Deep breaths, methacholine, and airway narrowing in healthy and mild asthmatic subjects

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RECENT STUDIES HAVE SHOWN that deep breaths taken before inhalation of methacholine attenuate the decrease in forced expiratory volume in 1 s and forced vital capacity in healthy but not in asthmatic subjects. We investigated whether this difference also exists by using measurements not preceded by full inflation, i.e., airway conductance, functional residual capacity, as well as flow and residual volume from partial forced expiration. We found that five deep breaths preceding a single dose of methacholine transiently attenuated the decrements in forced expiratory volume in 1 s and forced vital capacity in healthy (n = 8) but not in mild asthmatic (n = 10) subjects and increased the areas under the curve of changes in parameters not preceded by a full inflation over 40 min, during which further deep breaths were prohibited, without significant difference between healthy (n = 6) and mild asthmatic (n = 16) subjects. In conclusion, a series of deep breaths preceding methacholine inhalation significantly enhances bronchoconstrictor response similarly in mild asthmatic and healthy subjects but facilitates bronchodilatation on further full inflation in the latter.

METHODS

Subjects

A total of 11 healthy and 26 asthmatic subjects (Table 1) participated in the study after giving informed consent, as siently increases airway caliber, and the magnitude of this increase depends on the relative magnitude of the distending force of lung parenchyma and the constrictor force of airway smooth muscle (17). Therefore, any parameter derived from a full forced expiratory maneuver, including FEV1 and FVC, will depend on both airway smooth muscle shortening capacity and airway wall response to a deep breath.

It is reasonable to postulate that, if the lack of a bronchoprotective effect by multiple deep breaths taken before inhaling a constrictor agent in asthma is due to the inability to reduce the shortening capacity of airway smooth muscle, then the different effects of the deep breaths on airway response to MCh between asthmatic and normal subjects should be equally detected by measurements preceded or not preceded by a full lung inflation. By contrast, a bronchoprotective effect of deep breaths visible only with FEV1 and FVC would suggest a difference in distensibility of the airways in response to a single deep breath between normal and asthmatic subjects rather than in the shortening capacity of airway smooth muscle.

The present paper reports on two sequential studies aimed at addressing this point. In a first study, the effects of a single dose of inhaled MCh, preceded or not by a series of deep breaths, were compared at 1 and 10 min in healthy and mild asthmatic subjects by using measurements of airway caliber either requiring or not requiring full lung inflation. Although a bronchoprotective effect by deep breaths was observed with FEV1 and FVC in the healthy subjects, parameters not preceded by full inflation tended to change more with than without the deep breaths. To better investigate this effect, a second study was conducted by using a protocol without measurements requiring full lung inflation and extending the observation time to 40 min, during which deep breaths were carefully avoided.

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Table 1. Subjects’ demographic and physiological characteristics

<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th>Study 2</th>
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<tbody>
<tr>
<td>Healthy subjects</td>
<td>5/3</td>
<td>2/4</td>
</tr>
<tr>
<td>Asthmatic subjects</td>
<td>3/7</td>
<td>5/11</td>
</tr>
<tr>
<td>Age, yr</td>
<td>30 ± 5</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>FEV1, %predicted</td>
<td>96 ± 9</td>
<td>100 ± 8</td>
</tr>
<tr>
<td>FVC, %predicted</td>
<td>93 ± 11</td>
<td>105 ± 10</td>
</tr>
<tr>
<td>PD20FEV1, log µg</td>
<td>3.12 ± 0.15</td>
<td>1.71 ± 0.42</td>
</tr>
<tr>
<td>PD35sGaw, log µg</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SD. Three healthy subjects participated in both studies. FEV1, 1-s forced expiratory volume; FVC, forced vital capacity; sGaw, specific airway conductance; PD20FEV1, dose of methacholine causing a 20% decrease of FEV1 from control; PD35sGaw, dose of methacholine causing a 35% decrease of sGaw from control; ND, not determined; f/m, female/male. *P < 0.05 vs. healthy subjects.

Approved by the local ethics committee. Asthmatic subjects had mild intermittent disease (16). At the time of the study, they were in stable clinical conditions, with a FEV1 of 70% of predicted (19) without taking inhaled or oral steroids, cromolyn, antihistamine, or regular bronchodilators. Subjects using short-acting β2-agonists on demand were requested to avoid using them for 12 h before studies. Before entering the study, each asthmatic subject was tested for the effects of the deep breaths on airway caliber. To this purpose, specific airway conductance (sGaw) was measured (see below) before and again 2, 4, and 6 min after five deep breaths (i.e., from tidal breathing level to full inflation) taken within 30 s through the mouth without breath holding. Five asthmatic patients with a reduction of sGaw of >15% of the value recorded before the deep breaths were not included in the studies.

