Potent bronchoprotective effect of deep inspiration and its absence in asthma

TRISEVGENI KAPSALI,1 SOLBERT PERMUTT,2 BETH LAUBE,3 NICOLA SCICHLONE,1 AND ALKIS TOGIAS1,2

Divisions of 1Clinical Immunology and 2Respiratory and Critical Care Medicine, Department of Medicine, and 3Pediatric Pulmonary Division, Department of Pediatrics, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21224

Received 29 July 1999; accepted in final form 21 February 2000

Kapsali, Trisevgeni, Solbert Permutt, Beth Laube, Nicola Scichilone, and Alkis Togias. Potent bronchoprotective effect of deep inspiration and its absence in asthma. J Appl Physiol 89: 711–720, 2000.—In the absence of deep inspirations, healthy individuals develop bronchoconstriction with methacholine inhalation. One hypothesis is that deep inspiration results in bronchodilation. In this study, we tested an alternative hypothesis, that deep inspiration acts as a bronchoprotector. Single-dose methacholine bronchoprovocations were performed after 20 min of deep breath inhibition, in nine healthy subjects and in eight asthmatics, to establish the dose that reduces forced expiratory volume in 1 s by >15%. The provocation was repeated with two and five deep inspirations preceding methacholine. Additional studies were carried out to assess optimization and reproducibility of the protocol and to rule out the possibility that bronchoprotection may result from changes in airway geometry or from differential spasmogen deposition. In healthy subjects, five deep inspirations conferred 85% bronchoprotection. The bronchoprotective effect was reproducible and was not attributable to increased airway caliber or to differential deposition of methacholine. Deep inspirations did not protect the bronchi of asthmatics. We demonstrated that bronchoprotection is a potent physiologic function of lung inflation and established its absence, even in mild asthma. This observation deepens our understanding of airway dysfunction in asthma.

hyperresponsiveness; airway dysfunction; lung inflation; bronchoprovocation

Asthma is characterized by airway lability and inflammation. The former phenomenon is probably related to that of bronchial hyperresponsiveness, defined as increased obstructive response to various chemical and physical stimuli (22). Although hyperresponsiveness and airway inflammation are thought to be etiologically linked, the mechanism for such a link is elusive. It has become apparent to many investigators in this field that better understanding of the nature of airway hyperresponsiveness is warranted before the link to inflammation can be unveiled.

In 1995, Skloot et al. (25) showed that, when a methacholine inhalation challenge is carried out under conditions where deep inspirations are prohibited, the response to increasing methacholine concentrations is similar in asthmatic and healthy subjects. This work confirmed the previously reported effects of lung inflation in regulating bronchomotor tone (5, 8, 11, 21, 23). Furthermore, Skloot and co-workers (25) demonstrated that the effect of deep inspiration was absent or diminished in asthma, offering support to a hypothesis, originally suggested by Fish et al. (8), that airway hyperresponsiveness in asthma may merely represent reduced ability of a deep inspiration to relax the airways.

In the study of Skloot et al. (25), during the discussion of the potential mechanisms through which lung inflation benefits the airways, they referred to the work of Malmberg et al. (17) who, in an effort to develop an abbreviated methacholine bronchoprovocation for epidemiological studies, came across the observation that a deep inspiration before the administration of methacholine protected the airways from bronchoconstriction. In this work, it also appeared that the protective effect of deep inspiration was more pronounced than the effect obtained if deep inspiration took place after the administration of methacholine.

A bronchoprotective effect of lung inflation implies that the increase in lung volume triggers a process that renders airway smooth muscle resistant to contractile stimuli. In contrast, the presence of a bronchodilating effect alone does not allow one to differentiate between a process that alters the state of airway smooth muscle and a mere stretch relaxation effect. Consequently, understanding the process behind lung inflation-induced bronchoprotection may lead to the elucidation of the mechanism of airway hyperresponsiveness in asthma, provided that healthy subjects and asthmatics are profoundly different in this respect.

This paper describes a series of studies that we performed with the goals to 1) unequivocally establish the presence of a bronchoprotective effect of lung inflation and provide a methodology for quantitative
assessments and 2) compare healthy subjects to asthmatics.

**METHODS**

**Subjects**

We studied 8 asthmatics [age: 30.3 ± 9.1 (SD) yr, range 22–50 yr] and 12 nonasthmatics (33.5 ± 8.5 yr; range 24–54 yr). Asthmatic subjects had intermittent or mild persistent disease, according to National Asthma Education and Prevention Program guidelines (22), and were selected from a large database of allergic/asthmatic individuals recruited from the community by radio and newspaper advertising. We selected asthmatic subjects with high airway responsiveness, demonstrating a 20% reduction in forced expiratory volume in 1 s (FEV1) during a routine methacholine bronchoprovocation with a provocative concentration (PC20) < 1 mg/ml. The baseline FEV1 at the first evaluation in this study was 85.4 ± 8.2% (mean ± SD) predicted. Nonasthmatic subjects were all employees of the Johns Hopkins University. They reported no symptoms consistent with asthma and had never been diagnosed with asthma by a physician. In a routine methacholine bronchoprovocation screening, they received a concentration up to 75 mg/ml with <10% reduction in FEV1. All subjects were currently nonsmokers and had a smoking history of <5 pack-yr. They had not suffered an upper respiratory infection for at least 4 wk before evaluation. Asthmatic subjects were not on oral or inhaled steroids, cromolyn, nedocromil, methylxanthines, leukotriene modifiers, or long-acting β-agonists for at least 8 h before participating in the study. The study was approved by the Institutional Review Board of the Johns Hopkins Bayview Medical Center, and informed, written consent was obtained from each subject.

