our knowledge, no previous reports have examined the role of inactivating capsaicin-sensitive nerve fibers in pulmonary extracellular remodeling in a model of chronic allergic pulmonary inflammation.

We hypothesized that inactivating capsaicin-sensitive nerve fibers would modulate collagen and elastic fiber content in airways, vessels and lung tissues of guinea pigs with chronic pulmonary inflammation. To determine the functional importance of our findings, we also calculated the correlation between mechanical and histopathological parameters.

Material and Methods

All guinea pigs received humane care in compliance with the Guide for Care and Use of Laboratory Animals (NIH publication 85-23, revised 1985), and all experiments described in this study were approved by the Institutional Review Board of the Universidade de São Paulo (São Paulo, SP, Brazil).

Experimental groups

Animals received one of four treatments: a) capsaicin pretreatment and inhalations with normal saline (CAP-NS group, N = 6); b) capsaicin pretreatment and inhalations with ovalbumin solution (CAP-OVA group, N = 6); c) vehicle pretreatment and inhalations with normal saline (NS group, N = 6), or d) vehicle pretreatment and inhalations with ovalbumin solution (OVA group, N = 6).

Capsaicin-sensitive nerve inactivation

Capsaicin treatment consisted of a single dose of capsaicin (50 mg/kg, *sc*; Spectrum Chemical Corporation, USA), as previously described (2,3) (Figure 1). Each male Hartley guinea pig weighing 250-300 g received aminophylline (10 mg/kg, *ip*) and terbutaline (0.1 mg/kg, *sc*) and was anesthetized with ketamine (50 mg/kg, *im*) and xylazine (0.1 mg/kg, *im*). Capsaicin was suspended in a 50-mg/mL solution consisting of 80% normal saline, 10% ethanol and 10% Tween 80 (Sigma Chemical Co., USA). Guinea pigs received supplemental oxygen during anesthesia and recovery. In order to evaluate if capsaicin pretreatment reduced the lung content of substance P, we measured the lung content of substance P in lung homogenate by ELISA 72 h after the last inhalation. The lung homogenate was prepared as previously described by Martins et al. (7). We used the Substance P ELISA Kit (Abnova, USA) and ELISA was performed according to manufacturer instructions.

Induction of chronic allergic pulmonary inflammation

Fourteen days after capsaicin pretreatment, the guinea pigs were placed in a Plexiglas box (30 x 15 x 20 cm) coupled to an ultrasonic nebulizer (Soniclear, Brazil). A solution of ovalbumin (Grade V, Sigma Chemical Co.) diluted in 0.9% saline was prepared. The animals received seven inhalations over a period of 4 weeks with increasing concentrations of ovalbumin (1-5 mg/mL) to counteract tolerance (Figure 1). Control animals received aerosolized normal saline. The solution was continuously aerosolized into the

environment until respiratory distress (sneezing, coryza, cough, or retraction of the thoracic wall) occurred, or until 15 min had elapsed, as previously described (2,3).

Pulmonary mechanics evaluation

Seventy-two hours after the 7th inhalation, the animals were anesthetized with pentobarbital sodium (50 mg/kg, *ip*), tracheostomized and mechanically ventilated at 60 breaths/min with a tidal volume of 8 mL/kg using a Harvard 683 ventilator (Harvard Apparatus, USA). We performed 2-min challenges with either aerosolized ovalbumin (30 mg/mL, OVA and CAP-OVA groups) or normal saline (NS and CAP-NS groups) delivered into the breathing circuit through the air inlet of the ventilator. The tracheal pressure (Ptr) was measured with a Honeywell 142PC05D differential pressure transducer (Freeport, USA) connected to a side tap in the tracheal cannula. Airflow (V') was obtained by a pneumotachograph Fleish 4-0 (OEM Medical Inc., USA) connected to the tracheal cannula and to a Honeywell 163PC01D36 differential pressure transducer. Lung volume changes (V) were obtained by electronic integration of the airflow signal. Ptr, V' and V signals were collected before and after the OVA or NS challenge and were stored in a microcomputer. Nine to ten respiratory cycles were averaged to provide one data point (2,3). Respiratory system elastance (Ers) and resistance (Rrs) were obtained using the equation of motion of the respiratory system: $Ptr(t) = Ers \cdot V(t) + Rrs \cdot V'(t)$, where *t* is time.

Morphometric studies

At the end of the pulmonary mechanics evaluation, the anterior chest wall was opened and the lungs were washed with heparinized saline (1:40). A positive end-expiratory pressure of 5 cmH_2O was then applied to the respiratory system, and the airways were occluded at the end of expiration. Animals were exsanguinated via the abdominal aorta and lungs were removed *en bloc*, fixed with 4% buffered paraformaldehyde for 24 h and then transferred to 70% ethanol. Sections representing peripheral areas of the lungs were cut and processed for paraffin embedding. Histological sections (5 µm in thickness) were cut and submitted to morphometric analysis.

