Influence of Beclomethasone and Salmeterol on the Perception of Methacholine-Induced Bronchoconstriction*

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**Background:** Patient evaluation of asthma severity and medication needs is mostly based on respiratory symptoms and may be influenced by changes in perception of bronchoconstriction-induced sensations. However, the influence of asthma medication on the ability to perceive symptoms is still to be documented. This study evaluated the effects of short-term and regular use of salmeterol on the perception of methacholine-induced bronchoconstriction (MIB) in subjects with mild asthma, using inhaled salbutamol on an "as required" basis (n=15), and in subjects with moderate asthma, using daily inhaled beclomethasone (mean daily dose, 640 μg; n=15) in addition to salbutamol to control their asthma.

**Methods:** Methacholine challenges (MC) were performed at entry into the study, and then before, 1, and 12 h following inhalation of 50 μg of salmeterol or a placebo, after a 15-day baseline period; and after 4 weeks of twice daily use of those treatments. The measurements were then repeated with the alternate treatment after a 15-day washout period. Finally, a last MC was performed after another 15-day washout period. For each MC, the perception score of bronchoconstriction-associated breathlessness at 20% fall in FEV₁ (PS₂₀) was evaluated on a modified Borg scale from 0 to 10.

**Results:** Subjects using regular beclomethasone had a higher baseline PS₂₀ than those using only salbutamol (means: 3.06±0.06 and 2.01±0.07, p=0.0001). Short- and long-term use of salmeterol did not change significantly the PS₂₀ compared with placebo (p>0.05) in either group (with or without corticosteroid). Although there were some intraindividual variations, mean PS₂₀ did not vary significantly throughout the study.

**Conclusion:** These observations show that the perception of bronchoconstriction-associated breathlessness is not influenced by regular use of salmeterol. Patients using inhaled corticosteroids show a greater perception of MIB.

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**Key words:** asthma medication; breathlessness; long-acting β₂-agonists; perception of bronchoconstriction; salmeterol

**Abbreviations:** BD group=subjects using only bronchodilators to control their asthma; ICS group=subjects using a bronchodilator associated with inhaled corticosteroids to control their asthma; MIB=methacholine-induced bronchoconstriction; PC₂₀=provocative concentration of methacholine inducing a 20% fall in FEV₁; PS₂₀=perception score for breathlessness at 20% fall in FEV₁

Perception of bronchoconstriction in asthmatic patients seems influenced by many factors, including the speed of the asthmatic response, the provoking agent, and psychological factors. More recently, it has been suggested that airway inflammation could also modulate perception of bronchoconstriction. Roisman et al. found negative correlations between the perception of bradykinin-induced bronchoconstriction and both the magnitude of eosinophilic inflammation in airway mucosa and the degree of epithelial damage. Moreover, subjects receiving inhaled corticosteroids had a greater per-
ception of bradykinin-induced bronchoconstriction than those not using those agents.

Salmeterol is a long-acting β2-agonist providing bronchodilator and bronchoprotective effects lasting for >12 h.5–7 A decrease in bronchoprotection, however, has been observed after its prolonged use, although no significant increase in respiratory symptoms has been reported (to our knowledge).5–11 It seemed worthwhile, therefore, to determine whether salmeterol could influence (reduce) the perception of breathlessness associated with bronchoconstriction.

The purpose of this study was to evaluate the perception of breathlessness associated with methacholine-induced bronchoconstriction (MIB) after short-term (1 and 12 h postinhalation) or long-term (4 weeks of regular use) salmeterol therapy, when used or when not used concomitantly with inhaled corticosteroids. We also wanted to determine the influence of corticosteroids on the perception of MIB, to document the short- and long-term reproducibility of perception of MIB, and to investigate the possibility of a temporal adaptation to the perception of bronchospasm when these agents are used regularly. These observations were conducted during a recent study originally set to determine whether there was a tolerance to the protective effects of salmeterol on MIB in subjects using inhaled salbutamol “on demand” alone, as compared with those using regular inhaled beclomethasone in addition to salmeterol, to control their asthma.12

**Materials and Methods**

**Subjects**

Thirty subjects with mild-to-moderate asthma (12 male and 18 female), nonsmokers, aged 18 to 62 years (mean±SEM: 39.1±2.9 years) took part in this study. Fifteen were using only bronchodilators to control their asthma (BD group) and 15 others were using a bronchodilator associated with inhaled corticosteroids (ICS group).