**Study Design**

The experiments were separated into 4 phases.

**Phase 1: Screening.** The screening evaluation included a respiratory questionnaire, skin prick testing to a panel of common aeroallergens (mixed grasses, mixed trees, ragweed, *Alternaria, Cladosporium, D. farinae, D. pteronyssinus*, cat, dog, mixed cockroaches), and a methacholine challenge. The methacholine challenge was conducted routinely, i.e., after baseline maximal spirometric dilution was inhaled and new spirometric measurements were performed 3 min later, followed by bronchoprovocation with methacholine. The initial concentration of methacholine was 0.025 mg/ml and was increased by half-log increments (0.025, 0.075, 0.25, 0.75, etc.) until a 20% decrease in FEV1 from the postdiluent values was obtained or until the maximum concentration (75 mg/ml) was delivered. The PC20 of methacholine causing this fall was calculated by interpolation of the dose-response curve. Each dose of methacholine was inhaled with five deep breaths from functional residual capacity (FRC) to total lung capacity (TLC). Methacholine was delivered by a DeVilbiss 646 nebulizer (DeVilbiss, Somerset, PA) connected to a 30-psi compressed air source through a Rosenthal-French dosimeter that regulated the timing of each actuation to 0.6 s. At each dose level, a minimum of three forced spirometric maneuvers from TLC were performed, and the maneuver with the highest FEV1 was used for analysis.

**Phase 2: Establishment of a single-dose methacholine challenge.** The protocol we designed to demonstrate that deep inspiration acts as a bronchoprotector and to compare healthy subjects to asthmatics in this respect was a modification of that employed by Malmberg et al. (17). The protocol involves single-dose methacholine challenges. Therefore, before entering each subject into the main study, our task was to determine a single dose of methacholine capable of producing an adequate reduction in lung function. We chose 15% as the minimal desired reduction in FEV1. Both nonasthmatic and asthmatic subjects underwent the following protocol multiple times, each time separated by at least 1 day. At each occasion, a single dose of methacholine was administered, but that dose was increased every time until the dose that produced >15% reduction in FEV1 was established. In the beginning of every single-dose protocol, all subjects performed three reproducible maximal (full breath) expiratory maneuvers from TLC to residual volume (RV). The maneuver(s) yielding the best FEV1 and forced vital capacity (FVC) provided us with baseline measurements. After these three maneuvers, deep inspirations were prohibited for 20 min. At the end of this 20-min period, a single dose of methacholine was administered with five tidal inspirations (FRC to end inspiratory volume) using the dosimeter and nebulizer described above. The subjects were instructed to hold their breath for 5 s after each inspiration and to passively exhale thereafter. Three minutes later, three maximal expiratory maneuvers were again performed, and the outcome was compared with the baseline measurements. The concentration of methacholine delivered in the first single-dose bronchoprovocation was 10 mg/ml for nonasthmatics and 0.025 mg/ml for asthmatics. The subsequent single-dose methacholine provocations were performed with doubling dose incremental steps in nonasthmatics (20 and 40 mg/ml) and half-log incremental steps in asthmatics (0.075, 0.25, and 0.75 mg/ml).

**Phase 3: Determination of lung inflation-induced bronchoprotection and comparison of asthmatics to nonasthmatics.** Phase 3 included a total of three challenges (Fig. 1). Challenge 1 was a single dose of methacholine.
the same as the last challenge of phase 2, which identified the single dose of methacholine producing >15% reduction in FEV1. The same dose was also used in challenges 2 and 3, in which the subjects were instructed to take two and five deep inspirations, respectively, immediately before methacholine inhalation. Each deep inspiration consisted of an inhalation to TLC followed by passive exhalation.

**Phase 4: Control experiments. Reproducibility.** To test the reproducibility of the single-dose methacholine challenge model, five asthmatic and five nonasthmatic subjects underwent *challenge 1* (Fig. 1) on three consecutive days. In addition, the same five nonasthmatic subjects underwent *challenge 3* (Fig. 1) on three consecutive days to determine the reproducibility of the bronchoprotective effect of deep inspiration.

**Diluent inhalation challenge.** On another occasion, five asthmatic and five nonasthmatic subjects underwent *challenge 1* (Fig. 1), inhaling diluent instead of methacholine, to determine whether avoidance of deep inspiration alone can induce bronchoconstriction.

**Deep inspirations in the absence of methacholine.** On yet another occasion, all eight asthmatics underwent *challenge 3* (Fig. 1) without inhaling methacholine or saline, to assess whether deep inspirations themselves can induce bronchoconstriction. In addition, five nonasthmatic subjects participated in the same protocol with the intent to examine whether five deep inspirations would alter airway geometry after the 20-min quiet breathing period and whether such an effect would differentiate the two subject groups.