Airway inflammation

We examined H&E-stained slices to evaluate lung inflammation. We evaluated polymorphonuclear (PMN) cells around the airway (between the bronchial epithelium and the adventitia) using an integrating eyepiece (10^4 μm^2 of total area). We analyzed 10-20 fields per lung at 1000X magnification and reported the results as cells/unit area.

Pulmonary remodeling

Histological sections were stained with Sirius-Red (Direct Red 80, C.I. 35780, Aldrich, USA) for collagen fibers and with Weigert's Resorcin-Fuchsin for elastic fibers.

To analyze the collagen and elastic fiber content in the airways and lung vessels, we measured the total area of vascular or airway wall (from internal smooth muscle wall to external adventitia wall) and the area of collagen or elastic fibers (μm^2) in nine to ten vessels or airways/lung. We used polarized light to evaluate collagen at a magnification of 200X, using an image analysis system (Image J v.1.30). The collagen or elastic content in airways or vessels is reported as the quantity of collagen or elastic fibers in a specific frame divided by the total area of the frame (25,26).

To evaluate the collagen and elastic content in lung parenchyma, we employed conventional morphometry using a 100-point grid with a known area (62,500 μm^2 at a 400X magnification) attached to the eyepiece of the microscope. The volume proportion of collagen or elastic fibers in the lung tissue was determined by dividing the number of points hitting collagen or elastic fibers by the total number of points hitting alveolar septa. Ten fields of lung parenchyma per animal were analyzed randomly at 400X magnification, and results are reported as percentage (16). Both techniques were performed by two different researchers blind to the protocol design.

Statistical analysis

Data are reported as means ± SEM and were analyzed statistically by two-way analysis of variance, and multiple comparisons were made by the Holm-Sidak method. Spearman correlation was used to determine correlations between morphometric and functional parameters. P <

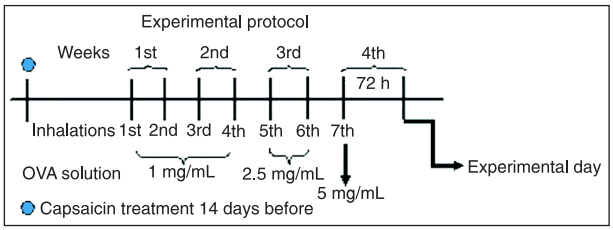

Figure 1. Timeline of the experimental protocol. Fourteen days before the beginning of the sensitization protocol, guinea pigs received a single dose of capsaicin (CAP, 50 mg/kg, *sc*) and were then submitted to 7 inhalations (2 per week for 4 weeks) with aerosols of normal saline (NS) or ovalbumin (OVA) solution with increasing doses of antigen. From the first to the 4th inhalation, the dose used was 1 mg/mL ovalbumin (2 weeks). For the 5th and 6th inhalations (in the 3rd week), animals inhaled 2.5 mg/mL ovalbumin. In the 7th inhalation (beginning of the 4th week), the dose of antigen used was 5 mg/mL. The solution of ovalbumin or saline was continuously aerosolized for 15 min or until respiratory distress occurred (sneezing, coryza, cough, or retraction of the thoracic wall). Seventy-two hours after the 7th inhalation, all guinea pigs were anesthetized, tracheostomized and mechanically ventilated. Then, animals received either the ovalbumin challenge (30 mg/mL; OVA and CAP-OVA groups) or saline inhalation (NS and CAP-NS groups) for 2 min. Finally, the animals were exsanguinated and the lungs removed.

0.05 values were considered to be significant. Statistical analysis was performed using the SigmaStat software (SPSS Inc., USA).

Results

Lung content of substance P

The present study confirmed our group's previous results (7), showing that pretreatment with capsaicin significantly reduced the content of substance P in lung homogenates (988.09 pg/g) when evaluated by ELISA, compared to untreated animals (23,670.78 pg/g; there was an approximate 88.5% reduction).