**Inclusion Criteria**

Patients were considered eligible for this study if they had a diagnosis of allergic or nonallergic asthma in accordance with the definition of the American Thoracic Society13 with a baseline provocative concentration of methacholine inducing a 20% fall in FEV1 (PC20) <8 mg/mL. FEV1 had to be >60% of the predicted value before bronchodilator administration. In the ICS group, the daily dose of inhaled corticosteroid had to be between 400 and 1,500 µg of beclomethasone equivalent, and it had to remain stable throughout the study.

The protocol was approved by the Laval and Sacré-Coeur Hospital Ethics Committees and all subjects signed informed consent forms.

**Study Design**

This was a double-blind, placebo-controlled, crossover study. After a baseline methacholine inhalation challenge and a 2-week run-in period, each subject in the two study groups randomly received either salmeterol, 50 µg, or a placebo twice daily during two treatment phases of 4 weeks each. A 2-week washout period separated the two treatments and another was done after the second treatment period at the end of the trial, when a final methacholine inhalation challenge was performed.

During the study, the subjects came to the laboratory on the entry day (day −14), on days 0, 28, 42, and 70 of the treatment period, and again at the end of the study (day 84). On first and last days (days 0, 28, 42, and 70) of active and placebo treatment periods, subjects underwent methacholine challenges 1 h prior to inhalation of study drugs (−1 h) and 1 and 12 h after. On days −14, 0, and 42, PC20, methacholine had to be within 1.6 doubling concentrations; otherwise a further 1-week washout period was done and the subject was restudied 1 week later.

During the treatment period, before their visits to the laboratory, the subjects stopped treatment with their study medication (salmeterol or placebo) for 48 h and their rescue medication (salbutamol) for 8 h.

**Methods**

Methacholine challenges were performed according to the method described by Juniper et al.14 For the determination of the PC20, the percent fall in FEV1 was calculated from the postsaline solution value.

The perception of MIB was evaluated at each MC using a perception score for breathlessness at 20% fall in FEV1 (PS20) on a modified "Borg scale" from 0 to 10.15 After inhalation of each methacholine concentration, perception scores for breathlessness were evaluated before each FEV1 measurement; as a positive correlation had been described previously between perception scores for breathlessness and fall in FEV1, the PS20 was determined by interpolation of the two last points on the perception/fall in FEV1 curve, corresponding to perception scores before and after the 20% fall in FEV1.15

**Analysis**

The two treatments were compared for the changes in perception of MIB at 1 and 12 h. Inferential analyses were done to assess comparability of the treatment groups. This is defined as the following ratios: PS20 (day 0 [1 or 12 h]/PS20 (day 0 [−1 h]) and PS20 (day 28 [1 or 12 h]/PS20 (day 28 [−1 h]) for the first treatment; and PS20 (day 42 [1 or 12 h]/PS20 (day 42 [−1 h]) and PS20 (day 70 [1 or 12 h]/PS20 (day 70 [−1 h]) for the second treatment. Repeated measures of crossover design have been used to compare treatment groups with respect to the perception of MIB at 1 and 12 h compared with predose (−1 h), before, and after 4 weeks of treatment.

**Results**

**Demographics and Pulmonary Function Tests**

At entry into the study, the two groups of subjects were comparable for gender and PC20 (p>0.05; Table 1). Subjects in the BD group were younger than those in the ICS group (respective mean ages, 31 and 45 years; p=0.01) and showed a trend toward
a shorter duration of asthma (9.5 vs 15.5 years; p>0.05). Fourteen subjects in the BD group and seven in the ICS group were atopic. At entry to the study, baseline FEV\textsubscript{1} was higher in the BD group than in the ICS group (87.1±3.8% predicted and 76.3±2.7% predicted; p=0.03). Mean baseline FEV\textsubscript{1} was not significantly different between treatment periods in either the BD (salmeterol; 85.3±3.7%, and placebo: 87.0±3.5%) or the ICS group (76.7±2.5% and 77.3±2.8%). Mean use of rescue medication during the run-in period was 0.63±0.07 inhalations per day in the BD group and 1.93±0.02 inhalations per day in the ICS group (p=0.03). According to their diary card records, all subjects were compliant to their medication and no increase in rescue medication was noted in either group throughout the study. A decrease in rescue medication of similar amplitude in both groups of subjects (threefold in the BD group and 3.2-fold in the ICS group) was observed while the subjects were receiving salmeterol.