**Effect of the length of the deep inspiration avoidance period.** To examine whether the 20-min deep inspiration avoidance period is the optimal time to obtain bronchoconstriction with single dose of methacholine, five nonasthmatic subjects underwent *challenge 1* (Fig. 1) on three consecutive days after 5, 10, and 20 min of deep inspiration restriction, respectively.

**Aerosol deposition pattern in the presence or absence of deep inspirations.** The aim of this experiment was to rule out the possibility that the distribution of aerosolized methacholine in the lungs is different when inhaled under conditions in which deep inspirations are prohibited than when five deep inspirations precede the aerosol administration. Five nonasthmatic subjects inhaled an aerosol generated from saline admixed with the radioisotope technetium-99m chelated to diethylenetriaminepentaacetic acid (DTPA) on two different visits. In accordance with the previous protocols, aerosol was generated by a DeVilbiss 646 nebulizer attached to a Rosenthal dosimeter through a compressed air tank set at 30 psi. On one visit, subjects followed the protocol of *challenge 1* (Fig. 1), and, on the other visit, they followed the *challenge 3* protocol. During each challenge, they inhaled the radioaerosol instead of methacholine, with five tidal inhalations. The order of these two visits was randomized. After the inhalation procedure, subjects sat with their backs to a large-field-of-view gamma camera (GE Maxicamera 400, St. Albans, Hertfordshire, UK), equipped with an all-purpose, parallel-hole collimator. This posterior lung image was stored on computer (SMV, Twinsburg, OH) and processed in terms of distribution homogeneity within the lungs, according to previously published methodologies (14–16). Deposition fraction was expressed in terms of radioactivity deposited in inner (I) vs. outer (O) zones and apical (A) vs. basal (B) zones of the lungs. Distribution was expressed in terms of the I-to-O and A-to-B ratios. For I/O, it was assumed that the I zone was comprised predominantly of larger, central airways, whereas the O zone was comprised of smaller airways and alveoli. Because these images were a two-dimensional representation of deposition, deposition in some smaller airways and alveoli also appeared in the I zone region of the image. However, because of the lung’s anatomy, it was assumed that the number of smaller airways and alveoli comprising the I region was significantly less than that found in the O region. For this reason, it was assumed that the two zones provided substantially different regional deposition information. Higher I/O indicated enhanced deposition of aerosol in the larger, central airways compared with the lung periphery. Higher A/B indicated enhanced deposition in the lung apex compared with the base. Lung images were also analyzed in terms of distribution homogeneity on a per-pixel-basis. Distribution homogeneity was quantified in terms of skew (an index of distribution symmetry), and higher skew values indicated an increase in distribution heterogeneity.

**Statistical Analysis**

On the basis of previous experience, spirometric pulmonary function outcomes follow normal distributions. Therefore, the pulmonary function data (FEV1 and FVC) obtained in this study were treated with parametric statistics. One-way ANOVA was used to compare 1) baseline lung function within each group for the three single-dose methacholine provocations of *phase 3*, 2) percent change in lung function from baseline within each group for the same single-dose challenges. One-sample *t*-tests were used to evaluate whether the changes in FEV1 or FVC from baseline were different than zero. Unpaired *t*-tests were used to compare the two subject groups in terms of 1) age, 2) baseline FEV1 percent predicted, 3) baseline FVC percent predicted, 4) baseline FEV1/FVC, 5) methacholine-induced changes in lung function at each of the three challenges, and 6) deep inspiration-induced changes in lung function in the absence of methacholine. Paired *t*-test was used to compare baseline FEV1/FVC to the respective predicted values within each subject group. Because of the small number of subjects, nonparametric statistics (Wilcoxon matched pairs, signed ranks test) were used to compare the results of the two procedures involved in the scintigraphic evaluations of lung aerosol deposition. Two-tailed *P* values <0.05 were considered statistically significant.

**RESULTS**

**Phase 1**

The demographic, skin test, and lung function/airway responsiveness data from the screening evaluation for the subjects that fulfilled the inclusion criteria and participated in the subsequent phases of the study are shown in Table 1. The two groups did not differ in terms of age (*P* = 0.42) and FVC percent predicted (*P* = 0.2); however, FEV1 percent predicted and FEV1/FVC were significantly different (*P* = 0.005 in both instances). In addition, the FEV1/FVC was not different from the predicted value in normal subjects but was statistically lower than the predicted value in asthmatics (*P* < 0.005). Three of the nonasthmatic subjects had positive skin tests (no more than 2 positive skin tests each), whereas all the asthmatics had multiple positive skin tests, most of which were clinically relevant to their lower airway disease. One of the three atopic
nonasthmatics had rhinitis symptoms that could be accounted for by a positive skin test; the other two were asymptomatic.

Phase 2

In the absence of deep inspirations, we were able to reduce FEV₁ by >15% in 10 of the 12 nonasthmatic and in all 8 asthmatic subjects using single doses of methacholine. The pulmonary responses of the nonasthmatics to the single doses of methacholine they received, as well as the doses of methacholine at which they experienced >15% reduction in FEV₁, are shown in Fig. 2. One nonasthmatic subject experienced >30% reduction in FEV₁ with 10 mg/ml and another three subjects with 20 mg/ml. Two nonasthmatic subjects experienced a <15% reduction in FEV₁ in 7.2 and 9.2%, respectively) with the highest methacholine concentration used (40 mg/ml) and were not included in the subsequent phases of the study. Asthmatics reached the targeted FEV₁ reduction with smaller single doses of methacholine (0.025–0.75 mg/ml).