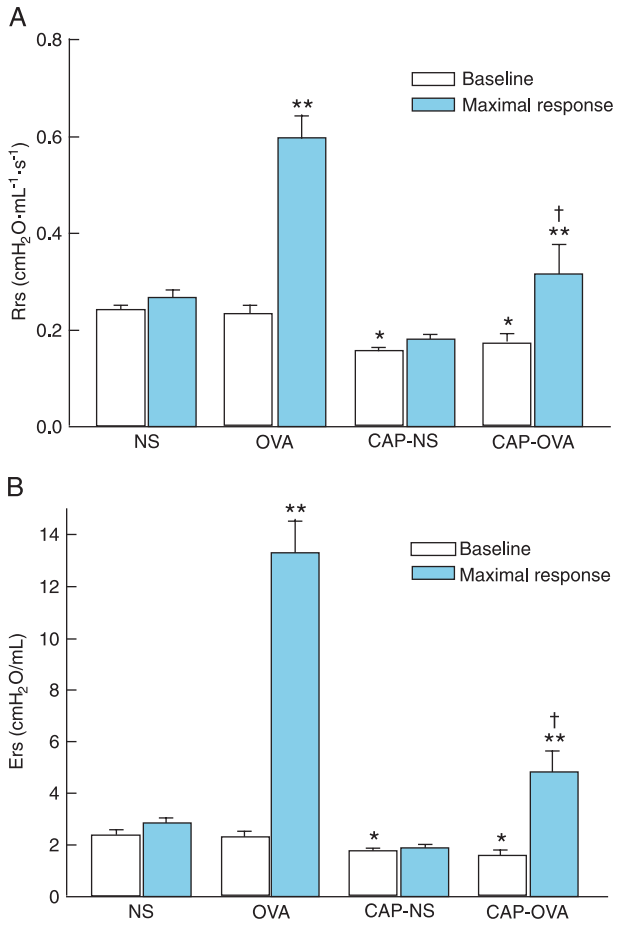

Figure 2. Lung mechanics. Data are reported as means and SEM of the baseline and maximal responses of respiratory system resistance (Rrs) and elastance (Ers) after antigen challenge. In the OVA group, guinea pigs were exposed to seven inhalations with ovalbumin. In the CAP-OVA group, animals received a high dose of capsaicin 14 days before the beginning of the ovalbumin inhalations. The control groups received inhalation with normal saline and pretreatment with vehicle (NS group) or capsaicin (CAP-NS group). *P < 0.001 compared to the baseline data of the NS and OVA groups, respectively; **P < 0.05 compared to maximal response of NS group; †P < 0.05 compared to maximal response of OVA group. Statistical analysis was done by two-way analysis of variance, and multiple comparisons were made using the Holm-Sidak method.

Evaluation of respiratory system mechanics

Evaluation of baseline lung mechanics revealed a reduction in both respiratory system resistance and elastance in animals pretreated with capsaicin compared to control (P < 0.001; Figure 2). Following an antigen challenge, ovalbumin-exposed animals presented significant increases in both respiratory system resistance (Figure 2A) and elastance (Figure 2B) compared to saline-exposed animals (P < 0.05). Capsaicin pretreatment also reduced the mechanical responses (P < 0.05 *vs* OVA; Figure 2). No difference was observed between the NS and CAP-NS groups.

Airway inflammation

To evaluate inflammation in the lungs, we quantified the number of PMN cells found around the airways. The ovalbumin-exposed animals had high PMN cell values (OVA = 20.23 ± 1.90; CAP-OVA = 12.62 ± 2.16) compared to saline-exposed animals (NS = 5.06 ± 1.91; CAP-NS = 3.55 ± 2.02; P < 0.001). In fact, capsaicin pretreatment reduced the quantity of PMN around the airways in ovalbumin-exposed animals (OVA x CAP-OVA, P < 0.05). These results confirmed previous studies from our group (2,3).

Qualitative evaluation of pulmonary remodeling

Figures 3A-F illustrates Picrosirius staining of collagen fibers. In airways and vessels (Figures 3A-C), the sections were observed using polarized light. In saline-exposed guinea pigs (Figure 3A) there was a weak red-orange birefringence in tissue sections coincident with the maintenance of the perivascular and peribronchial ECM architecture. In contrast, in ovalbumin-exposed animals, there was a diffuse increase of birefringence (Figure 3B) in both peribronchial and perivascular ECM. Similar results were observed in lung tissue (Figure 3D-F), with saline-exposed animals (Figure 3D) having fewer collagen fibers in the alveolar septa compared to ovalbumin-exposed animals (Figure 3E). In Figure 3C and F, capsaicin pretreatment of ovalbumin-exposed animals attenuated collagen deposition around the airways, vessel walls (Figure 3C) and lung tissue (Figure 3F). In Figure 3G-M, elastic fibers stained with Resorcin-Fuchsin around the airways and vessels (Figure 3G-I) and in lung tissue (Figure 3J-M). Qualitative analysis showed that ovalbumin-exposed animals (Figure 3H and L) had a greater elastic fiber content compared to saline-exposed animals (Figure 3G and J) in airways, vessels and lung tissue. Lung sections from animals exposed to ovalbumin and pretreated with capsaicin (Figure 3I and M) were similar to sections from saline-exposed animals. Airway and vessel photomicrographs were observed at 200X and lung tissue sections were observed at 400X.