Lung Function Measurements

Spirometry and flow-volume curves were obtained by using a mass flowmeter (SensorMedics, Yorba Linda, CA) and numerical integration of the flow signal. Airway resistance was measured by whole body plethysmography (Vmax 6200 Autobox, SensorMedics) while the subject was panting slightly >1.7 Hz. Immediately after each airway resistance measurement, thoracic gas volume (TGV) was measured by panting against a closed shutter at a frequency slightly <1 Hz, and sGaw was calculated as 1/(TGV × airway resistance). Functional residual capacity (FRC) was corrected for the difference between TGV and the end-expiratory volume of the four to six preceding tidal breaths. To obtain partial flow-volume curves superimposed at a constant absolute lung volume, TGV was first measured, and then a complete forced expiration initiated from end-tidal inspiration was performed soon after the reopening of the shutter. In each subject, partial forced expiratory flow (Vpart) was always measured at the same absolute lung volume (between 30 and 40% of control FVC), depending on the change in residual volume after partial expiration (RVpart).

Bronchial Challenges

Solutions of MCh were prepared by adding distilled water to dry powder MCh chloride (Laboratorio Farmaceutico Lo farma, Milan, Italy). Aerosols were delivered by a SM-1 Rosenthal breath-activated dosimeter (SensorMedics) driven by compressed air (30 lb./in.²) with 1-s actuations. Aerosol output at the mouth was 10 µl per actuation. Aerosols were inhaled during quiet tidal breathing in a sitting position. On screening day, the doses of MCh causing a reduction of sGaw by 35% (PD35sGaw) or FEV1 by 20% (PD20FEV1) were determined by standard incremental challenges. After 20 tidal inhalations of saline as a control, subjects inhaled double-increasing doses of MCh from 0.01 mg until a decrement of sGaw of ≥35% or of FEV1 of ≥20% was recorded 2 min after dosing. Dose increments were obtained by using three MCh concentrations (1, 10, and 50 mg/ml) with appropriate numbers of breaths (from 1 to 16). PD35sGaw and PD20FEV1 were calculated by interpolating between two adjacent points of log dose-response curves.

Experimental Procedures

Study 1. On 2 separate study days and in a random order, 10 asthmatic and 8 healthy subjects (Table 1) inhaled a single dose of MCh equal to twice the last dose inhaled in the standard incremental challenge to obtain the PD20FEV1, not preceded (day 1) or preceded (day 2) by five deep breaths (Fig. 1). The inhalation time lasted ~1 min. sGaw, FRC, Vpart, RVpart, FEV1, and FVC were measured in triplicate before a 10-min prohibition of deep breaths, whereas single measurements of sGaw, FRC, Vpart, and RVpart were taken again immediately before MCh inhalation. All measurements were then taken once at 1 and 10 min after MCh inhalation. Means of the triplicate measurement of sGaw, Vpart, RVpart, and FRC and the best FEV1-FVC combination were used as baseline values. In all circumstances, the full expiratory maneuver to measure FEV1 and FVC was preceded by measurement of sGaw, FRC, and partial expiratory maneuvers. Spontaneous deep breaths or sighs were strictly prohibited from 10 min before to 10 min after MCh inhalation.

Study 2. On 2 separate study days and in a random order, 16 asthmatic and 6 healthy subjects (Table 1) inhaled for ~1 min a single dose of MCh, equal to twice the last dose used in the standard incremental challenge to obtain the PD35sGaw, not preceded (day 1) or preceded (day 2) by five deep breaths (Fig. 1). PD35sGaw was used because PD20FEV1 in study 1 caused very large changes of all parameters not preceded by deep breath, which might have minimized or masked differences between conditions. sGaw, FRC, Vpart, RVpart, FEV1, and FVC were measured in triplicate before a 10-min prohibition of deep breaths, whereas single measurements of sGaw, FRC, Vpart, and RVpart were taken again immediately before MCh inhalation. Means of the triplicate measurement of sGaw, Vpart, RVpart, and FRC were taken as baseline values. Measurements of sGaw and FRC (and also of Vpart and RVpart in 8 asthmatic subjects) were taken every minute for the first 10 min and then at 15, 20, and 40 min after MCh inhalation. Spontaneous deep breaths or sighs were strictly prohibited during the entire test.

