Altered contractile sensitivity of isolated bronchial artery to phenylephrine in ovalbumin-sensitized rabbits

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Altered contractile sensitivity of isolated bronchial artery to phenylephrine in ovalbumin-sensitized rabbits. J. Appl. Physiol. 86(5): 1721–1727, 1999.—We tested the hypothesis that atopy and/or allergic lung inflammation enhances \( \alpha \)-adrenergic-agonist-mediated contractions of the bronchial artery. Bronchial arterial resistance vessels were isolated from rabbits that had undergone either systemic ovalbumin (OVA) sensitization followed by saline aerosol challenge (OVA/saline rabbits), or OVA sensitization followed by OVA aerosol challenge (OVA/OVA rabbits), or no sensitization followed by saline aerosol challenge (control rabbits). In OVA/OVA rabbits, bronchoalveolar lavage and lung histology revealed lymphocytic and eosinophilic inflammation. Arterial rings were contracted with phenylephrine (PE). In endothelium-intact arteries isolated from OVA/saline and OVA/OVA rabbits, PE responsiveness was enhanced compared with that of arteries isolated from controls. The nitric oxide synthase (NOS) inhibitor N\(^{\circ}\)-nitro-L-arginine methyl ester increased the contractile response to PE in all three experimental groups to a similar degree, suggesting that depressed NOS activity was not involved in the enhanced PE responsiveness in OVA/saline and OVA/OVA rabbits. After endothelium removal, arteries from OVA/saline and control rabbits showed similar PE responsiveness, indicating that the enhancement of PE responsiveness was endothelial dependent, possibly due to an endothelial constricting factor. In OVA/OVA rabbits, endothelium-denuded arteries showed decreased PE responsiveness compared with the other two groups; this difference was abolished by N\(^{\circ}\)-nitro-L-arginine methyl ester. We conclude that systemic sensitization with OVA per se enhances PE-induced contractions of isolated bronchial arteries in rabbits by an endothelium-dependent mechanism and that allergic lung inflammation attenuates this effect by increased nonendothelial NOS activity.

allegy; bronchoalveolar lavage; endothelium; nitric oxide; vasoconstriction

VASCULAR CONGESTION and edema and increased vascularity have been observed in the bronchi of patients with asthma and attributed to the effects of inflammatory mediators (9, 27). Asthmatic subjects have been shown to exhibit airway smooth muscle hyperresponsiveness to \( \alpha \)-adrenergic agonists. In atopic asthmatic subjects, antigen challenge potentiates \( \alpha \)-adrenergic airway smooth muscle hyperresponsiveness, a phenomenon that can last several weeks (26). The hyperresponsiveness to \( \alpha \)-adrenergic agonists seems to be associated with changes in the number of \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors (ARs), and the responses to the activation of these receptors seem to be abnormal (4, 24). It is therefore possible that bronchial vascular smooth muscle also exhibits hyperresponsiveness to \( \alpha \)-adrenergic agonists. This could be considered an adaptation to inflammatory vasodilatation and hypervascularity in the airway.

In the normal bronchus, norepinephrine released from adrenergic nerves is an important local regulator of vascular smooth muscle tone, and the activation of \( \alpha \)-ARs in the bronchial circulation in vivo by \( \alpha \)-adrenergic agonists has been shown to decrease bronchial arterial blood flow and increase bronchovascular resistance (3, 22).

In the last decade, the development of new techniques has allowed pharmacological investigation of small arteries in vitro, and we have been the first to perform contraction experiments on isolated rabbit bronchial arteries (28). We found that norepinephrine-induced contraction was mediated through activation of \( \alpha_1 \)- and \( \alpha_2 \)-ARs on both vascular smooth muscle and endothelium. Direct activation of the \( \alpha_1 \)- and \( \alpha_2 \)-ARs on vascular smooth muscle caused contraction, whereas activation of endothelial \( \alpha_1 \)- and \( \alpha_2 \)-ARs relaxed vascular smooth muscle through the release of nitric oxide (NO; \( \alpha_1 \)-AR) and a prostanoid (\( \alpha_2 \)-AR).