Quantitation of pulmonary remodeling

Measurements of pulmonary remodeling are shown in Figures 4, 5, and 6. There was an increase in collagen and elastic fiber content in airways (Figure 4), vessels (Figure 5) and lung tissue (Figure 6) of ovalbumin-exposed animals compared to saline-exposed animals ($P < 0.05$). Capsaicin

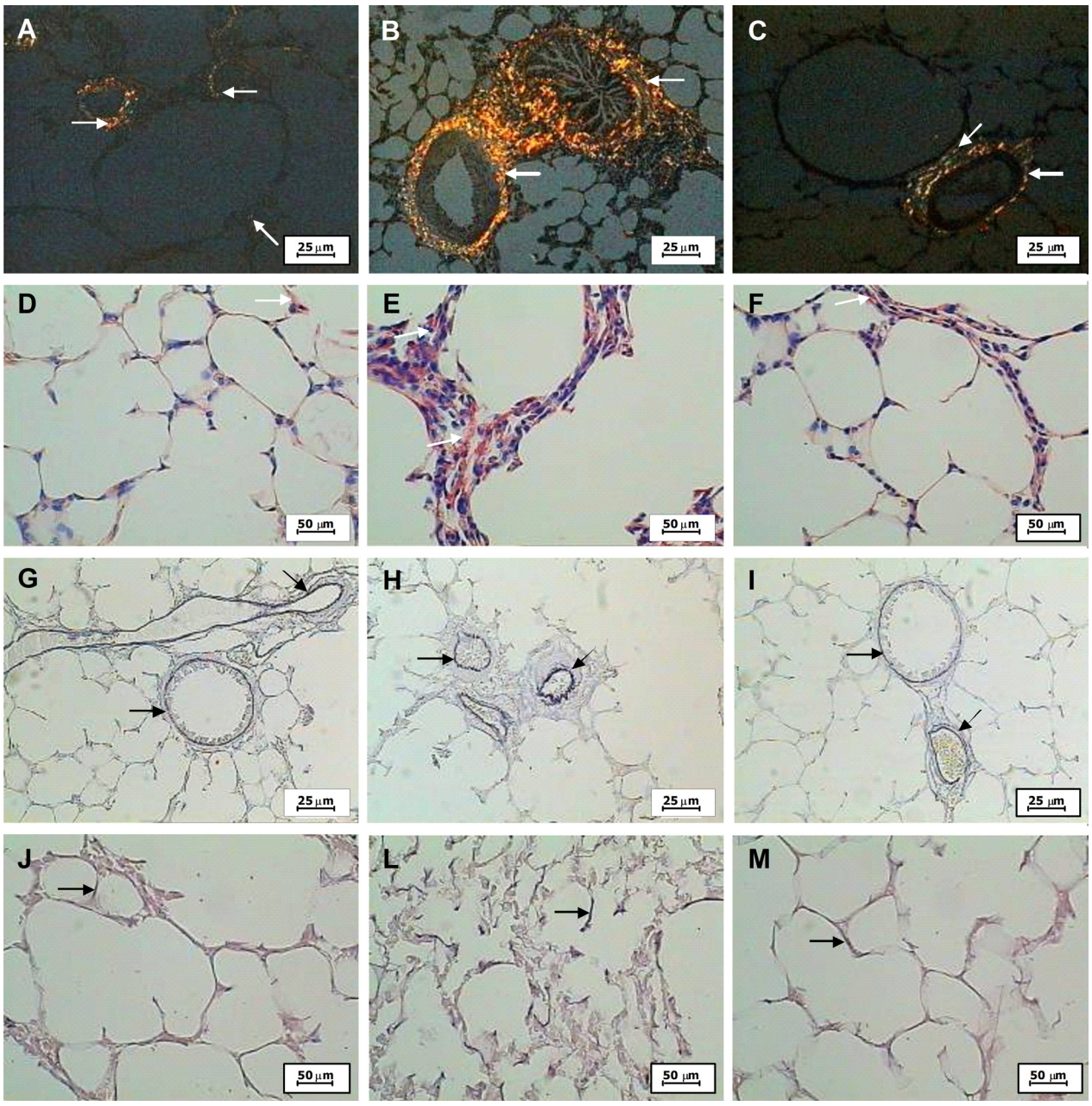

Figure 3. Lung remodeling. Non-cartilaginous airways, vessels and lung tissue obtained from saline-exposed (NS group = panels A, D, G, and J), ovalbumin-exposed (OVA group = panels B, E, H, and L), and ovalbumin-exposed and capsaicin-pretreated guinea pigs (CAP-OVA group = panels C, F, I, and M) were stained with Picrosirius and observed under polarized light (A-C) or not (D-F) or stained with Resorcin-Fuchsin (G-M). Saline-exposed animals showed a weak yellow-greenish birefringence along the walls in the tissue section (A and D), coinciding with the maintenance of the histoarchitecture of the extracellular matrix and scant elastic fibers (G and J). In contrast, tissue sections from the OVA group show an intense constriction and an increase in collagen and elastic fiber content in airways and vessel walls (B and H) as well as in lung tissue (E and L). Capsaicin pretreatment attenuated collagen (C and F) and elastic fiber deposition (I and M) in the airways, vessels (C and I) and lung tissue (F and M).

pretreatment reduced both collagen and elastic fiber content in the airways, vessels and lung tissue of ovalbumin-exposed animals (CAP-OVA group) compared to animals that received vehicle (OVA group, $P < 0.01$).

