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Effects of acute and chronic nitric oxide inhibition in an experimental model of chronic pulmonary allergic inflammation in guinea pigs

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Prado, Carla M., Edna A. Leick-Maldonado, David I. Kasahara, Vera L. Capelozzi, Milton A. Martins, and Iolanda F. L. C. Tibério. Effects of acute and chronic nitric oxide inhibition in an experimental model of chronic pulmonary allergic inflammation in guinea pigs. *Am J Physiol Lung Cell Mol Physiol* 289: L677–L683, 2005. First published June 3, 2005; doi:10.1152/ajplung.00010.2005.—Endogenously produced nitric oxide is a recognized regulator of physiological lung events, such as a neurotransmitter and a proinflammatory mediator. We tested the differences between chronic and acute nitric oxide inhibition by *N*^ω-nitro-L-arginine methyl ester (L-NAME) treatment in lung mechanics, inflammation, and airway remodeling in an experimental asthma model in guinea pigs. Both acute and chronic L-NAME treatment reduced exhaled nitric oxide in sensitized animals ($P < 0.001$). Chronic L-NAME treatment increased baseline and maximal responses after antigen challenge of respiratory system resistance and reduced peribronchial edema and mononuclear cells airway infiltration ($P < 0.05$). Acute administration of L-NAME increased maximal values of respiratory system elastance and reduced mononuclear cells and eosinophils in airway wall ($P < 0.05$). Chronic ovalbumin exposure resulted in airway wall thickening due to an increase in collagen content ($P < 0.005$). Chronic nitric oxide inhibition increased collagen deposition in airway wall in sensitized animals ($P < 0.05$). These data support the hypothesis that in this model nitric oxide acts as a bronchodilator, mainly in proximal airways. Furthermore, chronic nitric oxide inhibition was effective in reducing edema and mononuclear cells in airway wall. However, airway eosinophilic inflammation was unaltered by chronic L-NAME treatment. In addition, nitric oxide inhibition upregulates collagen deposition in airway walls.

experimental models of asthma; *N*^ω-nitro-L-arginine methyl ester; respiratory mechanics; airway collagen

NITRIC OXIDE (NO) is generated by a group of enzymes commonly referred to as nitric oxide synthases (NOS) classified into two categories based on the cell type that produces NO. NOS have been found in airway smooth muscle cells (27), nerves (33), epithelium (1), neutrophils (5), eosinophils (13), and mast cells (3).

Endogenously produced NO has been reported as a neurotransmitter of inhibitory nonadrenergic noncholinergic nervous system (2, 23) and may act as a proinflammatory mediator (12, 14, 31). Among its many roles, NO is an important controller of smooth muscle tone in vasculature (17, 24) and in airways (7, 8, 9, 25, 30).

Despite the large number of studies that has been performed, the precise role of NO in human asthma or animal models of allergic pulmonary inflammation is still unclear. NOS dysfunction

has been reported to exhibit both beneficial and deleterious effects, altering some relevant aspects of asthma pathology, mainly inflammatory cell recruitment and airway hyperresponsiveness (7, 8, 9, 25, 26, 31).

A second mechanism that might be involved in the pathophysiology of asthma is the bioavailability of L-arginine, the substrate for NOS. In fact, L-arginine is metabolized by both NOS and arginase, and these enzymes might affect the activity of each other through substrate competition (30). Recently, Meurs et al. (26) suggested that altered uptake of L-arginine and increased arginase activity might contribute to NOS dysfunction in asthma.

In the present study we compared the effects of acute and chronic administration of *N*^ω-nitro-L-arginine methyl ester (L-NAME), an unspecific inhibitor of NO production, in the modulation of airway inflammation and remodeling in an experimental model of chronic allergic pulmonary inflammation in guinea pigs (GP).

METHODS

Animals received humane care in compliance with the *Guide for Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1985), and the Local Ethical Committee approved the study.

Sensitization protocol. Male Hartley GP (300–400 g) were placed in a Plexiglas box (30 × 15 × 20 cm) coupled to an ultrasonic nebulizer (Soniclear, São Paulo, Brazil). An aerosol of ovalbumin solution (Sigma Chemical, St. Louis, MO) diluted in NaCl 0.9% (normal saline) was generated for 15 min or until respiratory distress occurred (onset of sneezing, coryza, cough, and/or indrawing of the thoracic wall). The observer who made the decision to withdraw the GP from the inhalation box was not aware of the treatment status of the animal. The time that GP were in contact to aerosol was termed inhalation time. This protocol was repeated twice a week for 4 wk (32) with increasing concentrations of ovalbumin (1–5 mg/ml) to overcome tolerance. Control animals were submitted to the same protocol with only normal saline.

