Adrenergic airway vascular smooth muscle responsiveness in healthy and asthmatic subjects

JERGIE BRIEVA AND ADAM WANNE
Division of Pulmonary and Critical Care Medicine, University of Miami School of Medicine at Mount Sinai Medical Center, Miami Beach, Florida 33140

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Brieva, Jorge, and Adam Wanner. Adrenergic airway vascular smooth muscle responsiveness in healthy and asthmatic subjects. J Appl Physiol 90: 665–669, 2001.—The purpose of the present study was to determine the responsiveness of airway vascular smooth muscle (AVSM) as assessed by airway mucosal blood flow (Qaw) to inhaled methoxamine (α₁-agonist; 0.6–2.3 mg) and albuterol (β₂-agonist; 0.2–1.2 mg) in healthy (n = 11; forced expiratory volume in 1 s, 92 ± 4 (SE) % of predicted) and asthmatic (n = 11, mean forced expiratory volume in 1 s, 81 ± 5%) adults. Mean baseline values for Qaw were 43.8 ± 0.7 and 54.3 ± 0.8 µl·min⁻¹·ml⁻¹ of anatomic dead space in healthy and asthmatic subjects, respectively (P < 0.05). After methoxamine inhalation, the maximal mean change in Qaw was −13.5 ± 1.0 µl·min⁻¹·ml⁻¹ in asthmatic and −7.1 ± 2.1 µl·min⁻¹·ml⁻¹ in healthy subjects (P < 0.05). After albuterol, the mean maximal change in Qaw was 3.0 ± 0.8 µl·min⁻¹·ml⁻¹ in asthmatic and 14.0 ± 1.1 µl·min⁻¹·ml⁻¹ in healthy subjects (P < 0.05). These results demonstrate that the contractile response of AVSM to α₁-adrenoceptor activation is enhanced and the dilator response of AVSM to β₂-adrenoceptor activation is blunted in asthmatic subjects.

bronchial blood flow; asthma; adrenergic agonists

BOTH AIRWAY SMOOTH MUSCLE and airway vascular smooth muscle (AVSM) express α-adrenergic and β-adrenergic receptors, but the receptor densities have been shown to differ between the two types of smooth muscle (2). Based on pharmacological observations, α-adrenergic receptors predominate on AVSM, whereas airway smooth muscle expresses mainly β-adrenergic receptors (9, 11). Contraction is mediated primarily by the α₁-adrenergic receptor (α₁-AR) and relaxation primarily by the β₂-adrenergic receptor (β₂-AR) (13, 19, 22).

There appear to be differences in the adrenergic responsiveness of airway smooth muscle between healthy and asthmatic subjects. For example, several investigators have reported that inhaled α₁-adrenergic agonists cause airflow obstruction in patients with asthma but not in healthy subjects (7, 23). Conversely, β₂-adrenergic agonist-induced bronchodilation may be blunted in some patients with asthma, although the clinical significance of this defect has been called into question (4, 6, 16, 24, 25). In contrast to airway smooth muscle, comparative data on α₁- and β₂-AR-mediated responses of ASVM have not been systematically examined in healthy and asthmatic subjects.

The aim of the present study was to determine whether the responsiveness of AVSM tone to α₁-AR and β₂-AR activation is altered in asthmatic subjects. We used airway mucosal blood flow (Qaw) as an index of AVSM tone, and inhaled methoxamine and albuterol as α₁-AR and β₂-AR activators, respectively.

METHODS

Test population. Twenty-two nonsmokers participated in the study. They denied having cardiovascular disease or taking vasoactive or anti-inflammatory medications. Subjects who had taken antibiotics or inhaled or systemic glucocorticoids and subjects who had an acute respiratory infection during the 6-wk period preceding the study were excluded. Eleven subjects were healthy (mean age 36.5 yr, range 29–46 yr; 9 women), and 11 had mild, intermittent asthma (mean age 33.1 yr, range 25–53 yr; 9 women) as defined by the American Thoracic Society and National Asthma Education Program (1, 18). The asthmatic subjects used a short-acting inhaled β-adrenergic agonist on demand as their only asthma treatment. The mean hourly β-adrenergic agonist use was 1.6 puffs (range 0–8). Nine of the 11 asthmatic subjects had a baseline forced expiratory volume in 1 s (FEV₁) <90% of predicted; the albuterol-induced mean increase in FEV₁ for all asthmatic subjects was 10.2 ± 2.5%. The two asthmatic subjects with FEV₁ >90% of predicted had previously demonstrated methacholine hyperresponsiveness. Cutaneous allergy testing was not performed. Historically, five asthmatic subjects denied having allergies, four reported known allergies (confirmed by previous skin testing in 3), and two were not sure. Informed consent was obtained from all subjects, and they received financial remuneration for their participation. Spirometry was carried out with an Essential Medic unit (model 6200, Autbox DL, Yorba Linda, CA). The highest FEV₁ of three forced vital capacity maneuvers was determined and expressed as an absolute value and as percent predicted (10).