Phase 3

The data from this phase of the study are depicted in Fig. 3. Only 9 out of the 10 nonasthmatics who reacted to a single-dose methacholine challenge participated in this phase of the study. During the challenge in which methacholine was given in the absence of deep inspirations, we were able to reduce FEV₁ to approximately the same extent in both groups: the mean ± SE percent reduction in FEV₁ from baseline was 30.4 ± 4.3% in nonasthmatics and 26.3 ± 4.4% in asthmatics, P = 0.5; the mean ± SE percent reduction in FVC was 17 ± 5.2% in nonasthmatics and 8.6 ± 2.9% in asthmatics, P = 0.2. When two and five deep inspirations preceded

Table 1. Demographic characteristics of the volunteers that participated in the experimental protocols

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>Race</th>
<th>Skin Test Reactivity</th>
<th>FEV₁ % Predicted</th>
<th>FVC % Predicted</th>
<th>FEV₁/FVC ×100</th>
<th>PC₂₀, mg/ml</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonasthmatics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>M</td>
<td>Cauc</td>
<td>–</td>
<td>104.6</td>
<td>113.4</td>
<td>80</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>M</td>
<td>Cauc</td>
<td>–</td>
<td>112</td>
<td>110.8</td>
<td>87</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>F</td>
<td>Cauc</td>
<td>–</td>
<td>106.3</td>
<td>114.5</td>
<td>78.9</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>M</td>
<td>Asian</td>
<td>–</td>
<td>94.8</td>
<td>94.1</td>
<td>85.8</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>M</td>
<td>Cauc</td>
<td>–</td>
<td>107.4</td>
<td>114.7</td>
<td>79</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>F</td>
<td>Cauc</td>
<td>+</td>
<td>116.6</td>
<td>117.3</td>
<td>83.7</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>F</td>
<td>Asian</td>
<td>–</td>
<td>78.5</td>
<td>82</td>
<td>77.3</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>F</td>
<td>Hyp</td>
<td>–</td>
<td>103.4</td>
<td>98.3</td>
<td>90</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>F</td>
<td>Cauc</td>
<td>+</td>
<td>112.7</td>
<td>122.7</td>
<td>75.9</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>F</td>
<td>Cauc</td>
<td>+</td>
<td>97.9</td>
<td>103.2</td>
<td>81.4</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>F</td>
<td>Cauc</td>
<td>–</td>
<td>101.3</td>
<td>102.9</td>
<td>85</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>31</td>
<td>M</td>
<td>Cauc</td>
<td>–</td>
<td>107.1</td>
<td>110.8</td>
<td>82.4</td>
<td>&gt;75</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>33.5 ± 8.5</td>
<td>103.6 ± 10.0</td>
<td>107.1 ± 11.4</td>
<td>82.2 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Asthmatics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>F</td>
<td>Cauc</td>
<td>+</td>
<td>94.9</td>
<td>93.2</td>
<td>88.2</td>
<td>&lt;1</td>
<td>prn Albuterol</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>M</td>
<td>Cauc</td>
<td>+</td>
<td>84.8</td>
<td>111</td>
<td>64.6</td>
<td>&lt;1</td>
<td>prn Albuterol</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>M</td>
<td>Cauc</td>
<td>+</td>
<td>70.7</td>
<td>93.2</td>
<td>61.8</td>
<td>&lt;1</td>
<td>prn Albuterol</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>F</td>
<td>Cauc</td>
<td>+</td>
<td>83.5</td>
<td>103.5</td>
<td>68.9</td>
<td>&lt;1</td>
<td>prn Albuterol</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>M</td>
<td>Cauc</td>
<td>+</td>
<td>86.6</td>
<td>109.6</td>
<td>68.2</td>
<td>&lt;1</td>
<td>prn Albuterol</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>M</td>
<td>AfrAm</td>
<td>+</td>
<td>79.8</td>
<td>89.4</td>
<td>77.8</td>
<td>&lt;1</td>
<td>prn Albuterol</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>M</td>
<td>Cauc</td>
<td>+</td>
<td>86.5</td>
<td>106.6</td>
<td>70.4</td>
<td>&lt;1</td>
<td>Salmeterol reg</td>
</tr>
<tr>
<td>8</td>
<td>31</td>
<td>F</td>
<td>Cauc</td>
<td>+</td>
<td>96.4</td>
<td>100.5</td>
<td>81.7</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30.3 ± 9.1</td>
<td>85.4 ± 8.2</td>
<td>100.9 ± 8.2</td>
<td>72.7 ± 9.0</td>
<td></td>
</tr>
</tbody>
</table>