Chronic L-NAME treatment. Chronic L-NAME treatment (60 mg·kg⁻¹·GP⁻¹·day⁻¹) was performed as previously described (14). In brief, GP received L-NAME dissolved in drinking water ad libitum chronically (OVA-LC or NS-LC groups, see *Experimental groups*), beginning 24 h after the fourth inhalation of either ovalbumin or normal saline to avoid interference with the sensitization. Control animals received sterile drinking water alone.

Acute L-NAME treatment. After the first measurement of exhaled NO (E_{NO}) and baseline respiratory mechanical parameters (see *Pulmonary mechanics evaluation*), GP received an intracardiac injection of L-NAME (50 mg/kg, Sigma Chemical) (28, 34) diluted in normal saline or corresponding volume of vehicle (1 ml/kg). Ten minutes

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after, animals received inhalation of a high dose of ovalbumin or normal saline (see *Measurement of E_{NO}*).

Experimental groups. Six groups of GP were used in the experimental protocol: 1) the first group inhaled normal saline and received sterile drinking water and intracardiac vehicle of L-NAME injection (NS group, $n = 10$); 2) the second group received aerosols of ovalbumin, sterile drinking water, and intracardiac vehicle of L-NAME injection (OVA group, $n = 11$); 3) the third group inhaled normal saline and received sterile drinking water and intracardiac L-NAME injection acutely (NS-LA group, $n = 8$); 4) the fourth group received aerosols of ovalbumin, sterile drinking water, and intracardiac L-NAME injection acutely (OVA-LA group, $n = 12$); 5) the fifth group inhaled normal saline and received L-NAME in drinking water chronically and intracardiac vehicle of L-NAME injection (NS-LC group, $n = 7$); 6) the sixth group received aerosols of ovalbumin, L-NAME in drinking water chronically, and intracardiac vehicle of L-NAME injection (OVA-LC group, $n = 9$).

Measurement of E_{NO} . Seventy-two hours after the seventh inhalation, GP 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 via a Harvard 683 ventilator (Harvard Apparatus, South Natick, MA). We measured E_{NO} levels at the expiratory output from a Mylar bag (22) during 5 min before acute L-NAME or vehicle treatment and after 10 min of the challenge with ovalbumin aerosol, corresponding to 20 min after acute L-NAME treatment. At this time point all animals had returned to baseline values of respiratory system resistance and elastance (Rrs and Ers, respectively).

E_{NO} was measured by chemiluminescence using a fast-responding analyzer (NOA 280; Sievers Instruments, Boulder, CO). The analyzer was calibrated with a certified 47 parts per billion (ppb) NO source (White Martins, São Paulo, Brazil) and zero NO filter (Sievers Instruments) before each measurement. At the inspiratory input a zero NO filter was attached to avoid environment contamination.

Pulmonary mechanics evaluation. Tracheal pressure (Ptr) was measured with a Honeywell 142PC05D (Freeport, IL) differential pressure transducer connected to a side tap in the tracheal cannula. Airflow (V') was obtained by a pneumotachograph Fleish 4-0 (OEM Medical) connected to the tracheal cannula and to a Honeywell 163PC01D36 differential pressure transducer. Lung volume changes (V) were obtained by electronic integration of the V' signal. Ptr, V' , and V signals were stored in a microcomputer. Nine to ten respiratory cycles were averaged to provide one data point (22, 28, 32).

Measurements of Ptr and V' were recorded 1) at baseline (immediately before intracardiac L-NAME or vehicle administration), 2) 10 min after acute L-NAME or vehicle treatment, 3) 1 min after ovalbumin (30 mg/ml) or saline challenge, 4) 3 min after the challenge, and 5) 5 min after challenge. Ers and Rrs were obtained from the equation of motion of the respiratory system: $Ptr(t) = Ers \cdot V(t) + Rrs \cdot V'(t)$, where t is time.

At the end of pulmonary mechanics and E_{NO} measurements, the anterior chest wall was removed, a positive end-expiratory pressure of 5 cmH₂O was applied to the respiratory system, and the airways were occluded at the end of expiration. GP were exsanguinated via the abdominal aorta, and lungs were removed en bloc.

Histology and morphometry. Lungs were fixed with buffered 4% paraformaldehyde and, after 24 h, transferred to 70% ethanol. Sections were processed for paraffin embedding and slices of 5 μ m were obtained and stained with hematoxylin-eosin. Morphometric analysis was performed with a microscope with an integrating eyepiece with 100-point and 50 lines, using a point-counting technique (15). To measure the amount of peribronchial edema, we selected transversely sectioned noncartilaginous airways that were magnified $\times 1,000$. The numbers of points of the integrating eyepiece falling on areas of edema were counted in three randomly selected areas of each airway wall (22). Mononuclear cells and eosinophils present in airway wall were counted in three randomly selected areas of the same airways

used to measure peribronchial edema and expressed as cells/unit area ($10^4 \mu\text{m}^2$) and edema index as the number of points overlying edema (μm^2).