The aim of the study was to use the isolated bronchial artery preparation to determine whether the contractile response to phenylephrine (PE; \( \alpha_1 \)-AR agonist) is altered in ovalbumin (OVA)-sensitized rabbits with or without subsequent OVA aerosol challenge. The study was based on the premise that OVA sensitization (atopy) and OVA sensitization followed by OVA aerosol challenge (allergic lung inflammation) have differential effects on \( \alpha_1 \)-adrenergic-induced contraction of vascular smooth muscle.

METHODS

OVA sensitization and challenge. New Zealand female albino rabbits (weight 6–8 lb.) were sensitized by subcutaneous injection of 1 ml of an OVA-containing emulsion (2.5 mg OVA in 1 part physiological saline and 1 part of Freund’s complete adjuvant). After 4 wk the injection was repeated. The experiments were carried out 2–4 wk later. Twenty-four hours before euthanasia, one group of rabbits received an aerosol challenge with a 5% solution of OVA in physiological saline (OVA/OVA). Another group of OVA-sensitized rabbits received an aerosol challenge with saline only (OVA/saline). A third group of rabbits was not sensitized but challenged with a saline aerosol before euthanasia (control). For the aerosol challenge, the rabbits were placed in a closed Plexiglas chamber (18 × 21 × 22 cm). One side of the chamber had an opening for tubing connected to a nebulizer (Raindrop, Puritan-Bennett, producing droplets with a mass median aerodynamic diameter of 3.6 μm); the other side of the chamber had...
AN OPENING (1 × 22 CM) FOR AIR EXCHANGE. THE NEBULIZER WAS CONNECTED TO A COMPRESSED AIR SOURCE AND REGULATED AT A FLOW RATE OF 8 L/ MIN. AT THIS SETTING, 3ML OF SOLUTION WERE NEBULIZED DURING A 20-MIN EXPOSURE TIME.