Table 1 shows the correlation coefficients between the morphometric and functional parameters in all experimental groups. We observed a correlation between the collagen fiber content (Table 1) of airways and baseline and maximal values of Ers ($R = 0.65$, $P < 0.02$; $R = 0.64$, $P < 0.02$). There was a positive and significant correlation between the collagen content of peribronchial vessels and the baseline and maximal responses of Rrs ($R = 0.48$, $P < 0.03$; $R = 0.52$, $P < 0.02$).

The elastic fiber content (Table 1) of the airways showed a positive correlation between the baseline and the maximal responses of both elastance ($R = 0.70$, $P < 0.001$; $R = 0.68$, $P = 0.001$, respectively) and resistance ($R = 0.54$, $P < 0.02$; $R = 0.81$, $P < 0.001$, respectively) of the respiratory system. The elastic fiber content of peribronchial vessels was also correlated with the baseline and maximal response of both elastance ($R = 0.63$, $P < 0.01$; $R = 0.64$, $P < 0.01$, respectively) and resistance ($R = 0.48$, $P < 0.04$; $R = 0.70$, $P < 0.001$, respectively) of the respiratory system.

We did not detect any correlations between the collagen content of lung tissue and the functional parameters. We detected a positive correlation between the elastic content of lung tissue and the baseline and maximal values of the resistance of the respiratory system ($R = 0.52$, $P < 0.03$; $R = 0.66$, $P < 0.003$).

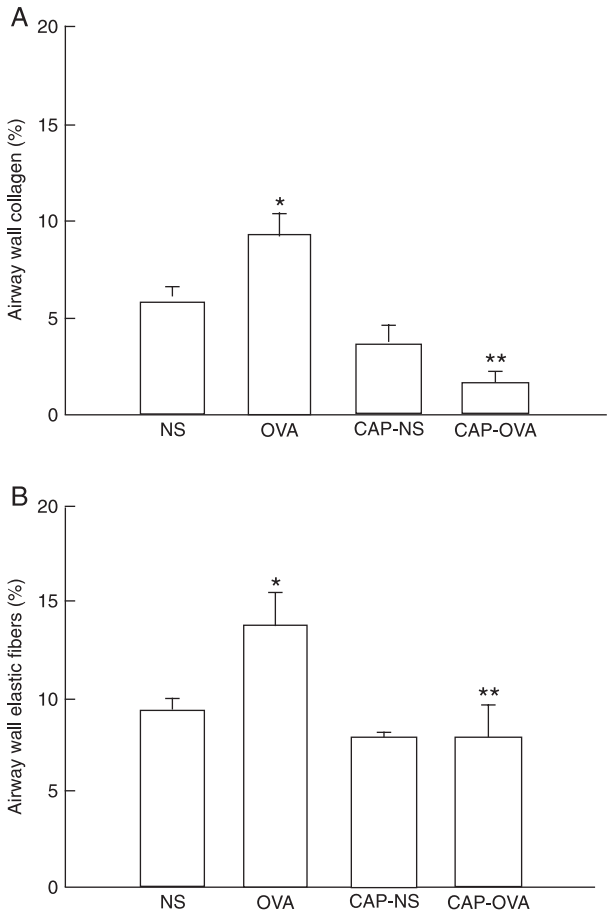

Figure 4. Airway remodeling. Data are reported as means and SEM of collagen (A) and elastic (B) content in airways reported as percentage. *P < 0.05 compared to normal saline-exposed animals (OVA x NS groups); **P < 0.01 compared to animals exposed to ovalbumin that received vehicle of capsaicin (OVA x CAP-OVA groups). Statistical analysis was done by two-way analysis of variance, and multiple comparisons were made using the Holm-Sidak method.