A histochemical method for cyanide-resistant eosinophil peroxidase (EPO) employing diaminobenzidine (Sigma Chemical), H₂O₂, and potassium cyanide (Sigma) was used to stain eosinophils (22, 32). Counterstaining with hematoxylin and eosin was employed to evidence cellular nuclei. EPO+ eosinophils were counted in the airway wall (between bronchial epithelium and adventitia). Ten to twenty fields were analyzed per lung, at a magnification of $\times 1,000$.

Airway remodeling. Histological sections were stained for collagen by 0.2% solution of Sirius red (Direct Red 80, C.I. 35780; Aldrich, Milwaukee, WI) (10). We measured the total area of airway wall and collagen fibers (μm^2) in 9–10 distal airways/lung, using polarized light at a magnification of $\times 200$, in an image analysis system (ImageJ version 1.30). The collagen content (%) was expressed as a relationship between the quantity of collagen fibers in a specific frame and the total area of the frame.

Statistical analysis. Values were analyzed by two-way analysis of variance, and multiple comparisons were made by Tukey test. A P value < 0.05 was considered significant (35).

RESULTS

Inhalation time. An observer who was not aware of the treatment status of the GP measured the time that each animal was able to stay in contact with antigen without respiratory symptoms, such as coughing, indrawing of the thoracic wall, and/or cyanosis. We called this time (in seconds) inhalation time. During the first four inhalations, we did not observe signs of respiratory distress in any of the animals studied. The inhalation time of ovalbumin-exposed animals during inhalations 5, 6, and 7 was significantly lower than saline-exposed groups ($P < 0.001$). Chronic L-NAME treatment did not modify the inhalation time in OVA-LC animals compared with OVA. Saline-exposed animals did not show any signs of respiratory distress in any of the inhalations (data not shown).

E_{NO} . Figure 1 shows the values of E_{NO} of the six groups studied. Baseline (preovalbumin or -saline challenge) E_{NO} was greater in OVA group compared with NS group ($P < 0.01$). Both acute (OVA-LA) and chronic (OVA-LC) L-NAME treatments reduced E_{NO} in ovalbumin ($P < 0.001$)- and saline-exposed animals (NS-LA and NS-LC, $P < 0.05$).

Pulmonary mechanics evaluation. There were no significant differences between baseline values of Rrs in saline- and

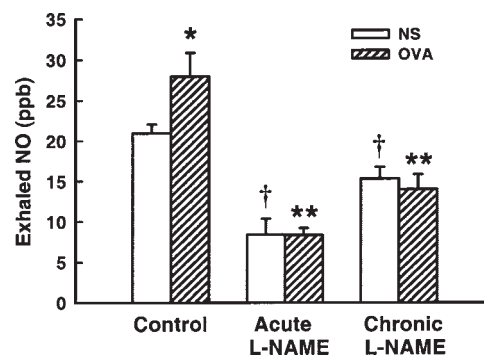


Fig. 1. Mean $\pm$ SE values of exhaled nitric oxide (E_{NO}) in guinea pigs previously exposed to 4 wk of inhalation with ovalbumin (OVA) or normal saline (NS) and treated acutely or chronically with N^G -nitro-L-arginine methyl ester (L-NAME) or vehicle. * $P < 0.01$ compared with NS-control; ** $P < 0.001$ compared with OVA-control; † $P < 0.05$ compared with NS-control.



ovalbumin-exposed animals (Fig. 2). Chronic L-NAME treatment increased baseline values of Rrs in both sensitized and nonsensitized animals ($P < 0.001$) compared with baseline values of OVA and NS groups. If one considers maximal responses of Rrs after ovalbumin challenge (Fig. 2B), all ovalbumin-exposed animals showed greater values compared with the corresponding saline-exposed animals ($P < 0.001$). There were no significant differences in maximal responses of Rrs between ovalbumin-exposed animals that received vehicle of L-NAME and ovalbumin-exposed animals that received acute L-NAME treatment (OVA and OVA-LA). However, chronic L-NAME treatment in ovalbumin-exposed animals increased maximal values of Rrs after challenge compared with ovalbumin-exposed animals that received vehicle of L-NAME and acute L-NAME treatment ($P < 0.01$).

Figure 3 shows the values of Ers. There were no significant differences among baseline values of Ers in saline-exposed and ovalbumin-exposed animals (Fig. 3A). Chronic L-NAME treatment reduced significantly the values of baseline Ers in saline-exposed animals ($P < 0.01$). Figure 3B shows that the values of maximal response of Ers after challenge in all ovalbumin-exposed animals were greater than the values found in saline-exposed animals ($P < 0.01$). When ovalbumin-exposed animals are considered, acute L-NAME treatment (OVA-LA) resulted in greater maximal values of Ers compared with ovalbumin-exposed animals that received only vehicle (OVA group) and chronic L-NAME treatment (OVA-LC, $P < 0.05$ for both).