BROCHIOALVEOLAR LAVAGE (BAL) AND LUNG HISTOLOGY. BAL AND LUNG HISTOLOGY WERE PERFORMED TO ASSESS THE INFLAMMATORY CELL STATUS OF THE RABBIT AIRWAY AND TO DOCUMENT LUNG INFLAMMATION IN OVA/OVA RABBITS. AFTER EUTHANASIA BY AN OVERDOSE OF PENTOBARBITAL SODIUM, A PLASTIC CATHETER (28 CM IN LENGTH AND 1.0 MM IN ID) WAS INSERTED VIA A TRACHEOSTOMY INTO THE TRACHEA OF THE RABBIT AS FAR AS THE TUBE WAS MOVING FREELY. BAL WAS PERFORMED BY SLOWLY INJECTING AND ASPIRATING THREE 5-ML ALIQUOTS OF HEPES SOLUTION (IN mM: 140 NaCl, 5 KCl, 1 MgCl₂, 10 glucose, and 10 hydroxyethylpiperazine-ethanesulfonic acid; pH 7.4) AT ROOM TEMPERATURE BY USING A 5-ML SYRINGE CONNECTED TO THE CATHETER. THE BAL RETURN WAS COLLECTED INTO A TEST TUBE PLACED ON ICE. MEAN BAL RETURN AVERAGED BETWEEN 58 AND 76% OF INSTILLED VOLUME. THE EFFLUENT COLLECTED FROM THE BAL WAS STRAINED THROUGH GAUZE TO REMOVE MUCUS AND CENTRIFUGED AT 420 G FOR 10 MIN AT 4°C. THE SUPERNATANT WAS DECANTED, AND THE CELLS WERE RESUSPENDED IN HEPES SOLUTION. AN ALIQUOT OF THIS RESUSPENSION WAS TRANSFERRED TO A HEMOCYTOmeter CHAMBER FOR CELL COUNTING. THE NUMBER OF Viable CELLS WAS ESTIMATED BY TRYpan BLUE EXCLUSION. A SECOND ALIQUOT OF THE CELL SUSPENSION WAS SPUN IN A CYTOSPIN (600 RPM FOR 10 MIN) AND STAINED BY DIFF-QUICK (BAXTER, SCIENTIFIC PRODUCTS) TO IDENTIFY CELL POPULATIONS. FOR DIFFERENTIAL CELL COUNTS, 500 CELLS PER SLIDE WERE IDENTIFIED (×100, OIL OBJECTIVE). CELL CATEGORIES INCLUDED EPITHELIAL CELLS, MACrophAGES, LYMPHOCYTES, NEUTROPHILS, BASOPHILS, EOSINOPHILS, AND MONOCYTES; UNIDENTIFIABLE CELLS WERE GROUPED INTO A CATEGORY TERMED "OTHER." IMMEDIATELY AFTER BAL, REPRESENTATIVE TISSUE SAMPLES WERE OBTAINED FROM BOTH LUNGS FOR HISTOLOGICAL ASSESSMENT. THREE TO FIVE SPECIMENS WERE TAKEN FROM EACH LUNG TO INCLUDE INTRAPULMONARY BRONCHI WITH DIFFERENT CALIBERS EXTENDING TO THE SUBPLEURAL AREAS. THE SAMPLES WERE IMMEDIATELY fixed in 10% FORMALIN IN PHOSPHATE BUFFER, EMBEDDED IN PARAFFIN, SECTIONED AT 4 μM, STAINED WITH HEMATOXYLIN AND EOSIN, AND EXAMINED IN A BLINDED MANNER. THE TISSUE SECTIONS WERE VIEWED WITH A BH2 OLYMPUS LIGHT MICROSCOPE EQUIPPED WITH DIFFERENTIAL INTERFERENCE CONTRAST OPTICS BY USING A CALIBRATED EYEPiece GRID (10 × 10 SQUARES) AND ×20 OBJECTIVE (TOTAL AREA 1,600 mm²). THE NUMBER AND DISTRIBUTION OF POLYMORPHONUCLEAR LEUKOCYTES, LYMPHOCYTES, AND EOSINOPHILS WERE ASSESSED IN THE BRONCHIAL WALL, LUNG PARENCHYMA, AND SUBPLEURAL AREAS. THREE FIELDS OF THE THREE TO FIVE SAMPLES OF EACH LOBE WERE EXAMINED IN FIVE ANIMALS PER EXPERIMENTAL GROUP. ALL THE FIELDS FROM A GIVEN ANIMAL WERE AVERAGED AND CONSIDERED REPRESENTATIVE OF THAT PARTICULAR ANIMAL. LUNG HISTOLOGY DATA WERE OBTAINED IN FIVE ANIMALS PER EXPERIMENTAL GROUP.


In some experiments, the endothelium of the bronchial artery was removed by rubbing the luminal surface of the vessel with a human hair. We have previously demonstrated that functionally this method successfully removes the endothelium in this preparation (28).

Dose-dependent contractions induced by PE were obtained by adding PE cumulatively in increasing concentrations. The contraction was expressed as percentage of the contraction induced by isotonic KCl (72.5 mM) solution (maximal contraction). The values of 72.5 mM KCl-induced contractions in six different conditions were not statistically different (NS): control rabbit, intact artery (1.246 ± 148 mg; n = 9) and denuded artery (853 ± 150 mg; n = 9); OVA/saline rabbit, intact artery (1.178 ± 150 mg; n = 9) and denuded artery (955 ± 150 mg; n = 9); OVA/OVA rabbit, intact artery (1.208 ± 124 mg; n = 13) and denuded artery (876 ± 123 mg; n = 13). In the same preparation and under control conditions, two subsequent dose-response curves (DRCs) for PE were reproducible; therefore, in experiments involving Nω-nitro-l-arginine methyl ester [l-NAME; NO synthase (NOS) inhibitor], the first DRC in the absence of l-NAME served as control for the second DRC in the presence of l-NAME. The l-NAME (10 μM) concentration used in this study was based on our previous observation that this concentration produces a maximal inhibition of PE-induced contraction in this preparation (28). PE concentration at half-maximal contraction (EC₅₀) and maximal contraction expressed as percentage of the contraction induced by 72.5 mM KCl (E₅₀) were determined. In some cases the DRC for PE did not level off, and thus the determination of E₅₀ and EC₅₀ was not feasible. Therefore, for the same experimental set of contraction data, the n values (number of rabbits) in Figs. 3 and 4 are higher than the n values for EC₅₀ and E₅₀.