Qaw. A soluble inert-gas uptake method was used to measure Qaw (15, 19, 21). The subjects were seated in front of a valve system that allowed them to inhale through a mouth-
piece (with the nasal passage occluded by a nose clip) room air or a gas mixture from a Teflon bag containing 10% dimethylether (DME), 5% helium, and balance oxygen and to exhale into a rolling seal spirometer (model 842; Ohio Instruments, Houston, TX). The subjects first inhaled room air to, and then exhaled 500 ml from, the total lung capacity position and subsequently inhaled rapidly the same volume of gas mixture from the Teflon bag. They held their breath for a predetermined duration and then exhaled into the spirometer through a critical flow orifice to standardize expiratory flow. The maneuver was performed with two breath-hold times each of 5, 10, 15, and 20 s in random order. During exhalation, the instantaneous concentrations of DME, nitrogen, and helium were measured at the airway opening with a mass spectrometer (Perkin-Elmer, Pomona, CA), along with the expired gas volume. The mass spectrometer inlet was not heated, and no corrections were made for water pressure. The resulting overestimation of DME concentration by measuring it at the airway opening was considered to be negligible (~0.3%). The mass spectrometer was also used to verify the gas concentration in the Teflon bag before inhalation of the gas mixture. Anatomic dead space (DS) was determined from the expired nitrogen concentration curve as described by Fowler and co-workers (12). The helium-corrected decrease in the DME concentration over time was obtained by least squares fit using the two measurements per gas for each of the four breath-hold times. This was done in the expired volume fraction corresponding to the DS minus the most proximal 50 ml. From the helium-corrected DME slope multiplied by the DS (V_{DME}), the mean DME concentration in the DS (F_{DME}), and the solubility coefficient for DME in blood and tissue (a), Qaw was calculated using Fick's principle (Qaw = V_{DME}/a·F_{DME}). Qaw was normalized for DS and expressed as microliters per minute per milliliter.

Protocol. The subjects were asked to come to the research laboratory in the morning of the study day without having had any coffee or caffeinated drinks. The subjects were asked to abstain from ingesting alcoholic beverages the night before. The asthmatic subjects were asked not to use their inhaled β-adrenergic agonist for at least 12 h before the study. After the measurement of baseline Qaw, the subjects inhaled albuterol on 1 experiment day and methoxamine on another. A dosimeter, consisting of a breath-activated sole-noid valve, which controlled flow of compressed air (45 lb/in.2) to a DeVilbiss 644 nebulizer, was used. The mass median aerodynamic diameter of the aerosol was 3.2 μm (geometric SD 2.0) as determined by a cascade impactor. Different solutions of albuterol or methoxamine in phosphate-buffered saline were freshly prepared. In a previous study, our laboratory (19) found that inhalation of phosphate-buffered saline aerosol had no effect on Qaw. In the present investigation, the subjects inhaled the aerosol from functional residual capacity to total lung capacity (inspiratory capacity). They took the required number of breaths of different drug solutions for the desired drug doses. During the first 0.6 s of each breath, 0.023 ml of solution was nebulized. Doses were 0.2, 0.4, 0.6, 0.8, and 1.2 mg for albuterol and 0.6, 1.2, 1.8, and 2.3 mg for methoxamine in all subjects except for albuterol doses in asthmatic subjects (0.6 and 1.2 mg). Repeat measurements of Qaw were made 15 min after each drug inhalation, and the interval between drug doses was 45 min. To monitor airway caliber, FEV1 was determined before each Qaw measurement.

Data analysis. The mass spectrometer and spirometer signals were fed through analog-to-digital converters to a computer and stored for data acquisition and calculation of Qaw. All Qaw data were analyzed after completion of the study. Statistical comparisons between groups were made with ANOVA. A P value of <0.05 was considered significant. The data are presented as means ± SE.

RESULTS

On the first experiment day, mean baseline Qaw was higher in asthmatic than in healthy subjects (54.3 ± 0.8 vs. 43.8 ± 0.7 μl·min⁻¹·ml⁻¹; P < 0.05). The baseline values were comparable on the second experiment day (Table 1). There was no difference in mean dead space between groups and between the 2 experiment days within groups. Methoxamine caused a decrease in Qaw at several doses in asthmatic subjects but only at the highest dose in healthy subjects (Fig. 1). The mean maximal change (Δmax) in Qaw was greater in asthmatic subjects (−13.5 ± 1.0 μl·min⁻¹·ml⁻¹) than in healthy subjects (−7.1 ± 2.1 μl·min⁻¹·ml⁻¹; P < 0.05; Fig. 2). After albuterol, mean Qaw remained essentially unchanged in the dose range of the study in asthmatic subjects and showed a dose-dependent increase in healthy subjects (Fig. 1). Mean Δmax in Qaw was considerably greater in healthy subjects than in asthmatic subjects (14 ± 1.1 vs. 3.0 ± 0.8 μl·min⁻¹·ml⁻¹; P < 0.05; Fig. 2).