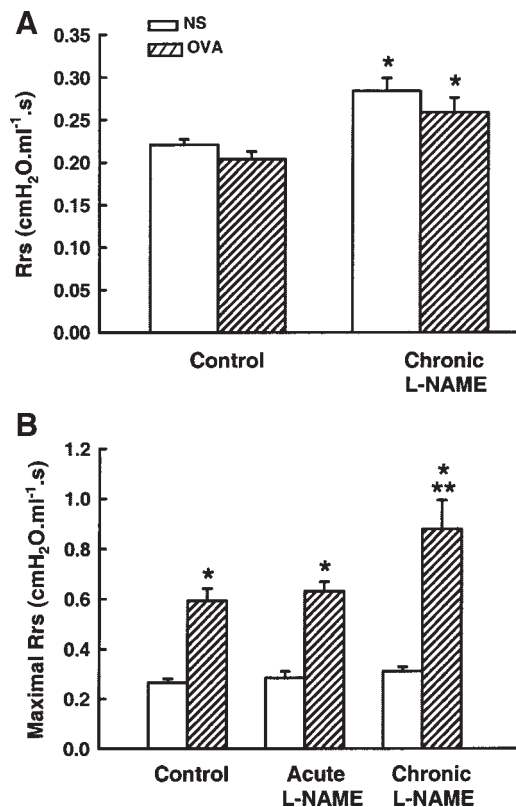


Fig. 2. Mean $\pm$ SE of baseline (A) and maximal (B) values of respiratory system resistance (Rrs) in guinea pigs previously exposed to 4 wk of inhalation with OVA or NS and then treated with L-NAME or vehicle. * $P < 0.001$ compared with respective NS groups; ** $P < 0.01$ compared with OVA-control and OVA-chronic-L-NAME.

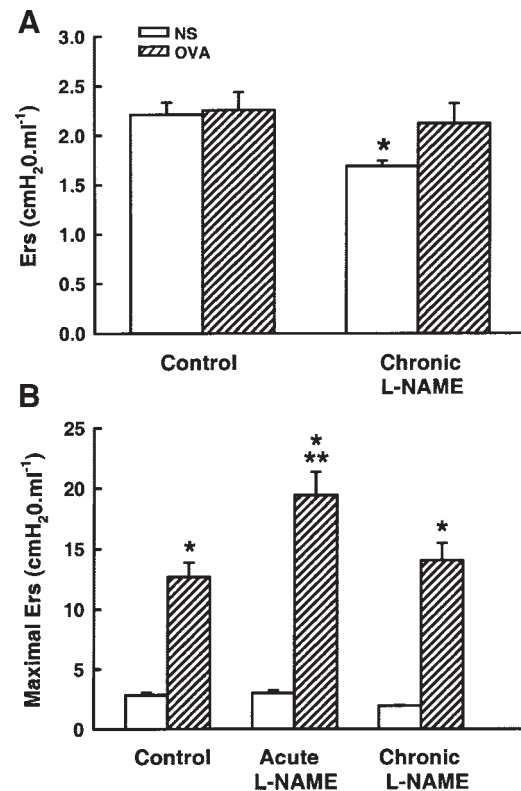


Fig. 3. Mean $\pm$ SE of baseline (A) and maximal (B) values of respiratory system elastance (Ers) in guinea pigs previously exposed to 4 wk of inhalation with OVA or NS and then treated with L-NAME or vehicle. * $P < 0.01$ compared with respective NS groups; ** $P < 0.05$ compared with OVA-control and OVA-chronic L-NAME (LC).

Peribronchial edema index. Figure 4 shows that ovalbumin-exposed animals had greater values of edema index (EI) compared with saline-exposed animals ($P < 0.05$). Chronic L-NAME treatment reduced EI in the OVA group compared with ovalbumin-exposed animals that received vehicle or acute L-NAME treatment ($P < 0.05$). There were no significant differences among saline-exposed groups.

Inflammatory cell evaluation. There was an increase in mononuclear cells around distal airways in ovalbumin-exposed

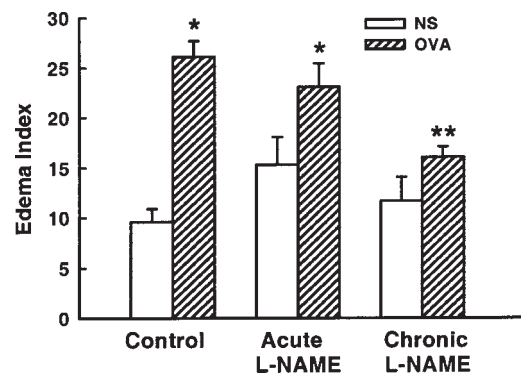


Fig. 4. Mean $\pm$ SE values of peribronchial edema index (EI, μm^2) in guinea pigs previously exposed to 4 wk of inhalation with OVA or NS and then acutely or chronically treated with L-NAME or vehicle. Animals received acute challenge with OVA or saline before lung harvest. * $P < 0.05$ compared with respective NS groups; ** $P < 0.05$ compared with OVA-control and OVA-acute L-NAME-treated animals.