At the beginning of each treatment period, mean PC\textsubscript{20} did not differ significantly between the BD (salmeterol: 2.2 mg/mL, placebo: 2.7 mg/mL) and ICS groups (2.1 mg/mL and 1.9 mg/mL). It did not change during the placebo treatment period, regardless of the time of day the methacholine challenge was done. The first inhalation of salmeterol afforded a significant protection against MIB in both groups up to 12 h as compared with the value at −1 h. This was evidenced by the increased number of double concentrations of methacholine that had to be inhaled in order to induce a 20% fall in FEV\textsubscript{1}. One hour after salmeterol, 3.3 and 2.0 more double concentrations of methacholine were necessary in the BD and the ICS group, respectively (both p<0.002). After 12 h, these numbers were, respectively, 2.5 (p≤0.002) and 1.2 (p=0.03) double concentrations. After 4 weeks of treatment, the protective effect of salmeterol was significantly attenuated compared with the corresponding value on the first day of the treatment, at 1 h (1.7 double concentrations, p=0.002) and at 12 h (1.1, p=0.04) in the BD group; the effect of salmeterol was reduced at 1 h (1.2, p>0.05) and was significantly attenuated at 12 h (0.2, p=0.01) in the ICS group.

**Perception of Bronchoconstriction-Associated Breathlessness (MIB)**

At entry to the study, mean baseline PS\textsubscript{20} was lower in the BD group (1.5±0.2) compared with the ICS group (3.2±0.4, p=0.0009). No significant correlation was found between the duration of asthma and the PS\textsubscript{20} evaluated at entry to the study (rs=0.264, p=0.15). PS\textsubscript{20} was significantly different from mean PS\textsubscript{20} on day 0 at 1 h before salmeterol (1.7±0.2) or placebo treatment (2.3±0.3) in the BD group (p=0.043); no significant difference was found between baseline PS\textsubscript{20} and mean PS\textsubscript{20} on day 0 at 1 h before salmeterol (3.2±0.3) or placebo treatment (2.8±0.3) in the ICS group (p>0.05). However, mean PS\textsubscript{20}, although it showed some variability within and between subjects, did not vary significantly throughout both treatment periods (salmeterol and placebo) in the BD or in the ICS group (p>0.05; Fig 1).

During the treatment periods, on the first day of treatment (acute effect, day 0 or 42), the PS\textsubscript{20} did not change significantly in either the BD or the ICS group, 1 h or 12 h after salmeterol or placebo as compared with 1 h before the treatment (p>0.05; Table 2 and Fig 2).

After 4 weeks of salmeterol or placebo (day 28 or 70), in the BD and in the ICS groups, no significant difference was found between the PS\textsubscript{20} measured 1 h

### Table 1—Subjects’ Characteristics*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Broncholators Only</th>
<th>Inhaled Corticosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>(10 F, 5 M)</td>
<td>(8 F, 7 M)</td>
</tr>
<tr>
<td>Age, y</td>
<td>31.0±3.4</td>
<td>45.3±4.3*</td>
</tr>
<tr>
<td>Asthma duration, y</td>
<td>9.5±1.6</td>
<td>15.5±3.1</td>
</tr>
<tr>
<td>Baseline FEV\textsubscript{1}, % predicted</td>
<td>87.1±3.8</td>
<td>76.3±2.7*</td>
</tr>
<tr>
<td>Baseline FVC, % predicted</td>
<td>93.7±3.2</td>
<td>99.4±4.0</td>
</tr>
<tr>
<td>Baseline PC\textsubscript{20}, mg/mL (geometric mean)</td>
<td>2.2±1.2</td>
<td>2.1±1.3</td>
</tr>
<tr>
<td>Baseline PS\textsubscript{20}</td>
<td>1.5±0.2</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Daily inhaled corticosteroid dose, µg</td>
<td>None</td>
<td>640±60</td>
</tr>
<tr>
<td>Rescue medication during run-in period, mean No. of inhalations per day</td>
<td>0.6±0.1</td>
<td>1.9±0.0*</td>
</tr>
</tbody>
</table>

*Mean±SEM. F=female; M= male.

*p=0.015.

*p=0.029.