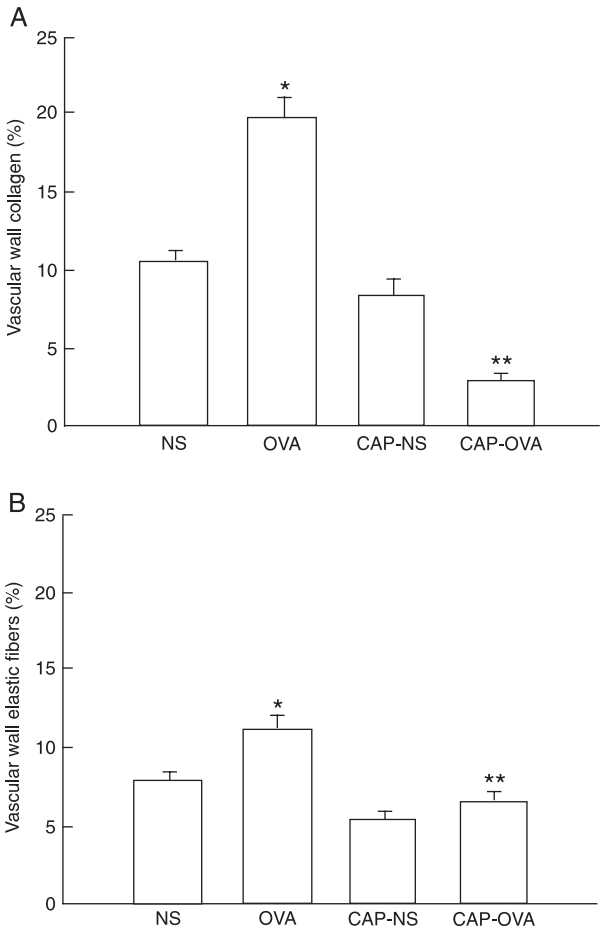

Figure 5. Lung vessel remodeling. The content of collagen (A) and elastic fibers (B) in the vascular wall of guinea pigs (means and SEM) submitted to seven inhalations of ovalbumin (OVA) or normal saline (NS) is represented. Data are reported as percentage. *P < 0.05 compared with NS-exposed guinea pigs; **P < 0.01 compared with OVA-exposed animals that received vehicle of capsaicin (OVA x CAP-OVA groups). Statistical analysis was done by two-way analysis of variance and multiple comparisons were made using the Holm-Sidak method.

Discussion

The present study demonstrates that the inactivation of capsaicin-sensitive nerve fibers by capsaicin pretreatment reduces collagen and elastic fiber deposition in airways, vessels and lung tissue. These effects could be attributed to neurokinin-modulated lung remodeling because 10 to 14 days after capsaicin pretreatment there was a greater than 90% reduction of substance P and neurokinin A in the lung (2,3,7). It has been well documented in the literature that pretreatment with high doses of capsaicin causes irreversible lesions in excitatory non-adrenergic, non-cholinergic fibers (27). This may explain why, as previously demonstrated, changes in C fibers appear to be permanent, and the lung content of substance P and neurokinin A remains reduced at the end of the protocol (2,3,7).

Substance P and neurokinin A belong to the neurokinin family and influence numerous respiratory functions under both normal and pathological conditions, including the regulation of airway smooth muscle tone, vascular tone, mucus secretion, immune functions, and inflammatory cell recruitment (2-5,28). We have shown that this experimental model presents similarities related to the physiopathology of asthma. Chronically ovalbumin-exposed guinea pigs demonstrate hyperresponsiveness to methacholine and an intense bronchoconstriction after antigen challenge. In addition, animals exposed to ovalbumin also show an increase in the number of eosinophils and CD4+ lymphocytes in both bronchoalveolar lavage and lung tissue, including those that express inducible and neuronal nitric oxide synthase (iNOS and nNOS, respectively) (2,3,16,26).

In view of the importance of neurokinins in the control of smooth muscle tone, we confirmed data (2,3) reporting a sharp and significant effect of capsaicin-sensitive nerve fiber inactivation on the attenuation of the bronchoconstriction response associated with a reduction of airway inflammation.

Pulmonary remodeling is mainly characterized by structural and functional changes in lung constituents, including airway smooth muscle, epithelium, blood vessels, and mucus glands (10,17,29). In addition, airway microvasculature changes have long been recognized as a feature of asthma and represent one of the histopathological alterations related to the pulmonary repair process that occurs in chronic lung