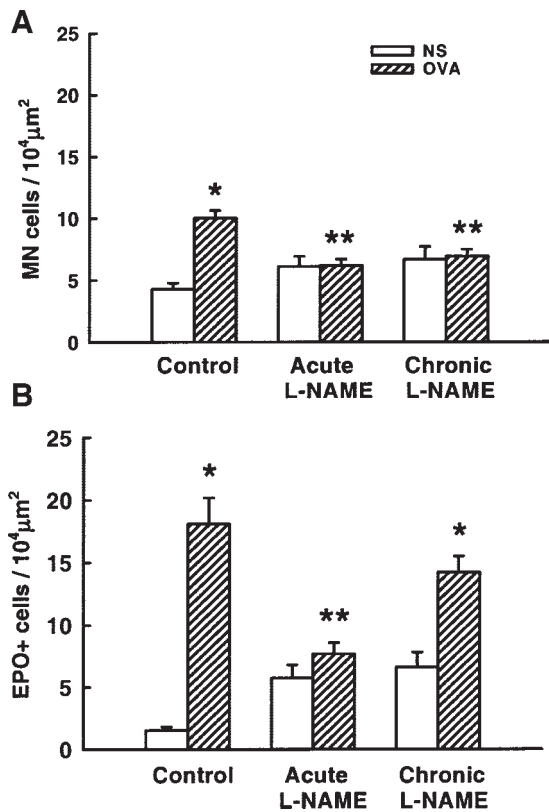
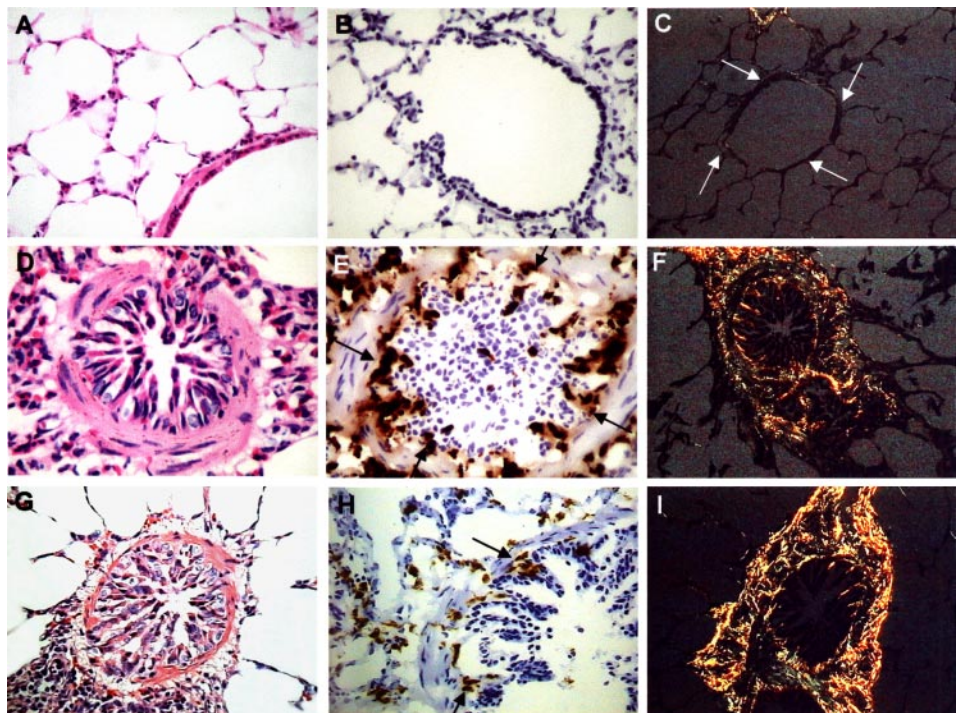


Fig. 5. Mean $\pm$ SE of mononuclear (MN) and eosinophil peroxidase-positive (EPO+) eosinophils around distal airways in animals previously exposed to 4 wk of inhalation with OVA or NS and then acutely or chronically L-NAME-treated or vehicle-treated animals. Animals received acute challenge with ovalbumin or saline before lung harvest. Results are expressed per unit of area ($10^4 \mu\text{m}^2$). * $P < 0.01$ compared with respective NS groups; ** $P < 0.001$ compared with OVA-control animals.