Baseline mean FEV1 was lower in asthmatic than in healthy subjects on both experiment days (Table 1). The mean Δmax values in FEV1 after methoxamine and albuterol are shown in Fig. 3. The drug-induced changes in DS were minimal and statistically not different for the two adrenergic agents in either group (Fig. 4).

Mean systemic blood pressure and pulse rate remained unchanged throughout the experiment with both drugs and in both groups of subjects.

### Table 1. Baseline measurements

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<thead>
<tr>
<th></th>
<th>First Experiment Day</th>
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<th>Second Experiment Day</th>
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<tbody>
<tr>
<td></td>
<td>FEV1, liters</td>
<td>FEV1, %predicted</td>
<td>Qaw, μl·min⁻¹·ml⁻¹</td>
<td>DS, ml</td>
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<tr>
<td>Healthy</td>
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<tr>
<td>(n = 11)</td>
<td>2.92 ± 0.19</td>
<td>92 ± 4</td>
<td>43.8 ± 0.7</td>
<td>158 ± 10</td>
</tr>
<tr>
<td>Asthma</td>
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<tr>
<td>(n = 11)</td>
<td>2.68 ± 0.31*</td>
<td>81 ± 5*</td>
<td>54.3 ± 0.8*</td>
<td>165 ± 20</td>
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<tr>
<td></td>
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<td></td>
<td>2.62 ± 0.31*</td>
<td>55.9 ± 0.7*</td>
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<td>146 ± 16</td>
<td>171 ± 15</td>
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Values are means ± SE for n subjects. FEV1, forced expired volume in 1 s; Qaw, airway mucosal blood flow; DS, anatomic dead space. Predicted values are from Ref. 10. *P < 0.05 vs. healthy subjects.
DISCUSSION

This investigation disclosed enhanced $\alpha_1$-AR-mediated contraction and blunted $\beta_2$-AR-mediated relaxation of AVSM in asthmatic compared with healthy subjects. We selected patients with mild asthma for several reasons. First, we wanted to minimize the difference in baseline $Q_{aw}$ between the asthmatic and healthy subjects because baseline $Q_{aw}$ could have influenced the response to the adrenergic agonists. Marked vasodilation associated with more severe asthma could further attenuate vasodilation by albuterol, although this has thus far not been demonstrated experimentally. In our study, mean baseline $Q_{aw}$ was only 24% higher in asthmatic subjects than healthy subjects. This difference is unlikely to explain the observed differential responses to methoxamine and albuterol. Because baseline $Q_{aw}$ varied among subjects, the methoxamine- and albuterol-induced changes in $Q_{aw}$ were expressed in absolute terms rather than as percent baseline. Second, the choice of mild asthmatic subjects who did not use $\beta$-adrenergic agonists regularly circumvented the problem of agonist-induced tolerance to albuterol. Third, we were able to find a sufficient number of glucocorticosteroid-naive patients by restricting the study to mild asthmatic subjects. Our laboratory has previously made the observation that glucocorticosteroids restore blunted $\beta$-adrenergic AVSM responsiveness (8). Finally, the magnitude of methoxamine- and albuterol-induced changes in airway caliber was minimized by studying individuals with a near-normal baseline FEV1. As a result, the maximum mean FEV1 did not exceed 250 ml after either drug. The relatively small changes in FEV1 and normalizing $Q_{aw}$ for DS minimized a potential influence of airflow caliber on the measurement of $Q_{aw}$. Furthermore, inhaled cholinergic agonists have been shown to cause bronchoconstriction without changing airway blood flow in sheep, indicating that airway blood flow is independent of airway smooth muscle tone (9a, 21a). Finally, DS was only minimally affected by the small changes in FEV1 induced by methoxamine and albuterol in the present study.

Our laboratory (19) has previously shown that, in healthy subjects, the vasoactive effects of a single in-
hale dose of methoxamine and albuterol are transient, waning by 30 min postchallenge. Because 45 min elapsed between drug inhalations in the present study, the dose-response curves were not cumulative for Q̇aw. The drug doses used in this experiment (0.6–2.3 mg for methoxamine, 0.2–1.2 mg for albuterol), for which we used solutions nebulized only during inspiration by a dosimeter, are more comparable to those of metered dose inhalers than constant-flow nebulizers, in which a considerable fraction of the nebulized dose is wasted during the expiratory phase (17). Within the dose ranges of this study, differences in adrenergic responsiveness were demonstrated without adverse drug reactions. Similarly, significant systemic vascular changes as assessed by systemic blood pressure and heart rate were not present at the drug doses used. Palpitations, tachycardia, and tremor were observed at doses of albuterol exceeding 1.2 mg in preliminary experiments. The maximum dose was, therefore, set at 1.2 mg in the protocol.