HEPES WAS USED AS THE NORMAL INCUBATION SOLUTION. WHEN SOLUTIONS WITH HIGHER EXTRACELLULAR K⁺ CONCENTRATION WERE USED, THE EXTRA KCl WAS SUBTRACTED FROM THE CONCENTRATION OF NaCl TO MAINTAIN THE OSMOLARITY AND Cl⁻ CONCENTRATION.

Drugs. The following drugs were used: OVA AND Freund’s complete adjuvant (Sigma Chemical, St. Louis, MO) and l-NAME AND PE (Research Biochemicals International, Natick, MA).

Statistical analysis. For comparisons to baseline values within a group, statistical analysis was performed either by independent tests or by paired Student’s t-tests. For comparisons among the three groups, ANOVA was used. After significant differences were found among the three groups, a paired comparison of OVA/saline and OVA/OVA groups with the control groups was tested by Dunnett’s test. P < 0.05 was considered as significant. The data were expressed as means ± SE, unless stated otherwise.

RESULTS

Inflammatory cell influx. To characterize the stage of inflammation in the airways and lung tissue, cell counts of inflammatory cells in BAL and histological lungs slices were performed.

The total cell numbers in BAL were similar in control, OVA/saline, and OVA/OVA rabbits, and the cell profiles were similar in control and OVA/saline rabbits (Table 1, Fig. 1). In OVA/OVA rabbits, the percentage of macrophages was diminished and the percentage of lymphocytes and eosinophils was increased. Collectively, lymphocytes and eosinophils constituted 9 ± 3% of the total cell count in controls, 8 ± 2% in OVA/saline rabbits (P = NS vs. control), and 37 ± 5% in OVA/OVA rabbits (P < 0.001 vs. control).

In histological lung sections of both OVA/saline and OVA/OVA rabbits there was a tendency toward higher
lymphocyte counts (27 ± 6 and 25 ± 6 cells/field, respectively) compared with control rabbits (16 ± 6 cells/field; Fig. 2). The eosinophil counts were similar in OVA/saline and control rabbits (1.5 ± 0.4 and 5.6 ± 3.0 cells/field, respectively) but were substantially higher in OVA/OVA rabbits (21 ± 5 cells/field). In all sensitized rabbits, inflammatory cell infiltration was found at different levels of cartilaginous airways as well as lung parenchyma. Inflammatory cells usually were located in the vicinity of vessels in the subepithelial area and also in the adventitia of the muscular pulmonary arteries. However, histological sections from isolated bronchial arteries, as they were prepared for contraction experiments, had no or only an occasional eosinophil.

PE-induced contractions of bronchial artery. In intact arteries from OVA/saline and OVA/OVA rabbits, the DRC for PE-induced contractions was shifted to the left (indicating enhanced PE responsiveness) compared with intact arteries from control rabbits (Fig. 3). The EC50 value was significantly lower in OVA/OVA rabbits than in controls, whereas the E_max was not significantly higher; in OVA/saline rabbits, the slight differences in the PE-induced contractility were not found to be statistically different from those of the control rabbits (Table 2). In intact arteries, L-NAME (10 µM) increased the response to PE in all three experimental groups (Fig. 3). In control rabbits, L-NAME induced a small shift in the EC50 value and a significant potentiation of E_max to 77 ±14% (Table 2). In OVA/saline and OVA/OVA rabbits, L-NAME shifted the EC 50 values to 0.5 ±0.1 and 0.6 ±0.1 µM, respectively, and increased the corresponding E_max values to 117 ±11% in OVA/saline and 102 ±11% in OVA/OVA rabbits. Thus the L-NAME induced potentiation of PE responsiveness was similar in the three groups of rabbits. However, in the presence of L-NAME, the difference in the PE-induced response