Fig. 6. Samples obtained from saline-exposed, OVA-exposed vehicle, and ovalbumin-exposed L-NAME-treated noncartilaginous guinea pig airways were stained with hematoxylin and eosin (A, D, G), EPO (B, E, H), and picrosirius and were observed under polarized light (C, F, I). Saline-exposed groups show the weak yellow-greenish birefringence of the walls in tissue section (arrows, C) coincident with the maintenance of the histoarchitecture of the noncartilaginous airway extracellular matrix in hematoxylin and eosin preparations (A) and scant number of EPO+ eosinophil (B). In contrast, ovalbumin-exposed vehicle-treated airways show an intense bronchoconstriction associated to peribronchial edema (D), EPO+ eosinophil infiltration (arrows, E), and an increase of birefringence in the airway wall (F). After L-NAME treatment, noncartilaginous airways showed maintenance of the EPO+ eosinophil (arrows, H) and an intense decrease of peribronchial edema (G) coincident with the increase of collagen content in extracellular matrix (I). Magnification: C, F, G, I, $\times 200$; A, B, D, E, H, $\times 400$.



compared with respective saline-exposed animals (Fig. 5A). Both acute and chronic L-NAME treatments reduced mononuclear cells in ovalbumin-exposed animals (OVA-LA and OVA-LC groups) ($P < 0.001$) compared with ovalbumin-exposed and non-L-NAME-treated animals (OVA group).

Figure 5B shows that EPO+ eosinophils were increased in ovalbumin-exposed animals compared with respective saline-exposed animals ($P < 0.01$ for all comparisons). Only acute L-NAME treatment reduced EPO+ eosinophils in ovalbumin-exposed animals compared with ovalbumin-exposed non-L-NAME-treated animals ($P < 0.001$).

Figure 6 shows representative photomicrographs of airways stained with hematoxylin and eosin (Fig. 6, A, D, and G), for EPO+ eosinophils (Fig. 6, B, E, and H) and with polarized picrosirius (Fig. 6, C, F, and I) from GP who inhaled normal saline (Fig. 6, A, B, and C) and were chronically exposed to ovalbumin with vehicle (Fig. 6, D, E, and F) or chronically treated with L-NAME (Fig. 6, G, H, and I). Airways of ovalbumin-exposed GP have a prominent peribronchial edema and inflammatory infiltration (Fig. 6D) with a great number of EPO+ eosinophils (Fig. 6E). In addition, ovalbumin-exposed animals treated with vehicle show an increase in airway wall area (Fig. 6, D and F) and collagen deposition (Fig. 6F). L-NAME treatment in ovalbumin-exposed animals attenuated peribronchial edema formation and mononuclear cells (Fig. 6G) and increased collagen content in the airway wall (Fig. 6I).

Characterization of airway remodeling. Figure 6, C, F, and I, shows the collagenous fibers in a saline-exposed and ovalbumin-exposed animal stained with picrosirius and observed under polarized light. The saline-exposed group showed the weak red-orange birefringence of peribronchial interstitium in tissue sections (Fig. 6C) coincident with the maintenance of the architecture of the peribronchiolar extracellular matrix (ECM). In contrast, an ovalbumin-exposed animal shows a diffuse increase of birefringence (Fig. 6F) in peribronchiolar ECM

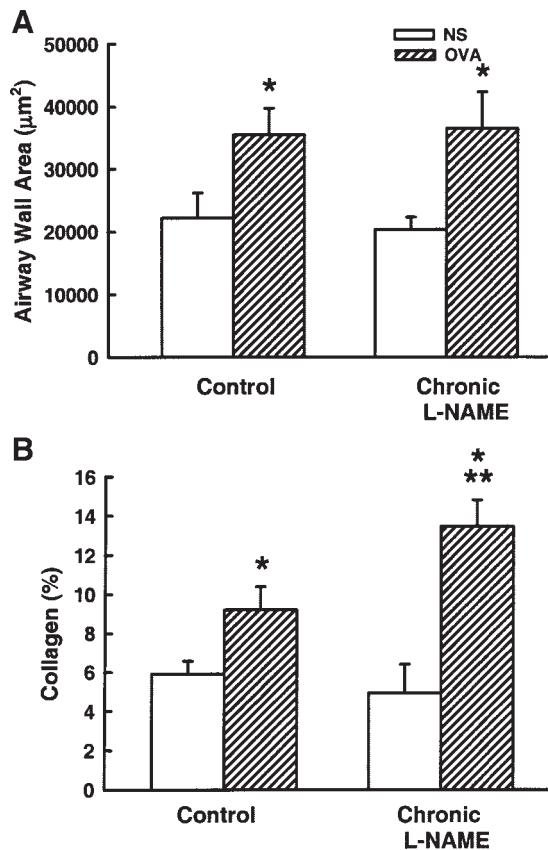


Fig. 7. Means $\pm$ SE of total area (A) and collagen content (B) in airway wall from the 4 groups studied. * $P < 0.01$ compared with NS-control; ** $P < 0.05$ compared with OVA-control animals.

seen in hematoxylin and eosin preparations (Fig. 6D). Figure 6A shows a saline-exposed animal in which the architectural pattern of the collagen content is preserved. Figure 6I shows that chronic L-NAME treatment increased collagen deposition around distal airway wall.