Statistical Analysis

A mixed between-within groups ANOVA with Duncan’s post hoc comparisons was used for both studies. For study 1, the dependent variables were the percent changes of FEV1, FVC, FRC, Vpart, RVpart, and sGaw, whereas groups, study days, and observation times were the independent factors. For study 2, the dependent variables were the areas under the curves (AUC) of percent changes in Vpart and sGaw or the absolute changes in FRC and RVpart vs. time, whereas groups and study days were the independent factors. Values of P < 0.05 were considered statistically significant. Data are presented as means ± SD.
RESULTS

Study 1

There were no significant differences in baseline lung function between study days (Table 2). Furthermore, there were no significant differences in FRC, V̇part, RV-part, and sGaw before and after the 10-min deep-breath prohibition (P > 0.1 for all comparisons).

In the healthy subjects (Fig. 2A), the decrements of FEV₁ and FVC induced by MCh inhalation on the day without deep breaths were 37 ± 14 and 28 ± 15% after 1 min, respectively, and 37 ± 13 and 28 ± 14% after 10 min, respectively. On the day with five deep breaths, the decreases of both FEV₁ (27 ± 13%) and FVC (13 ± 9%) were significantly less (P < 0.05 and P < 0.01).

Table 2. Baseline data of study 1

<table>
<thead>
<tr>
<th></th>
<th>Healthy Subjects</th>
<th>Asthmatic Subjects</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>FEV₁, liter</td>
<td>3.42 ± 0.73</td>
<td>3.38 ± 0.65</td>
</tr>
<tr>
<td>FVC, liter</td>
<td>3.95 ± 0.92</td>
<td>3.98 ± 0.93</td>
</tr>
<tr>
<td>FRC, liter</td>
<td>3.01 ± 0.57</td>
<td>3.06 ± 0.72</td>
</tr>
<tr>
<td>V̇part, liter</td>
<td>1.73 ± 0.52</td>
<td>1.66 ± 0.56</td>
</tr>
<tr>
<td>sGaw, cmH₂O⁻¹·s⁻¹</td>
<td>0.24 ± 0.04</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>RV-part, liter</td>
<td>2.98 ± 0.96</td>
<td>3.00 ± 0.92</td>
</tr>
</tbody>
</table>

Values are means ± SD. Days 1 and 2 are the days when methacholine was not preceded or was preceded by 5 deep breaths, respectively. FRC, functional residual capacity; RV-part, residual volume of the partial forced expiration; V̇part, forced expiratory flow from partial flow-volume loop. None of the differences between days was statistically significant (P > 0.1 for all comparisons).
respectively) after 1 min, but not after 10 min (35 ± 17% for FEV₁ and 28 ± 14% for FVC), than on the day without deep breaths. By contrast, the increments in RVpart and FRC and the decrements of sGaw and V part were similar with or without the deep breaths at either 1 or 10 min after MCh (P > 0.4 for all comparisons).

In the asthmatic subjects (Fig. 2B), there were no significant differences in decrements of FEV₁, FVC, sGaw, and V part, or in increments in RVpart and FRC between days with and without deep breaths, either after 1 or 10 min (P > 0.1 for all comparisons).

Study 2

Mean baseline sGaw and FRC were not significantly different between study days either in asthmatic or healthy subjects (Table 3). Furthermore, there were no significant differences in FRC, sGaw, V part, and RVpart before and after the 10-min deep-breath prohibition (P > 0.1 for all comparisons).

The decrease of sGaw over time (Fig. 3) was greater on the day with than on the day without deep breaths, both in asthmatic (AUC: 1,970 ± 651 vs. 1,395 ± 612) and healthy (AUC: 1,485 ± 475 vs. 1,053 ± 403) subjects. Although the difference across all days achieved statistical significance (P < 0.01) only in the asthmatic group, there was no significant interaction between study days and groups (P = 0.6), suggesting that the effect of the deep breaths was not significantly