Table 1. Effect of OVA sensitization and aerosol challenge on bronchoalveolar fluid cell count

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Control (9)</th>
<th>OVA/saline (8)</th>
<th>OVA/OVA (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial</td>
<td>8 ± 3</td>
<td>12 ± 6</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Macrophage</td>
<td>75 ± 7</td>
<td>78 ± 7</td>
<td>38 ± 5†</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>9 ± 3</td>
<td>8 ± 2</td>
<td>16 ± 2*</td>
</tr>
<tr>
<td>Polymorphnuclear leukocyte</td>
<td>4 ± 4</td>
<td>0.6 ± 0.5</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.06 ± 0.03</td>
<td>0.3 ± 0.1</td>
<td>21 ± 4†</td>
</tr>
<tr>
<td>Basophil</td>
<td>0 ± 0</td>
<td>0.04 ± 0.04</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.8 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Other</td>
<td>0 ± 0</td>
<td>0.01 ± 0.01</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Values are means ± SE for no. of rabbits in parentheses. Control: no sensitization, saline aerosol challenge; OVA/saline: ovalbumin sensitization, saline aerosol challenge; OVA/OVA: OVA-sensitization, OVA aerosol challenge. *P < 0.05, †P < 0.001 vs. control.
of OVA/saline and OVA/OVA rabbits in comparison to control rabbits was further enhanced.

Endothelium removal enhanced PE responsiveness in control and OVA/saline rabbits and removed the difference between these two groups (Fig. 4A). In control rabbits, the EC$_{50}$ value for PE was decreased to 0.5 ± 0.1 µM (n = 6; P < 0.05 vs. intact) and the E$_{max}$ value increased to 95 ± 7% (P < 0.001 vs. intact). In OVA/saline rabbits, endothelium denudation decreased the EC$_{50}$ value for PE to 0.73 ± 0.02 µM (n = 7; P < 0.01 vs. intact) and caused a slight increase in the E$_{max}$ value (73 ± 6%; P < 0.05 vs. intact). In OVA/OVA rabbits, on the other hand, endothelium denudation did not shift the DRC significantly, and the EC$_{50}$ value was not decreased (n = 8; 0.9 ± 0.1 µM; P = NS vs. intact) and the E$_{max}$ value was only slightly increased (74 ± 9%; P < 0.05 vs. intact); in other words, in endothelium-denuded arteries of OVA/OVA rabbits, the EC$_{50}$ was higher than the corresponding EC$_{50}$ of control rabbits (P < 0.01.) Thus endothelium denudation abolished the

<table>
<thead>
<tr>
<th>L-NAME (10 µm)</th>
<th>EC$_{50}$, µM</th>
<th>E$_{max}$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.1 ± 0.2</td>
<td>28 ± 7</td>
</tr>
<tr>
<td>OVA/saline</td>
<td>1.9 ± 0.2</td>
<td>47 ± 6</td>
</tr>
<tr>
<td>OVA/OVA</td>
<td>1.3 ± 0.2$^a$</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>Presence</td>
<td>1.5 ± 0.3$^c$</td>
<td>77 ± 14$^a$</td>
</tr>
<tr>
<td>Control</td>
<td>0.5 ± 0.1$^{b,e}$</td>
<td>117 ± 8$^{a,e}$</td>
</tr>
<tr>
<td>OVA/saline</td>
<td>0.6 ± 0.1$^{b,d}$</td>
<td>102 ± 11$^{a,e}$</td>
</tr>
<tr>
<td>OVA/OVA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of 9, 6, and 8 experiments in control, OVA/saline, and OVA/OVA rabbits, respectively. L-NAME, N$^G$-nitro-L-arginine methyl ester; EC$_{50}$, phenylephrine concentration at half-maximal contraction; E$_{max}$, maximal contraction expressed as percentage of 72.5 mM KCl-induced contraction. $^a$P < 0.05, $^b$P < 0.01 vs. control. $^c$P < 0.05, $^d$P < 0.01, $^e$P < 0.001 vs. absence of L-NAME.
differential PE sensitivity in OVA/saline and control rabbits and paradoxically reduced the PE sensitivity in OVA/OVA rabbits compared with the other two groups.