Measurements of the collagen fibers are presented in Fig. 7. The density of the collagen fibers was significantly higher in ovalbumin-exposed animals (Fig. 7A) compared with saline-exposed animals ($P < 0.01$), and chronic L-NAME treatment did not change these responses. Considering collagen content, we found that ovalbumin-exposed animals also showed greater values of percentages of collagen (Fig. 7B) compared with the NS group ($P < 0.01$). Chronic NO inhibition group showed a similar pattern, with an increase in the amount of collagen content in airway wall compared with ovalbumin-exposed animals that received only vehicle of L-NAME ($P < 0.05$).

DISCUSSION

The precise role of NO in human asthma and in animal models of chronic allergic airway inflammation has not yet been fully determined. As for the possible importance of modulation of NO and arginase pathways as a therapeutic strategy for improving asthma control, the evaluation of the effects of chronic treatment with L-NAME in experimental models of chronic allergic inflammation is of primary importance. Most studies using chronic NO inhibition evaluated cardiovascular effects, and there are few studies in acute

asthma model that evaluated chronic effects of L-NAME treatment (14).

We showed that acute inhibition of NO production resulted in an increase in Ers, whereas chronic inhibition resulted in an increase in Rrs. Because respiratory resistance reflects mainly larger airways and respiratory elastance reflects mainly distal airways and/or lung parenchyma, in our experimental model the chronic bronchodilator effects of NO were related to actions predominantly in larger airways. Because acute NO inhibition induced an intense blood extravasation in lung parenchyma it is possible that the alterations observed in Ers were due to these changes in lung vasculature and not only in small airways.

We also observed that baseline values of Rrs of chronic L-NAME-treated GP were greater than those observed in nontreated animals. These data suggest that besides NO-related bronchodilator effects in inflammatory situations, NO may modulate proximal airway tonus in physiological situations. In agreement with this hypothesis, previous studies suggest that approximately half of inhibitory nonadrenergic noncholinergic response *in vitro* is mediated by NO released from peripheral nerves by neuronal NOS (23). Interestingly, Dupuy et al. (9) showed that inhaled gaseous NO is a potent bronchodilator in GP, acting in the distal airways only in high doses. In our study, in contrast to the effects observed in Rrs, values of baseline Ers in chronic L-NAME-treated animals were reduced compared with nontreated ones. One possible explanation may be related to effects on vascular components of lung parenchyma and to the interdependence between distal airways and alveolar-capillary wall.

Acute responses to antigen exposure were evaluated by the time that GP were able to be in contact with the aerosol of ovalbumin (inhalation time). In this context, repeated exposures to increasing concentrations of ovalbumin progressively reduced these measurements after the fifth inhalation, and L-NAME treatment did not modify this response. We reasoned that acute response to antigen challenge depends on several mechanisms including antigen immunization, smooth muscle alterations, and mast cell degranulation that may not be expressively modified by L-NAME treatment. It is important to note that, in our experimental model, chronic L-NAME treatment did not interfere with sensitization to ovalbumin. We performed passive cutaneous anaphylaxis test to evaluate the formation of specific anaphylactic IgG₁ antibodies after the seventh inhalation of ovalbumin, as previously described (32). Both OVA and OVA-LC GP had a similar title of IgG₁ antibodies (1:640).

E_{NO} is an important inflammatory marker and has been used to control asthma in humans (20, 21) and in experimental models of lung inflammation (7, 11, 25). We have previously shown that there is an increase in E_{NO} in this experimental model of chronic asthma in GP (22), and our present data confirm these previous findings. In addition, we also certified the efficiency of L-NAME treatment since E_{NO} was reduced in ~70% and 50% in acutely and chronically treated animals, respectively, as was observed by other authors (7, 25). Is important to note that the values of E_{NO} in saline-exposed animals (20 ppb) are similar to values found in other studies by our group (22, 28) and others (25) in GP.

We confirmed previous results that in this experimental model of chronic pulmonary inflammation there are peribron-



chial edema formation and lymphocytic and eosinophilic recruitment around distal airways (22, 32). Although chronic L-NAME inhibition reduced vascular leakage and secondary peribronchial edema formation, the acute treatment did not interfere with this response. We may hypothesize that edema formation secondary to inflammatory stimuli is progressively established and a single dose of L-NAME was not sufficient to block this response. There are several studies showing that NO plays a role as a vasodilator and that this function is probably derived from NO from constitutive isoforms (15, 24, 27).