Methoxamine caused bronchoconstriction in asthmatic subjects. The lowest mean FEV₁ value was 74.5 ± 4.1% of predicted, and it is, therefore, unlikely that methoxamine caused hypoxia that was severe enough to influence Q̇aw.

α₁-Adrenergic responsiveness. Our study showed airway vascular hyperresponsiveness to methoxamine in asthmatic subjects, similar to the previously demonstrated airway hyperresponsiveness to α₁-adrenergic agonists (7, 23). The mechanisms responsible for the asthma-associated smooth muscle hyperresponsiveness are not known. With respect to airway smooth muscle, methoxamine-induced vasoconstriction could have reduced the washout of methoxamine from the airway tissue, leading to increased airway smooth muscle contraction. In addition, α₁-AR density has been reported to be increased in patients with obstructive lung disease (5); this may explain or contribute to the enhanced α₁-adrenergic smooth muscle responsive-

ness, although it is not known which lung cells over-express α₁-ARs.

It is tempting to attribute the α₁-adrenergic AVSM hyperresponsiveness to airway inflammation, systemic sensitization, or both. This has not been studied in humans. However, experiments conducted in animal models of allergic sensitization and airway inflammation support this notion. There are several possible mechanisms of inflammation-induced α₁-adrenergic hyperresponsiveness, including increased α₁-AR expression and function or altered postreceptor signal transduction in AVSM and airway vascular endothelium, altered inactivation or cellular uptake of α₁-adrenergic agonists, or a combination thereof. Some of these possibilities have been investigated. For example, it has been reported that antigen-sensitized and airway-challenged guinea pigs have an increased pulmonary α₁-AR density (3). In addition, Zschauer et al. (26) showed that the contractile sensitivity of AVSM to phenylephrine increased in bronchial artery rings removed from ovalbumin-sensitized rabbits. In that model, the α₁-adrenergic hyperresponsiveness induced by systemic sensitization alone was related to an endothelial contractile factor. The putative inflammatory products responsible for the potentiation of α₁-AR-mediated AVSM contractions in asthma remain to be identified.

β₂-Adrenergic responsiveness. We found a marked attenuation of β₂-AR-mediated relaxation of AVSM in asthmatic subjects compared with healthy subjects. Healthy subjects had a dose-related increase in Q̇aw, whereas in asthmatic subjects, Q̇aw remained unchanged within the nebulized dose range of albuterol (0.2–1.2 mg). It is possible that higher doses of albuterol would have increased Q̇aw in asthmatic subjects as well, but we decided against exceeding a nebulized dose of 1.2 mg to avoid acute toxic drug effects.

Although the assessment of adrenergic airway smooth muscle responsiveness was not the objective of our investigation, the monitoring of FEV₁ disclosed that, in contrast to AVSM, airway smooth muscle responsiveness to albuterol was not blunted, in keeping with other reports (8, 9). The reason for the blunted AVSM responsiveness to albuterol in our asthmatic subjects is not known but may involve β₂-ARs. Pulmonary β₂-AR density has been reported to be decreased in patients with obstructive airway disease (5). Possibly AVSM cells or endothelial cells are more susceptible than airway smooth muscle cells, resulting in a demonstrable blunting of albuterol responsiveness in the former but not the latter. Another hypothesis is that airway smooth muscle has a greater β₂-AR reserve and that asthma-related loss of β₂-AR density is of no or little functional consequence. In contrast to airway smooth muscle, a reduction of β₂-AR density on blood lymphocytes has been found in patients with asthma, and this was accompanied by a decreased β₂-adrenergic responsiveness as assessed by cyclic AMP production (14, 20). In this regard, AVSM seems to resemble blood lymphocytes more than airway smooth muscle.
In a previous study, we showed that the vasodilator response in the airway to 180 μg of albuterol administered by inhalation was restored by a 2-wk treatment with an inhaled glucocorticosteroid (8). Assuming that this effect of the glucocorticosteroid was related to its anti-inflammatory action, the observation suggests that the attenuated β2-adrenergic responsiveness of AVSM is a consequence of the asthma-associated airway inflammation.

In summary, the results of this study demonstrate an enhanced adrenergic constrictor response and blunted adrenergic dilator response of the airway circulation in patients with asthma. This could be considered an adrenergic adaptation to the asthma-associated inflammatory vasodilation in the airway.

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REFERENCES