In control and OVA/saline rabbits, L-NAME (10 µM) had no effect on the PE-induced contraction of endothelium-denuded arteries (Fig. 4B). In control rabbits, the EC50 and Emax values were 0.8 ± 0.3 µM and 71 ± 13% in the absence of L-NAME and were 0.9 ± 0.4 µM and 79 ± 14% in the presence of L-NAME (P = NS; n = 6). In OVA/saline rabbits, the corresponding EC50 and Emax values were 0.7 ± 0.2 µM and 93 ± 9% in the presence of L-NAME and were 0.8 ± 0.2 µM and 72 ± 7% in the absence of L-NAME (P = NS; n = 7). In OVA/OVA rabbits, L-NAME shifted the PE DRC to the left so that it was no longer different from the DRC in control rabbits (Fig. 4B). The EC50 and Emax values were 1.2 ± 0.2 µM and 85 ± 6% in the absence of L-NAME and were 0.7 ± 0.1 µM and 97 ± 5% in the presence of L-NAME (P < 0.05 for both, n = 9). This indicates attenuation of PE responsiveness by NOS activation in bronchial vascular smooth muscle in OVA/OVA rabbits.

**DISCUSSION**

BAL and lung histology disclosed lymphocyte and especially eosinophil recruitment to the lung in OVA/OVA rabbits but not in OVA/saline rabbits. This is in keeping with earlier observations that, 24 h after antigen challenge, rabbits exhibit a predominantly eosinophilic lung inflammation (2, 5, 10, 19, 23). The experiments in intact arteries demonstrated that OVA sensitization, especially with subsequent OVA aerosol challenge, slightly enhanced the contractile sensitivity to PE. L-NAME increased the contractile sensitivity to PE in all three experimental groups, confirming our earlier observations that NO was released during α1-AR activation (28). However, L-NAME did not eliminate, but enhanced, the difference in PE responsiveness between sensitized (OVA/saline and OVA/OVA) and nonsensitized (control) rabbits. This indicates that the potentiation of PE contraction by OVA sensitization and OVA sensitization plus OVA aerosol challenge was not due to a downregulation of NOS. Endothelium removal abolished the enhanced PE responsiveness of arteries from OVA/saline rabbits in comparison to control rabbits. This suggests that the OVA sensitization-induced upregulation of PE responsiveness was mediated by the endothelium.

Surprisingly, the response to PE was diminished in endothelium-denuded arteries from OVA/OVA rabbits. In the presence of L-NAME, the PE responsiveness of arteries from these rabbits was enhanced back to the level of the other two groups.

Taken together, these results indicate that 1) the response to PE is increased by systemic OVA sensitization; 2) this effect is endothelium dependent but does not involve a depressed NOS activity; and 3) allergic inflammation in the lung induced by OVA aerosol challenge in OVA-sensitized rabbits causes endothelium-independent attenuation of the PE-induced response, suggesting a NO synthase activation in vascular smooth muscle cells. We have previously shown that α1-AR activation causes NO release from endothelium in rabbit bronchial artery (28). This was confirmed in the present study in which L-NAME failed to potentiate PE responsiveness in endothelium-denuded arteries from control rabbits. We therefore believe that the L-NAME sensitive downregulation of PE responsiveness in endothelium-denuded arteries from OVA/OVA rabbits was due either to the appearance of α1-AR-dependent NOS activity or α1-AR-independent NOS activity in vascular smooth muscle. Irrespective of the underlying mechanisms, an atopy potentiates α1-AR-mediated contraction of rabbit bronchial arteries, and allergic inflammation blunts the effects of an atopy.

Effect of systemic OVA sensitization. The immunologic mechanisms whereby systemic sensitization to OVA increased PE-induced contractions of the bronchial artery are not known. It may reflect a generalized systemic hyperresponsiveness to PE. Earlier studies have shown that inflammation in general can increase the vascular response to α1-AR activation (12, 13, 25) and that in allergic asthma the superficial dermal blood vessels are also affected (25). Systemic OVA sensitization has been shown to induce hypersensitivity to norepinephrine in isolated dog pulmonary arteries (14).