We described the differences in the kinetics of lymphocytic and eosinophilic airway recruitment attributable to experimental allergic inflammation and the acute and chronic NO inhibition. The findings observed in acutely L-NAME-treated animals identify in distal airways an expressive reduction of both mononuclear cells and EPO+ eosinophils. Therefore, it was somewhat surprising to find such an expressive acute effect resulting from NO inhibition. In previous studies using this model, we showed, in both bronchoalveolar lavage (BAL) and lung tissues, an increase in eosinophils and CD4+ lymphocytes immediately after acute ovalbumin challenge. In fact, in this experimental model, eosinophils are 70–75% of inflammatory cells in BAL fluid. This acute challenge was also performed 72 h after the last exposition to inhaled ovalbumin, the time corresponding to the late response. In addition, the lungs were removed 20 min after acute antigen challenge. Some other authors have also observed the acute effect of NO inhibitors' treatment in inflammatory cell recruitment (12, 31).

However, chronic L-NAME treatment only altered mononuclear cell airway infiltration, suggesting that NO acts as a proinflammatory mediator influencing the recruitment of this type of cells.

Although there are several studies that show a role of NO in inflammatory cell recruitment, NO effects in eosinophil recruitment are still a matter of controversy. On one hand, some authors showed that acute treatment with nonselective inhibitors of NO reduced allergen-induced eosinophilia (12, 18). On the other hand, Eynott et al. (11) demonstrated that in a model of acute asthma in rats, the inhibition of NO by SC51 [inducible NOS (iNOS) inhibitor] reduced only neutrophils. Blease et al. (4), studying a murine model of fungal asthma, demonstrated that, despite an effective treatment with a single dose of L-NAME with nitrite levels reduction in BAL, there is an increase in peribronchial and BAL eosinophils. Ferreira et al. (14) showed that chronic L-NAME treatment reduced eosinophil in a model of acute inflammation. It is possible that different protocols of antigen sensitization, antigen challenge, type of inhibitors used, and different species and parameters evaluated contributed to these apparently conflicting data.

We observed that, in this animal model, chronic inflammation is associated with airway wall area and an increase in collagen content in the bronchial wall. Furthermore, chronic L-NAME treatment resulted in an amplification of this remodeling process concerning collagen deposition. It is possible that NO has a protective effect in the remodeling process. Despite some evidence suggesting that NO has some role in collagen deposition (16, 19, 30), there are few studies on the effects of NO in the remodeling process in experimental models of asthma. Kenyon et al. (19) showed, in an experimental model of allergic pulmonary inflammation, that iNOS-knockout mice present an increased in airway collagen content in airways.

These authors suggested that the absence of NO could be involved in the downregulation of multiple genes related to apoptotic pathways, such as BCL-2 and caspases, and that it would affect myofibroblasts.

Hogaboam et al. (16), in a model of nonfibrotic lung granuloma, showed that NO from constitutive and/or inducible isoforms regulates the fibrotic process, evidenced in this study by collagen deposition. These authors suggested that NO might either affect the pulmonary fibroblast or play a role in upregulation of profibrotic cytokine synthesis in the lung. These cytokines prevent metalloproteinase synthesis involved in the cleavage of collagen fibers. Other studies suggest that NO inhibition directly regulates fibroblast activation (6, 36). These effects may be related to enhancing chemokine receptor expression (CCR2 and CCR3) for profibrotic chemokines (monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 α , respectively) in fibroblasts cultured from L-NAME-treated mice (16).

The possible role of NO inhibition in contributing to collagen deposition could also be related to the enhanced bioavailability of L-arginine that was metabolized by arginases. Overproduction of a downstream product of this reaction called ornithine might provoke the proliferation of mesenchymal cells and collagen deposition (30). In this regard, Meurs et al. (26) also suggested that enhanced arginase activity contribute to allergen-induced deficiency of constitutive NOS (cNOS)-derived NO, presumably by competition with cNOS for the common substrate, L-arginine. In addition, arginase I and II are constitutively expressed in the airways, particularly in the bronchial epithelium and in peribronchial connective tissue fibroblasts (29).

In conclusion, our data suggest that NO acts as a bronchodilator, mainly in proximal airways. Furthermore, chronic NO inhibition was effective in reducing edema and mononuclear cells in airway wall. However, airway eosinophilic inflammation was unaltered by chronic L-NAME treatment. In addition, NO inhibition upregulates collagen deposition in airway wall in this experimental model of chronic allergic pulmonary inflammation.

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GRANTS

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