*p=0.031.
before and 1 or 12 h after salmeterol or placebo (Fig 3). Throughout the treatment periods, there was no significant difference in PS20 between those receiving salmeterol and those receiving placebo treatment in the BD or ICS group (p > 0.05). While short- and long-term salmeterol use was not associated with significant changes in PS20 compared with placebo in the BD and in ICS groups (p > 0.05), at any time measured, subjects from the ICS group had a higher PS20 than those of the BD group (Fig 1; p = 0.0001). At the end of the 2-week run-in period, there was no significant correlation between PC20 and PS20 (rs = 0.127, p = 0.05).

Perception of bronchoconstriction-associated

**Table 2—Perception Scores at 20% Fall in FEV1**

<table>
<thead>
<tr>
<th>Visits</th>
<th>Day 0 or 42</th>
<th>Day 28 or 70</th>
<th>Time</th>
<th>1h</th>
<th>12h</th>
<th>1h</th>
<th>12h</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BD only</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>2.3</td>
<td>1.7</td>
<td>2.3</td>
<td>1.7</td>
<td>&gt;0.05*</td>
<td>3.0</td>
<td>1.6</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>&gt;0.05*</td>
<td>1.9</td>
<td>2.1</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td><strong>ICS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>&gt;0.05*</td>
<td>3.0</td>
<td>3.0</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>2.7</td>
<td>3.4</td>
<td>3.4</td>
<td>3.4</td>
<td>&gt;0.05*</td>
<td>3.0</td>
<td>2.9</td>
<td>&gt;0.05*</td>
</tr>
</tbody>
</table>

*Treatment vs baseline comparison.
1Salmeterol vs placebo comparison.
1BD vs ICS comparison, p = 0.0001 for all values.

**Discussion**

This study shows that salmeterol did not significantly change perception of MIB compared with placebo, either short term or after a period of 4 weeks of daily use, in asthmatic subjects either using bronchodilators alone or using them with inhaled corticosteroids. To our knowledge, only one previous study looked at the perception of induced-bronchoconstriction in asthmatic patients and the effect of corticosteroid treatment, and no other study specifically compared the effects of long-acting β2-agonists on the perception of bronchoconstriction.4 Our results suggest that salmeterol does not impair the perception of bronchospasm and confirms that inhaled corticosteroid use seems associated with a greater perception of asthma symptoms.

A previous study by Noseda et al16 compared the
perceived effect on shortness of breath of an acute inhalation of bronchodilator in asthmatic and COPD patients, showing in this case an improvement of the perception of shortness of breath on a visual analog scale following the inhalation. However, this improvement corresponded to an improvement in FEV₁ and authors did not compare the perception of breathlessness for a similar degree of bronchoconstriction with and without bronchodilator. More studies are needed, therefore, to determine the influence of asthma drugs on perception of MIB or for a given degree of change in pulmonary function.

At all time points of the study, subjects of the ICS group had a greater perception score for a given fall in FEV₁ than did subjects receiving bronchodilators only, as reported by Roisman et al.⁴ Subjects in the ICS group were slightly older than those of the BD group. Age has been reported to have an influence on the degree of perception of methacholine-induced breathlessness, and we previously noted an age-related increase in perception of breathlessness in asthmatic subjects although the correlation was weak.¹⁵ In the present study, however, no significant correlation was found between age and perception of breathlessness in the whole group of subjects or in the ICS or BD group considered separately. On the other hand, Connolly et al.¹⁷ had observed a reduced awareness of methacholine-induced bronchoconstriction in elderly subjects. Finally, in the study of Roisman et al.⁴ the mean ages of the subjects with and without inhaled corticosteroids were similar, and those in the ICS group had a greater perception of bradykinin-induced bronchoconstriction.

The subjects in the ICS group used a greater amount of rescue medication than those in the BD group prior to treatment. This could have contributed to influencing the degree of perception of methacholine-induced breathlessness.

In the present study, at the end of the run-in period, atopic subjects had a reduced perception of breathlessness compared with nonatopic subjects. As there were more atopic subjects in the BD group than in the ICS group, this could have contributed to the lower perception observed in the BD group, maybe as a result of ongoing allergen-induced inflammation. At the end of the run-in period, however, no significant difference was observed in the perception of breathlessness in the ICS group whether they were atopic or not.