In our study the increase in the sensitivity to α1-AR activation was endothelium dependent. Endothelium is known to release a variety of smooth muscle-relaxing and -contracting factors. Theoretically, there would be two ways whereby sensitization could cause endothelium-dependent differences in the contractile response to PE: 1) by inhibiting the release of relaxing factors or 2) by releasing contractile factors. In rabbit bronchial artery, the major endothelium-derived relaxing factor released by α1-AR activation is NO (28). However, in the present study, the response of sensitized intact arteries to PE in OVA-sensitized rabbits was further enhanced by L-NAME, thereby excluding diminished NOS activity as the cause of the increased contractile responsiveness. Therefore, it is more plausible that the OVA-sensitization-induced increase in PE responsiveness could be due to one or more endothelium-derived contractile factors.

Endothelial cells can be activated by several stimuli, including cytokines, which seem to play pivotal roles in the control of vascular tone and structure. For example, interleukin (IL)-1 and tumor necrosis factor (TNF) have been shown to increase endothelin-1 (ET-1) mRNA expression and ET-1 secretion (8, 17, 20). Increased endothelin levels in BAL have been reported in patients with asthma (16, 18). Other known endothelium-derived contractile factors could also be involved (e.g., cyclooxygenase, lipoxygenase, and phospholipase D products). The identification of putative endothelium-derived contractile factors will be the object of future studies.

The mechanism whereby systemic sensitization induces the α-AR hyperresponsiveness is not known. Systemic sensitization has been reported to alter the
 mechanical properties of both airway and pulmonary arteries and veins to histamine in dogs (1, 15). However, our findings provide the first evidence that sensitization might not, against earlier beliefs, have a direct effect on the mechanical properties of the vascular smooth muscle but rather exhibits its influence through functional changes in endothelium. In the present study, the cell differentiation data in BAL and lung histology in OVA/saline rabbits did not differ significantly from the control rabbits, suggesting that the hyperresponsiveness to PE-induced effect was not dependent on an increased number of inflammatory cells in the lung at the time of assessment.

Allergic lung inflammation. In OVA/OVA rabbits, the presence of allergic lung inflammation was demonstrated by increased numbers of lymphocytes and eosinophils in BAL and lung histology. In endothelium-denuded arteries of these rabbits, antigen challenge produced a diminished PE responsiveness, and this appeared to be due to increased NOS activity. Several studies have demonstrated that cultured vascular smooth muscle can produce NO in inflammation mimicking conditions and thus regulate the contractile sensitivity to other mediators. For example, cytokines such as IL-6, IL-1, and TNF are known to activate the synthesis of NO and/or prostacyclin in various vascular smooth muscle cells and attenuate the contractile sensitivity to α1-AR activation (6, 11, 21). There is increasing evidence that NO synthesis is enhanced in a variety of diseases, asthmatic inflammation included, and several classical signs of inflammation are reversed by NOS inhibitors (7). NO is synthesized by a family of NOS, which are either constitutive and calcium dependent (cNOS; e.g., neurons, endothelium) or inducible and calcium independent (iNOS; e.g., macrophages, fibroblasts, smooth muscle cells, neutrophils). iNOSs respond to receptor stimulation and produce small amount of NO, whereas iNOSs respond to a variety of inflammatory factors and produce NO in large amounts. L-NAME, which was used for blocking NOS inhibitors in this study, is a nonselective inhibitor of NOS and blocks both cNOS and iNOS. Thus the augmented NOS activity in our OVA/OVA rabbits may have involved either NOS in vascular smooth muscle cells.

In summary, the results of this study indicate that in rabbits antigen sensitization induced hypersensitivity of the bronchial artery to PE is mediated by endothelium and does not involve inhibition of endothelial NOS. Possibly, the hypersensitivity to PE is related to release of endothelium derived contractile factor(s). The results also show that allergic lung inflammation may regulate NOS in vascular smooth muscle.

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