FEV₁, forced expired volume in 1 s; FVC, forced vital capacity; M, male; F, female; Cauc, Caucasian; Hisp, Hispanic; AfrAm, African-American; PC₂₀, concentration of inhaled methacholine causing 20% reduction in FEV₁ from the post diluent inhalation value under routine conditions; prn, as needed; reg, regular. ANOVA comparisons between groups: age, P = 0.42; FEV₁, P = 0.005; FVC, P = 0.20; FEV₁/FVC, P = 0.005.
the methacholine administration, the effect on lung function was different in the two groups: deep inspirations before the inhalation of methacholine had a striking protective effect in the nonasthmatic subjects (ANOVA between the challenges for FEV1, \( P < 0.0001 \); for FVC, \( P = 0.0008 \)) but had no effect in asthmatics (ANOVA between the challenges for FEV1, \( P = 0.38 \); for FVC, \( P = 0.32 \)). With two deep inspirations, the mean \( \pm SE \) percent reduction in FEV1 from baseline was 11.4 \( \pm 2.2 \) and 36.6 \( \pm 3.8 \%) in nonasthmatics and asthmatics, respectively, \( P < 0.0001 \); the mean \( \pm SE \) percent reduction in FVC was -2.3 \( \pm 3.8 \) and 16.8 \( \pm 3.1 \%) in nonasthmatics and asthmatics, respectively, \( P = 0.002 \). With 5 deep inspirations prior to the methacholine inhalation, the mean \( \pm SEM \% \) reduction in FEV1 was 4.6 \( \pm 0.9 \) in nonasthmatics and 34.9 \( \pm 7.6 \%) in asthmatics, \( P < 0.0001 \); the mean \( \pm SE \) percent reduction in FVC was -8 \( \pm 3.2 \%) in nonasthmatics and 17.2 \( \pm 6.4 \%) in asthmatics, \( P = 0.002 \). It is of interest that, in the nonasthmatics, the FVC was higher than baseline when deep inspirations preceded the methacholine administration; the improvement in FVC over baseline was statistically significant with five deep inspirations before methacholine inhalation. Within each group, baseline lung function did not differ between the challenges (ANOVA for nonasthmatics: \( P = 0.2 \); for asthmatics, \( P = 0.29 \)).

**Phase 4**

**Reproducibility.** In the nonasthmatics, during the three trials in which no deep inspirations were allowed before inhalation of methacholine, the standard deviation (SD) of the reduction in lung function represented, in all cases, \(<30\%\) of the average reduction [average coefficient of variation (CV) = 25\%]. Between-subject variability was low. In the trials in which five deep inspirations preceded the administration of methacholine, the average CV was higher (78\%). This was not surprising as the mean reduction in FEV1 was low because of bronchoprotection. In asthmatics, the average CV of methacholine-induced reduction in FEV1 was 33\%. Although this was somewhat higher than in the nonasthmatics, the difference in the CVs between the groups was not statistically significant (\( P = 0.56 \)).

**Diluent inhalation challenge.** No reduction in spirometric values was obtained with saline inhalation, in the absence of deep inspirations, in nonasthmatics or asthmatics. Mean FEV1 values at baseline and after the inhalation of the diluent were 3.70 \( \pm 0.33 \) and 3.68 \( \pm 0.34 \) for nonasthmatics and 3.22 \( \pm 0.60 \) and 3.24 \( \pm 0.70 \) for asthmatics, respectively. These data indicate that merely breathing at low lung volume does not induce bronchoconstriction.

**Deep inspirations in the absence of methacholine.** In asthmatics, five deep inspirations alone caused no reduction in FEV1 or FVC. If anything, a small improvement from baseline in both FEV1 and FVC was noted (Table 2). This trend was also observed in the group of healthy subjects, but it was statistically significant only in the asthmatic group and only for FVC (\( P = 0.04 \)). Nevertheless, no significant differences were found between the two groups in the deep inspiration-induced spirometric changes from baseline. Midmaximal expiratory flow (MMEF) did not follow the improvement trends observed with FEV1 and FVC. In fact, the values of this outcome appeared to decline, after the five deep inspirations, to an equal degree in both asthmatics and nonasthmatics; however, the changes in MMEF were not statistically significant in either subject group (\( P = 0.13 \) and \( P = 0.35 \) in asthmatics and nonasthmatics, respectively).

**Effect of the length of the deep inspiration avoidance period.** Having established the concentration of methacholine at which a >15\% reduction in FEV1 was developed, after five of the nonasthmatic subjects abstained from deep breaths for 20 min, we examined whether...
the same effect would still be obtained if the deep inspiration avoidance period was diminished to 10 and 5 min. The results of this experiment are presented in Fig. 4. Two subjects showed similar reductions (≥15%) in FEV\textsubscript{1} on all three challenges. In the remaining three subjects, only the 20-min deep breath prohibition period resulted in the anticipated reduction in lung function. These data are in support of those of Moore et al. (19), who suggested that time away from TLC is important to the bronchospastic effect of methacholine in healthy subjects and also indicate that the 20-min deep breath avoidance period allows the induction of bronchospasm in most healthy subjects under this simple, single-dose bronchoprovocation protocol. However, we did not test whether longer periods would have a more pronounced effect.