Although mean baseline FEV₁ was significantly lower in the ICS group than in the BD only group, this would hardly explain the difference in perception of breathlessness, since, in a previous study on 150 asthmatic subjects, we found no significant correlation between the PS₂₀ and baseline FEV₁.¹⁵ Burdon et al.¹⁸ had previously found a reduced

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**Figure 3.** Left: the baseline PS₂₀ was unchanged before (day 0 or 42) and after 4 weeks (day 28 or 70) of regular use of inhaled salmeterol; this was observed in both BD and ICS groups. Right: the PS₂₀ measured 1 h or 12 h following salmeterol inhalation was similar whether it followed a single dose or a 4-week use of salmeterol.
perception of MIB in asthmatic subjects with residual baseline airflow, although these subjects had lower mean baseline FEV₁ than our subjects.

At the end of the 2-week run-in period, there was no significant correlation between PC₂₀ and PS₂₀. This observation is in agreement with our previous report. However, this does not exclude the possibility that for marked airway responsiveness, there may be a lower degree of perception as was suggested by Burdon et al in the past.

Subjects receiving corticosteroids had a longer duration of asthma than those receiving bronchodilators only. However, this difference in duration of asthma, which was not statistically significant between the two groups, may modulate perception of bronchoconstriction but did not seem a major determinant since no significant correlation was found between the duration of asthma and the PS₂₀ evaluated at entry in the study.

Among the different factors that may influence perception of asthma symptoms, airway inflammation and anti-inflammatory medications have been proposed. In the present study, we made no measurements of airway inflammation and thus, we cannot tell if the difference in perception between the subjects from the ICS group and the BD group are due to a lesser degree of inflammation in the group treated with inhaled corticosteroids. However, we still lack information on how asthma medications can modulate perception of respiratory symptoms. This is of the utmost importance, as most of the time, patients adjust their medication according to respiratory symptoms and rescue medication needs. If a medication was reducing perception, such an adjustment could lead to insufficient treatment of residual airflow obstruction.

In this regard, Roisman et al suggested that the asthmatic inflammatory process, with its eosinophilic airway inflammation and epithelial damage, could reduce perception of bronchoconstriction, possibly due to damage to sensory receptors in the airways. Our results are in keeping with those observations, as we showed that patients using corticosteroids had significantly higher symptom scores for a given degree of fall in expiratory flows compared with subjects using only bronchodilators on demand.

The reason corticosteroid users have greater perception scores compared with nonusers is still uncertain. It may be due to reduced damage to airway sensory nerve endings compared with asthmatic subjects not receiving corticosteroids. It has been shown that even in the mildest asthma, and even in "preasthmatic" conditions, significant airway inflammation and epithelial damage occurred. However, inhaled corticosteroid use may lead to restoration of the integrity of airway epithelium. Corticosteroids may therefore potentially improve perception of respiratory symptoms, both by reducing the inflammatory process and improving the epithelial structure. Other mechanisms, however, are possible and should be documented further.

However, long-acting β-adrenoceptor agonists such as salmeterol provide a marked improvement of asthma symptoms in most subjects. Although there is still more work to be done in this area, these agents are presently considered to have no clinically significant anti-inflammatory properties; some authors have even expressed the fear that they may allow increased airway inflammation, particularly in subjects not using corticosteroids. It therefore seemed of interest to determine whether salmeterol could influence the perception of breathlessness associated with bronchoconstriction in both users and nonusers of inhaled corticosteroids, following long-term regular use. Long-term studies will be of interest, but our data are quite reassuring, as they show that there was no significant change in perception of bronchoconstriction-induced breathlessness in both groups after 4 weeks of regular intake of salmeterol.

In conclusion, short-term and sustained use of salmeterol did not significantly change the perception of methacholine-induced breathlessness compared with placebo, even if there was a loss in the protective effect of salmeterol over time. However, although other factors, such as the age of subjects, atopic status, baseline FEV₁, duration of asthma, and the amount of rescue medication used may also have influenced the perception of breathlessness, they did not seem by themselves to be major determinants of perception of MIB, although inhaled corticosteroid use was associated with improved asthma symptoms perception.

These observations have clinical relevance and suggest that asthmatic subjects using no bronchial anti-inflammatory agent perceive less acutely respiratory symptoms than those currently using them, while long-term salmeterol use does not modify baseline perception of respiratory sensations.

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