### Table 3. Baseline data of study 2

<table>
<thead>
<tr>
<th></th>
<th>Healthy Subjects</th>
<th>Asthmatic Subjects</th>
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</thead>
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<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>FRC, liter</td>
<td>2.85 ± 0.70</td>
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<td></td>
<td>3.03 ± 0.78</td>
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<tr>
<td>RVpart, liter</td>
<td>ND</td>
<td>1.87 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>1.93 ± 0.39</td>
</tr>
<tr>
<td>sGaw, cm H₂O⁻¹·s⁻¹</td>
<td>0.26 ± 0.07</td>
<td>0.20 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>0.27 ± 0.08</td>
<td>0.21 ± 0.08</td>
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<tr>
<td>V part, l/s</td>
<td>ND</td>
<td>3.02 ± 1.49</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>2.93 ± 1.59</td>
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Values are means ± SD. None of the differences between days was statistically significant (P > 0.1 for all comparisons).
different in the two groups. Similarly, the increase of FRC (Fig. 4) over time was greater on the day with than on the day without deep breaths in asthmatic subjects (AUC: 13.89 ± 11.03 vs. 5.34 ± 7.07, P < 0.01) and at several times in the healthy group (AUC: 5.34 ± 3.83 vs. 2.03 ± 5.70, P = 0.05). As for sGaw, there was no significant interaction between study days and groups (P > 0.2).

In the subgroup of asthmatic subjects in whom V̇part and RVpart were also measured, the percent decrease of V̇part (Fig. 5A) and the absolute increase in RVpart (Fig. 5B) over time were significantly greater on the day with than on the day without deep breaths (V̇part: AUC, 2,526 ± 648 vs. 1,939 ± 570, P < 0.05; RVpart: AUC, 26.57 ± 16.3 vs. 7.88 ± 3.20, P < 0.05).

DISCUSSION

The results of the present study can be summarized as follows. First, the bronchoprotective effect of deep breaths taken before a single dose of MCh was only visible in healthy subjects by using parameters preceded by full inflation (FEV1 and FVC). Second, prior deep breaths enhanced the bronchoconstrictor response to MCh in both healthy and asthmatic subjects, as inferred from changes in parameters not requiring full lung inflation.

Two recent studies (10, 21) reported that a series of five deep breaths taken before the inhalation of a single dose of MCh dramatically and exclusively protected healthy humans from airway narrowing. The conclusion was based on the fact that the decrements of FEV1 and FVC after a bolus of MCh were significantly less when the constrictor agent was preceded by deep breaths than when it was not. The results of the present study confirm that prior deep breaths significantly blunt the decrease of FEV1 and FVC measured 1 min after MCh in healthy subjects, and this protec-
The bronchoprotective effect of deep breaths observed in healthy subjects by Kapsali et al. (10) was interpreted as suggesting a depression of the airway smooth muscle contractile machinery at the levels of the cross-bridge cycling rate (3) or plastic adaptation of the contractile filaments within the smooth muscle cell (5). The fact that FEV1 and FVC decreased less when MCh was preceded by deep breaths than when it was not does not, however, necessarily imply a reduced capacity of airway smooth muscle to shorten because of the possible effects of the full lung inflation required by these measurements. The finding of study 1 that the deep breaths blunted the decrease of FEV1 and FVC but not of measurements not preceded by a full inflation, such as Vpart, RVpart, FRC, and sGaw, suggests a mechanism facilitating the distensibility of contracted airways rather than inhibiting airway smooth muscle shortening. In healthy subjects, the disappearance of the effect of prior deep breaths on the decrease of FEV1 and FVC after 10 min of prohibition of further deep breaths may reflect a progressive increase in airway smooth muscle stiffness when the contractile apparatus is no more urged to change configuration with large lung inflations (3, 5). This is consistent with in vitro data showing that the tone of airway smooth muscle is fully recovered under static conditions within 8 min after a large stretch (4). The difference in the effect of the deep breaths on FEV1 and FVC between healthy and asthmatic subjects, but not on the parameters not preceded by a full inflation, may be interpreted as reflecting greater airway stiffness in asthma (7, 8). According to the latch-bridge theory (15), unstretched airway smooth muscle goes into a “frozen” state characterized by less hysteresivity and greater stiffness (3). Therefore, a reduced ability of the full inflations to distend constricted airways would reflect a greater proportion of latch bridges formed in asthma during prohibition of the deep inspirations preceding MCh inhalation. In healthy subjects, the multiple deep breaths would convert more latch bridges into rapidly cycling cross bridges, thus increasing airway smooth muscle hysteresivity and distensibility (3). Another theory that can explain a reduced stiffness after the deep breaths is a change in the configuration of the contractile elements inside the cell from a parallel to a serial arrangement (5). Whether the cytoskeletal configuration of asthmatic airway smooth muscle is different from normal is, however, unknown. Alternative hypotheses that possibly help explain these findings are a prevalent distribution of airway obstruction in the most peripheral parts of the lung (1) and more extensive airway closure (9), as well as a decreased airway-to-parenchyma interdependence in asthmatic compared with normal subjects (6). All of these functional conditions are expected to require extra distending forces to achieve a given degree of bronchodilatation.