**Methacholine aerosol deposition pattern in the presence or absence of deep inspirations.** The data from this protocol are presented in Table 3 and show that no significant differences in regional aerosol distribution (I/O, P = 0.49; A/B, P = 0.52), distribution homogeneity (skew, P = 0.2), or deposition fraction (P = 0.2) were found between the radioaerosol inhalations that were preceded by deep inspirations and those that were not.

**DISCUSSION**

In 1995, Skloot et al. (25) demonstrated that, in the absence of deep inspirations, methacholine inhalation by nonasthmatic individuals led to severe bronchoconstriction at concentrations that were conventionally regarded as within the asthmatic range (<8 mg/ml). In that study, they found that the airway responsiveness of nonasthmatic subjects, when assessed by partial (from end-tidal inspiratory to RV) forced expiratory maneuvers, was not much different from that of asthmatics. Those observations indicated that lung inflation (through deep inspiration) had a potent beneficial effect on human airways and that this effect was markedly diminished or absent in asthma. Skloot et al. (25) proposed that lung inflation induced bronchodilation and speculated that, in asthma, the phenomenon of airway hyperresponsiveness to a direct spasmogen, such as methacholine, was merely a manifestation of the lack of the bronchodilatory ability of deep breaths. On the basis of the striking effect of deep inspirations on airways reponsiveness, it was expected that, after the induction of severe bronchoconstriction in the absence of deep inspirations, a sequence of deep breaths would rapidly return healthy airways to their baseline, nonconstricted state. The findings of that study disproved this hypothesis. Although the first deep breath taken after the end of the methacholine provocation produced a stronger bronchodilator response in the nonasthmatics, the overall ability of three deep inspirations to induce bronchodilation in this setting was not different between asthmatic and nonasthmatic subjects (25). Given that other investigators have documented the bronchodilatory ability of deep inspiration in experimental designs in which a full spirometric maneuver follows a partial maneuver (26), the explanation Skloot and colleagues offered for their finding was that the potency of this bronchodilatory effect was significantly diminished in the face of preestablished significant smooth muscle contraction (25). This explanation, however, also raised some questions as to whether the striking difference between asthmatic and nonasthmatic subject, with respect to the beneficial effect of deep inspiration, could be solely attributed to a bronchodilator effect, which appeared quite susceptible to increased smooth muscle tone. This uncertainty led to the generation of another hypothesis, that deep inspirations not only have a bronchodilatory but also, most importantly, a bronchoprotective, effect on the airways.

The significance of demonstrating that deep inspiration acts as a bronchoprotector lies in the fact that it can lead to new testable hypotheses regarding the

**Table 2. Effect of 5 deep inspirations on spirometric outcomes after a 20-min quiet breathing period, in healthy controls and asthmatic subjects**

<table>
<thead>
<tr>
<th></th>
<th>FEV\textsubscript{1} Baseline, liters</th>
<th>FEV\textsubscript{1} Post 5 DIs, liters</th>
<th>FEV\textsubscript{1} %change</th>
<th>FVC Baseline, liters</th>
<th>FVC Post 5 DIs, liters</th>
<th>FVC %change</th>
<th>MMEF Baseline, l/s</th>
<th>MMEF Post 5 DIs, l/s</th>
<th>MMEF %change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthmatics</td>
<td>8 3.22 ± 0.14</td>
<td>3.36 ± 0.11</td>
<td>4.91 ± 2.69</td>
<td>4.68 ± 0.32</td>
<td>5.07 ± 0.28*</td>
<td>9.72 ± 4.32</td>
<td>2.27 ± 0.16</td>
<td>2.08 ± 0.16</td>
<td>6.27 ± 8.57</td>
</tr>
<tr>
<td>Nonasthmatics</td>
<td>5 3.32 ± 0.47</td>
<td>3.77 ± 0.49</td>
<td>1.33 ± 0.99</td>
<td>4.08 ± 0.55</td>
<td>4.20 ± 0.58</td>
<td>2.93 ± 1.43</td>
<td>3.36 ± 0.61</td>
<td>3.23 ± 0.62</td>
<td>4.80 ± 2.13</td>
</tr>
</tbody>
</table>

Values are means ± SE. Baseline measurements were performed before the beginning of the 20-min quiet breathing [avoidance of deep inspirations (DIs)] period. For the columns with %changes in spirometric outcomes, a positive number indicates a decline, whereas a negative number indicates an improvement over baseline. MMEF, *In asthmatics, 5 DIs induced a statistically significant improvement over baseline (P = 0.04).
mechanism through which lung inflation operates. It also allows us to speculate that stretching of the airway, which should occur as a result of lung inflation, activates a biochemical or biomechanical process that changes the resting state of the airway smooth muscle or operates in functional antagonism against bronchoconstrictive stimuli. If deep inspiration acted as a bronchodilator alone, it would have been difficult to support such a hypothesis; instead, we would have most probably attributed the bronchodilation to mechanical breaking of actinomyosin bridges in airway smooth muscle. The second major finding of this investigation is related to the magnitude of the bronchoprotective effect of deep inspiration in healthy airways. Bronchoprotection of this level rivals the best pharmacological agents currently available for the prevention of bronchospasm. From this perspective, understanding of the mechanism of lung inflation-induced bronchoprotection has the potential of opening new pathways in the development of more potent therapies for this disease.