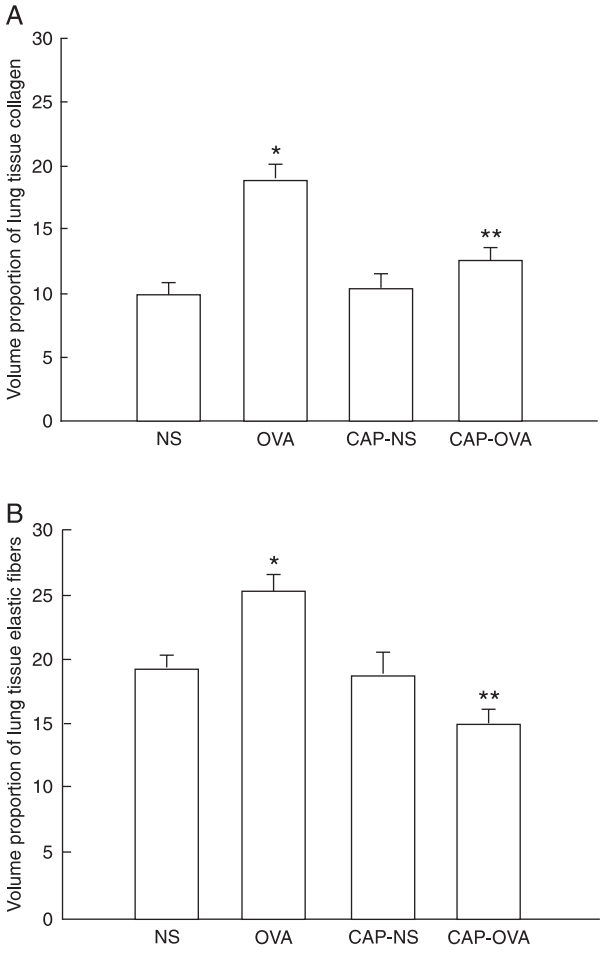

Figure 6. Lung parenchyma remodeling. The content of collagen (A) and elastic fibers (B) in lung tissue (alveolar septa) of guinea pigs (means and SEM) submitted to seven inhalations of ovalbumin (OVA) or normal saline (NS) are represented. Results are reported as percentage. *P < 0.05 compared with NS-exposed guinea pigs; **P < 0.01 compared with OVA-exposed animals that received vehicle of capsaicin (OVA x CAP-OVA groups). Statistical analysis was done by two-way analysis of variance and multiple comparisons were made using the Holm-Sidak method.

Table 1. Correlation matrix between functional parameters and collagen and elastic fibers in airways, vessels and lung tissue.

	Airways	Vessels	Lung tissue
Collagen fibers			
Rrsbas	0.47 (ns)	0.48 (0.027)	0.09 (ns)
Rrsmax	0.46 (ns)	0.52 (0.016)	0.25 (ns)
Ersbas	0.65 (0.011)	0.29 (ns)	0.23 (ns)
Ersmax	0.64 (0.013)	0.39 (ns)	0.18 (ns)
Elastic fibers			
Rrsbas	0.53 (0.02)	0.47 (0.040)	0.52 (0.027)
Rrsmax	0.81 (0.000)	0.70 (0.00)	0.66 (0.003)
Ersbas	0.70 (0.000)	0.63 (0.004)	0.09 (ns)
Ersmax	0.68 (0.001)	0.64 (0.003)	0.43 (ns)

The Spearman correlation, R, was used to determine the correlations between morphometric and functional parameters. *P values are shown in parentheses. ns = nonsignificant; Rrsbas = baseline respiratory system resistance; Rrsmax = maximal respiratory system resistance after ovalbumin challenge; Ersbas = baseline respiratory system elastance; Ersmax = maximal respiratory system elastance after ovalbumin challenge.

inflammatory diseases (13,14). These vascular changes contribute to thickening of the airway wall (30), leading to the narrowing of the bronchial lumen and to the progressive loss of lung function and irreversibility of pulmonary constriction observed in some chronic lung inflammatory diseases (10,11).

Corticosteroids, the most effective anti-asthma drugs (31,32), do not always work to reduce the tissue and vascular pulmonary remodeling. In addition, the mechanisms and possible therapeutic implications of blood vessel alterations are just beginning to be elucidated. For these reasons, chronic *in vivo* studies that evaluate new mechanisms and therapeutic approaches are still necessary.

The present study demonstrated a possible role for capsaicin-sensitive nerves in the modulation of lung remodeling. Inactivation of these afferent sensory nerve terminals in ovalbumin-exposed animals attenuated the increase of collagen and elastic fiber content in airways, vessels and lung tissues exposed to chronic inflammatory stimuli. These data suggest that excitatory non-adrenergic, non-cholinergic nervous system mediators are an important pathway involved in ECM remodeling in this animal model.

It is important to note that the effects observed in this study could also be attributed to direct effects of capsaicin stimulation of vanilloid receptors. The resulting functional impairment of C fibers is probably due to a combination of capsaicin receptor loss, blockage of nerve conduction and inhibition of voltage-sensitive cation channels (1,4). Capsaicin pretreatment resulted in an 85% reduction of substance P content in lung homogenates (data not shown) and other studies have shown that this protocol also attenuates neurokinin A to the same extent (7). Taken together, these data suggest that the differences observed in CAP-OVA animals can be related to the absence of neurokinins, particularly substance P and neurokinin A.

Few studies have evaluated the effects of neurokinins on lung remodeling. Bowden et al. (33) studied *Mycoplasma pulmonis* infection in rats, which evoked a neurogenic inflammation and showed a 3-fold increase in substance P-induced plasma leakage. This vasculature hyperreactivity may be due to an increase in the expression of NK1 receptors on endothelial cells in remodeled vessels (34). De Swert et al. (21) showed that goblet cell hyperplasia induced by repeated ovalbumin exposure was reduced in mice lacking the tachykinin NK1 receptors.

Regarding the effects of neurokinins on ECM components, Ko et al. (35) demonstrated that 24 h after capsaicin infusion there is a reduction of about 30% in the number of fibroblasts in a cell culture model. Others have shown that bradykinin is able to induce fibroblast proliferation and collagen production and can increase the fibroblast-mediated contraction of released collagen gels (24).