Another important finding of this study is that deep breaths taken before inhalation of MCh enhanced the bronchoconstrictor response, as assessed by any parameter not preceded by a full lung inflation (FRC, Vpart, RVpart, and sGaw) recorded over time after MCh. This effect was similar in healthy and asthmatic subjects. The data of the present study do not allow us to give a conclusive explanation for this finding, but some speculation can be made about possible mechanisms. In some asthmatic patients, a sustained bronchoconstriction may develop after taking one or more full inflations (13, 18), an effect that has been attributed to a myogenic response triggered by the stretching of airway smooth muscle (22, 23). In vitro, this behavior was observed after passive sensitization with allergenic serum (14) or chemical agents able to convert airway smooth muscle from multiunit to single-unit conditions (23). In vivo, this phenomenon seems to occur only in a minority of subjects with rather severe asthma. In the present study, no subject showing even a slight decrease in sGaw after five deep breaths was included. This and the observation that the deep breaths taken before MCh inhalation tended to increase the bronchoconstrictor response even in normal subjects seem to rule out a myogenic response as a major determinant of the present findings. Nevertheless, we acknowledge that mechanical stretching can increase the permeability of the smooth muscle cell membrane to ions, including calcium (2, 11), and in addition cause release of mediators and/or activation of neural pathways. Although not perhaps sufficient to trigger a contractile response in unstimulated smooth muscle, these mechanisms could interfere with the response to MCh. In this scenario, the net effect of the deep breaths would result from the balance between mechanisms causing airway smooth muscle contraction and mechanisms causing bronchodilatation. Alternatively, the greater response to MCh after the series of deep breaths might have been due to mechanical unloading of the airways. Because of imperfect rheological properties, the elastic recoil of lung parenchyma decreases after a deep inspiration and needs time to recover (20). Therefore, the external load on airway smooth muscle will be transiently reduced after a deep breath. If multiple deep breaths cause a steplike reduction of lung elastic recoil, then the airway smooth muscle might be even more unloaded and for a longer time. Therefore, when tidal breathing is resumed after the deep breaths, the cyclic stretching imposed by lung parenchyma is less, thus possibly resulting in more rapid transition to a frozen state (3) or adaptation of the contractile apparatus to a shorter length (4). Under these conditions, exposure of the airways to a constrictor agent followed by a long prohibition of deep breaths after MCh could have resulted in greater airway narrowing. Finally, we acknowledge that a series of deep breaths before a constrictor agent may have increased mucosal permeability to the agent itself, thus causing an increased response similarly in both groups.

In the subgroup of asthmatic subjects in whom Vpart and RVpart were also measured, the same enhancing
effect of deep breaths on airway narrowing was observed as with sGaw and FRC. This rules out that the effect of deep breaths on MCh-induced changes in sGaw was limited to large or extrathoracic airways. The consistently greater decrements in Vp and sGaw and the increments in RVpart and FRC when MCh was preceded by five deep breaths would suggest enhanced airway narrowing both downstream and upstream from the flow-limiting segment, greater airway closure, and remarkable lung hyperinflation as a reaction of the respiratory system to airway narrowing.

An additional observation that may have practical implications is that, in healthy subjects, the decrease of FEV₁ with a single MCh dose equal to twice the last dose used to obtain the PD₉₀FEV₁ was much greater than 20% when the deep breaths were prohibited. This difference probably reflects the protective effect of the multiple full inflation maneuvers made to measure FEV₁ during the standard cumulative challenge on the screening day.

In conclusion, the present study reveals new and more complex features of airway responses to repeated deep breaths. In both asthmatic and healthy subjects, deep breaths taken before inhaling MCh surprisingly enhanced the bronchoconstrictor response. The major difference between healthy and asthmatic subjects in this respect seems to be the ability of the deep breaths to increase the distensibility of contracted airways in healthy patients.

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