To determine whether deep breaths confer bronchoprotection, we developed and tested the validity and reproducibility of a single-dose methacholine provocation protocol, a modification of the protocol previously employed by Malmberg and colleagues (17). Using this protocol, we confirmed the work of these investigators and demonstrated that, after abstaining from deep inspirations for 20 min, nonasthmatic subjects can develop remarkable airway obstruction, some with even a relatively low single dose of methacholine.

With the use of this model, our data fully supported our hypotheses that deep inspirations before the administration of methacholine had a striking protective effect in nonasthmatic subjects and the magnitude of this effect was a function of the number of deep inspirations preceding the methacholine administration. In contrast, the response of asthmatics to methacholine activates a biochemical or biomechanical process that changes the resting state of the airway smooth muscle and demonstrated that, after abstaining from deep inspirations, according to our present findings, conformed protection to the airways of the nonasthmatics and did not allow for bronchoconstriction to develop.

In view of previous work (25), it may appear somewhat peculiar that we chose to perform maximal spirometric maneuvers, as opposed to partial ones, to measure the outcome of modified, single-dose methacholine provocations. One can argue that our approach...
in the present study risked a distorted result because deep inspiration is inherent in the maneuver that is supposed to evaluate the effect of a spasmogen in the absence of deep inspirations. Such a risk would be expected to be greater in the nonasthmatic group, in which the single deep breath, taken during spirometry, could possibly partially reverse the spasmogen-induced obstruction (25). Our decision to employ maximal maneuvers was based on two observations. First, the reliability and reproducibility of maximal maneuvers is higher than that of partial ones. Second, the preliminary work of Skloot et al. (25) showed that, even by incorporating the maximal maneuver, it was still possible to demonstrate significant reductions in FEV\textsubscript{1} in nonasthmatic subjects. In fact, if we consider the cumulative dose of methacholine delivered from the nebulizer during a bronchoprovocation with stepwise, half-log increasing concentrations, such as the one used in the study by Skloot et al. (25), the single concentration of 20 mg/ml of methacholine [at which most of our volunteers reacted in the current study with 15–53% reductions of FEV\textsubscript{1} (Fig. 2)] is equivalent to an ~10 mg/ml top concentration of the stepwise protocol. In their study, Skloot et al. (25) reported a mean reduction in FEV\textsubscript{1} of 36%, with an average top concentration of methacholine of 10.8 mg/ml. Although it did not prevent us from addressing our hypothesis, the incorporation of the full (deep) breath spirometric maneuver in the present study increased the difference between nonasthmatic and asthmatic subjects, with respect to the single dose of methacholine at which the targeted 15% reduction in FEV\textsubscript{1} from baseline was achieved. The single, deep breath produced bronchodilation in the nonasthmatic subjects but not in the asthmatics, in whom the bronchodilatory ability of deep inspiration is probably low (25). We believe that this is why the two groups were quite different in terms of methacholine responsiveness in the present protocol, whereas their responsiveness in the study of Skloot et al. (25) was close.

The various control experiments incorporated in this body of work validate our methodology and rule out certain potential explanations for the observed bronchoprotection in the healthy state. 1) The reproducibility of both the reduction in lung function induced by the single dose of methacholine and of the bronchoprotective effect of deep inspiration in nonasthmatics was good. The average intra-individual CV of the bronchoconstrictive effect of the single-dose methacholine challenge (25 and 33% in healthy and asthmatic subjects, respectively) was better than the average intra-individual CV of the log PD\textsubscript{20} obtained by routine, incremental provocation with methacholine, which is ~50%. 2) By performing a saline inhalation challenge in both asthmatics and nonasthmatics, we showed that a 20-min prohibition of deep inspirations alone did not induce bronchoconstriction. 3) We also showed that, in the mild asthmatics of our study, five deep breaths alone do not induce bronchoconstriction; therefore, the bronchoconstriction we observed in asthmatics, in the presence of deep inspirations, was not influenced by the deep inspirations themselves. On the contrary, both asthmatics and nonasthmatics showed some improvement in FEV\textsubscript{1} and in FVC when five deep inspirations were taken after the 20-min quiet breathing period. This improvement was only significant in the asthmatics and only in the case of FVC (Table 2). Because bronchoprotection by deep inspiration was only found in the nonasthmatics, the above findings indicate that the bronchoprotective effect is not related to any change in airway geometry induced by deep inspiration. 4) The bronchoprotective effect of deep inspiration in healthy individuals was so impressive, that we questioned whether it could involve an artifact related to the administration of the aerosol. For this reason, we designed the radiolabeled aerosol study to examine whether the five deep breaths that preceded the inhalation of methacholine could result in substantial changes in airway deposition pattern. The data we collected failed to detect any change in aerosol distribution induced by the preceding deep breaths. Therefore, it is highly unlikely that the bronchoprotective effect of lung inflation is due to differential distribution of aerosolized methacholine in the lungs.