Since non-adrenergic-, non-cholinergic-sensitive nerve terminals are present in the adventitia and smooth muscle airway and vascular walls, both epithelial and endothelial lesions can increase the levels of transforming growth factor-beta (TGF-β), which stimulates fibroblasts to produce more collagen and elastic fibers (36). In addition, another explanation is related to the direct or indirect effects of neurokinins on other growth factors such as vascular endothelial growth factor and fibroblast growth factor (37).

Another possible explanation of the results reported here is related to the effects of neurokinins on eosinophilic and lymphocytic pulmonary recruitment (3,5). To confirm that neurokinins induce inflammation, the present study evaluated the number of PMN cells around the airways. Corroborating previous data from our group (2,3), capsaicin-pretreated animals exposed to ovalbumin showed a reduction in the density of PMN cells around the airways compared to the animals treated with vehicle. It is important to note that Tibério et al. (3) used this same experimental model and showed that 90% of the PMN cells around the airways are eosinophils. These and other inflammatory cells play an important role in the activation or in the inhibition of the local production of metalloproteinases, including collagenase-2 and other ECM components, such as fibroblasts.

Although inflammation and remodeling are interdependent processes that may modulate the long-term evolution of asthma and contribute to the decline of respiratory function experienced by asthmatic patients (38), some investigators have argued that the process of ECM remodeling might be beneficial by protecting against bronchoconstriction (39). In the present study, we observed a strong and positive correlation between the intensity of maximal responses to antigen challenge and the content of elastic and collagen fibers both in airways and vessel walls, suggesting that ECM remodeling has a negative effect on lung mechanics.

To better understand our findings, it is important to emphasize the intracellular events involved in lung remodeling. SMADs are a class of proteins that modulate the activity of TGF-β and as such have been intensely investigated. Fueki et al. (40) demonstrated that the stimulation of cultured epithelial cells with substance P, neurokinin A and calcitonin gene-related peptide induced a decrease in Smad 7 protein and an increase in TGF-β. Since the Smad 7 pathway acts in the transcription inhibition pathway for collagen, versican and biglycan, these results support the hypothesis that neurokinins play a key role in airway remodeling and that these effects could be mediated by TGF-β.

The importance of the lung parenchyma in asthma physiopathology has been recently recognized, particularly for the most severely affected asthmatic patients (15,17). It is important to note that the effects of capsaicin pretreatment on collagen and elastic fibers in alveolar septa are less intense than the effects on airways and vessels. Because NK1 receptors are present on the post-capillary venular endothelium of the airways but not on that of the alveoli (34), the reduction in alveolar collagen and elastic fibers in the septa induced by capsaicin pretreatment may be induced by the reduced release of secondary mediators.

As mentioned above, in the present study, we obtained new insights about the connection between the remodeling alterations of distal airways and peribronchial vessels and the mechanical pulmonary repercussions induced by chronic inflammation. In fact, we observed a strong and significant correlation between mechanical responses in the elastance of the respiratory system and the collagen and elastic fiber content in the airways. On the other hand, changes in respiratory system resistance were only positively and significantly correlated with the elastic fiber content of the airways, showing the importance of the remodeling of this ECM component for these responses. It is important to reiterate that we noted a statistical tendency to a positive correlation of collagen fiber deposition in the airways and resistance of the respiratory system (Rrs base: R = 0.46, P = 0.08 and Rrs maximal: R = 0.46, P = 0.09).

Although the major determinant of lung mechanical alteration is the airway smooth muscle function, the extracellular alterations of peribronchial vessels had significant repercussions on the mechanical pulmonary responses. In fact, the resistance responses of the respiratory system were positively associated with the collagen and elastic fiber content of the peribronchial vessels. However, the repercussions of the responses of respiratory system elastance were mainly associated with the elastic fiber content of the peribronchial vessels. We did not find any other evidence supporting the importance of the vascular vessel components in lung mechanical responses.

Although we observed a modulation of capsaicin-sensitive nerve inactivation in alveolar wall extracellular remodeling, these findings were not correlated with the responses of respiratory system elastance. We think that the protocol used for the mechanical evaluation may be less sensitive to the possible impact of lung parenchyma remodeling changes on functional parameters.

The present study has some limitations. We used capsaicin pretreatment, which depleted both substance P and neurokinin A. In order to evaluate the precise mechanisms involved, the use of specific receptor antagonists for each neurokinin are needed in future studies. In addition, although we cannot extrapolate our data to human beings directly, our results support the importance of neurokinins in lung inflammatory responses, indicating the need for new studies in order to evaluate whether treatment with specific neurokinin receptor antagonists can control pulmonary changes in the ECM, which were mainly observed in the severe asthma group.

The results obtained in the present study suggest that capsaicin-sensitive nerve terminals are involved in collagen and elastic fiber deposition in the airways, vessels and lung tissue of guinea pigs with chronic allergic pulmonary inflammation. Collectively, these data support the idea that extracellular remodeling of airways and peribronchial vessels is one of the determinants of the mechanical pulmonary responses induced by chronic inflammation.

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