An interesting observation was the increase over baseline in FVC that occurred when the nonasthmatic subjects performed two or five deep inspirations before administration of methacholine. As this was a serendipitous finding, it is difficult to interpret. If we were to attempt an interpretation, however, it would be important to note that similar increases in FVC (and not in FEV\textsubscript{1} or MMEF) were observed in the asthmatics, as well as in nonasthmatics, when five deep inspiration maneuvers were performed in the absence of methacholine (phase 4, control experiment C; Table 2). In the absence of deep inspirations, some air spaces may become atelectatic. The deep breaths taken after the 20-min quiet breathing period may recruit these spaces, which now become filled with air. In the absence of spasmogen, the air that was recruited into these spaces is expirable as part of the FVC. Because the same deep breaths in healthy subjects prevent any methacholine-induced airway constriction from taking place, the air that was recruited in the previously atelectatic spaces continues to be expirable in the presence of spasmogen, and the FVC continues to be increased compared with baseline. However, in asthmatics, methacholine induces bronchospasm, resulting in early airway closure, air trapping, and reduction in FVC.

Two major hypotheses can be raised regarding the mechanism by which lung inflation confers protection on the nonasthmatic lung from a spasmogenic stimulus. The first hypothesis is that this phenomenon results from a direct effect of stretch on airway smooth muscle. Airways are capable of complete closure at very high transmural pressures in vitro (12, 20). Gunst et al. (13) found that maximal, and even submaximal, doses of methacholine could produce airway closure in bronchi at constant transmural pressures in excess of 25 cmH\textsubscript{2}O. The closure was completely prevented and the response to methacholine was markedly reduced if
the bronchi were subjected to volume oscillations during exposure to methacholine. Thus the in vitro work suggests that volume oscillations during exposure to methacholine can prevent closure, but, once closure occurs, large forces must be generated to overcome the closure. These in vitro observations may provide an explanation for deep inspiration in nonasthmatic subjects markedly attenuating the effect of inhaled methacholine. If healthy subjects do not invoke this method of attenuation, as in the case of the 20-min deep breath prohibition period in our protocol, more constriction takes place and persists, even after deep inspirations. Gunst et al. (13) proposed that depression of contractility during length oscillations is a function of the plasticity of the organization of contractile filaments within airway smooth muscle cells, which allows contractile element length to be reset in relation to smooth muscle cell length as a result of changes in the length to which the muscle is stretched (24). Fredberg and colleagues (9, 10) explain the depression of contractility during length oscillations by a different mechanism. During the initial activation of the smooth muscle, there is a period of rapid cross-bridge cycling and rapid velocity of shortening. With prolonged stimulation, there is slower cycling of cross bridges, and the nature of the cross bridges changes to the latch or frozen state. Also, absence of stretch allows the system to move closer to the frozen state. By taking deep breaths before the administration of methacholine, nonasthmatic subjects place their airway smooth muscle in a condition that is most resistant to the development of latch bridges. It is important to point out that several differences exist between the experimental settings of the in vitro studies performed by Gunst et al. (13) and Fredberg et al. (9, 10) and the in vivo settings of our work. These investigators examined airway smooth muscle after it was exposed to methacholine and investigated the effects of tidal oscillations on methacholine responsiveness. In our experiments, the deep inspirations induce presumably much stronger smooth muscle stretch, and they precede the administration of latch bridges.

The second hypothesis is that airway stretch activates a process that antagonizes a bronchoconstrictive stimulus in a functional way. For example, airway stretch may activate neural pathways that could lead to inhibition of cholinergic tonic activity (18), NANC bronchodilation (6, 7), or even release of a bronchodilator such as nitric oxide from nonneural sources (1).

Whatever the bronchoprotective effect of deep inspiration is in nonasthmatic subjects, the question remains as to why it is absent or markedly attenuated in asthma. As long as elastic recoil is normal, TLC is not altered in asthma, indicating that asthmatics do not have reduced ability to inflate their lungs. Therefore, in asthma, the function of lung inflation may theoretically become defective at three levels: 1) lung inflation may not be capable of stretching the airway; 2) airway stretch may take place, but its effect on airway smooth muscle is blunted because some elements of airway inflammation defunctionalize this effect; and 3) an intrinsic abnormality of the asthmatic smooth muscle prevents airway stretch from functioning in a bronchoprotective manner. In studies using high-resolution computerized tomography to measure airway area at FRC and TLC in the absence or presence of a bronchoconstrictive stimulus, Brown et al. (2) reported that nonasthmatic subjects do not differ from mild asthmatics in their ability to increase airway area with lung inflation. These observations indicate that the second or third possibilities offer more plausible explanations for the lack of a beneficial effect of deep inspiration in asthma.

In conclusion, we report the presence of a previously suggested, but uncharacterized, physiologic respiratory function, that of bronchoprotection against smooth muscle spasmogens, which is conferred by deep breaths. This function is dose dependent and has remarkable potency. The bronchoprotective effect of deep inspiration is absent in asthma and may be a pivotal pathophysiologic abnormality in this disease. Investigation of the cause of this asthma dysfunction may offer the long-awaited link between airway inflammation and airway lability in asthma.

This work was supported by National Institutes of Health Grant RO1-HL-61277.

T. Kapsali is the recipient of the George Behrakis Hellenic Fellowship in Respiratory Allergy at the Johns Hopkins Asthma and Allergy Center and of the American Academy of Allergy, Asthma and Clinical Immunology Zeneca Asthma Research Award.

REFERENCES


