Interpretation of Cough Provoked by Airway Challenges*

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Study objective: To analyze the cough response to three airway challenges in order to clarify whether the recording of the provoked coughs would be beneficial in the management of asthma.

Design: A prospective study.

Setting: University hospital.

Participants: Fifteen healthy subjects, 16 steroid-naïve subjects with asthma, and 16 subjects with steroid-treated asthma.

Interventions: Inhalation challenges with isotonic histamine, hypertonic saline solution, and hypertonic histamine, using an ultrasonic nebulizer and 2-min tidal breathing method.

Measurements: Airflow parameters were measured with a spirometer, and the coughs were recorded manually.

Results: Coughing during the isotonic histamine challenge was associated with the degree of the bronchoconstriction induced. When this was taken into account, the healthy subjects coughed as frequently as the asthmatic subjects. During the two hypertonic challenges, the asthmatic subjects coughed more frequently than did the healthy subjects when the induced bronchoconstriction had not yet developed. At that stage of the hypertonic saline solution challenge, the mean coughing frequency was 0.7 coughs per minute (95% confidence interval [CI], 0.03 to 1.3 coughs per minute) for the healthy subjects, 2.7 coughs per minute (95% CI, 0.8 to 4.5 coughs per minute) for the steroid-naïve asthmatic subjects, and 1.3 coughs per minute (95% CI, 0.6 to 1.9 coughs per minute) for the steroid-treated asthmatic subjects (p = 0.018). For the hypertonic histamine challenge, the respective values were 0.8 coughs per minute (95% CI, 0.4 to 1.2 coughs per minute), 3.6 coughs per minute (95% CI, 2.4 to 4.9 coughs per minute), and 2.1 coughs per minute (95% CI, 1.0 to 3.1 coughs per minute; p = 0.001). This cough did not correlate with airway hyperresponsiveness.

Conclusions: Coughing during isotonic histamine challenge seems to be a manifestation of bronchoconstriction, and recording of the coughs may not provide additional information to airflow measurements. Frequent coughing during hypertonic saline solution and hypertonic histamine challenges in the absence of bronchoconstriction is a pathologic phenomenon. Sensitivity to the cough-provoking effect of hypertonic challenges seems to be enhanced in patients with asthma but unrelated to airway hyperresponsiveness. Therefore, the recording of the provoked coughs during these challenges may add to the information obtained from airflow measurements.

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Key words: asthma; bronchial hyperresponsiveness; cough; histamine; hypertonic saline solution

Abbreviations: CCR = coughs/concentration ratio; Cf = coughing frequency; Cfund5 = coughing frequency at that stage of the challenge when the fall in FEV₁ is < 5% from baseline; CI = confidence interval; PC₂₀ = provocative concentration of a substance causing a 20% fall in FEV₁

Airway challenges have traditionally been used in the assessment of the tendency of the bronchi to narrow in response to an exogenous stimulus. These tests fall into two categories, those that act directly on smooth muscle and those that cause the airways to narrow indirectly by a release of endogenous mediators. Recently, it has been proposed that...
airway challenge with mannitol, which is an indirect hypertonic stimulus, could also be used to study the mechanisms of asthmatic cough. In that study, asthmatic subjects coughed much more frequently than healthy subjects per dose of mannitol, and 6 months of treatment with inhaled budesonide statistically significantly decreased the sensitivity of asthmatic patients to the cough-provoking effects of mannitol. Mannitol-induced bronchoconstriction and cough probably have different pathways in asthma, and, therefore, the recording of both provoked coughs and induced bronchoconstriction during this challenge may give supplementary information about the patient’s disease.

Several other types of airway challenges, including histamine and hypertonic saline solution challenges, also provoke cough. At present, the clinical significance of the cough response to these challenges is unknown, and the number of provoked coughs are not routinely recorded. Histamine is a direct smooth muscle contractor, and smooth muscle contraction itself is able to provoke cough. Some animal studies have suggested that histamine might also act directly on cough receptors to increase their activity. Hypertonic saline solution is an indirect, hypertonic stimulus like mannitol, and it could be assumed that the mechanisms of hypertonic saline solution-provoked cough and mannitol-provoked cough are similar.

In the present study, we analyzed the cough response to three types of airway challenges in order to clarify whether the recording of the provoked coughs would be beneficial in the management of asthma. Fifteen healthy subjects, 16 steroid-naive subjects with asthma, and 16 subjects with steroid-treated asthma were challenged with isotonic histamine, hypertonic saline solution, and hypertonic histamine. Hypertonic histamine challenge is a novel method for assessing airway responsiveness, and can be regarded as the combination of a direct and an indirect airway challenge. When analyzing the cough response to the challenges, special attention was paid to the degree of induced bronchoconstriction.

**Materials and Methods**

**Study Design**

A detailed description of the study population and the challenges has been published previously, and this is another analysis of the same data set. In the previous publication, the airway responsiveness to the challenges was described, whereas the present publication describes the cough responses. All subjects underwent the following three airway challenges: the isotonic histamine challenge; the hypertonic saline solution challenge; and the hypertonic histamine challenge. The challenges were performed in a random order, with at least 2 nights between the challenges, approximately at the same time of the day, within 3 weeks. The study was not blinded. The asthmatic subjects also underwent a second hypertonic histamine challenge to study its repeatability.

**Subjects**

The study was completed by 32 asthmatic subjects who met the American Thoracic Society criteria for asthma. In 16 of these subjects, the diagnosis had been recently established, and they had never used any corticosteroid preparations. The other 16 asthmatic subjects had used inhaled corticosteroids for a mean duration of 7 months (range, 3 to 60 months) with a mean daily dose of 797 g (range, 400 to 1,600 g). Most of the asthmatic subjects had moderate persistent asthma, according to the Global Initiative for Asthma classification. In addition, there were 15 healthy volunteers who had no chronic respiratory diseases or symptoms and were life-long nonsmokers. Subjects had refrained from taking aerosol short-acting g-agonists for 6 h, aerosol long-acting g-agonists for 48 h, and antihistamine and leukotriene receptor antagonists for 3 days before undergoing the challenges. Inhaled corticosteroids were not consumed on the study days. The Finnish National Agency of Medicines and the Institutional Ethics Committee approved this study, and all subjects provided their informed written consent for participation in the study.

**The Challenges**

A hand-held ultrasonic nebulizer (Omron U1; Omron LTD; Tokyo, Japan) was used in all challenges with a measured output of 0.44 to 0.48 mL/min. Also, the protocol was identical between the challenges, with variation only of the inhaled solution. Before the isotonic histamine challenge, the spirometry (model M9449; Medikro Ltd; Kuopio, Finland) was performed in triplicate according to the American Thoracic Society guidelines, and the largest FEV1 value was used as the baseline value. Then, the subjects inhaled a standard isotonic phosphate-buffered saline solution (osmolality, 292 mOsm/kg, measured by freezing-point depression [The Advanced Micro Osmometer 3300-BK; Advanced Instruments, Inc; Norwood, MA]) for 2 min using tidal volume breathing. The coughs were manually recorded during the 90-s observation period after the inhalation. Spirometry was performed in duplicate at 90 s from the end of the inhalation, and the larger of the two values was used in the analysis. If the FEV1 had not fallen ≥20%, the inhalation of the next solution began 2 min after the end of the previous inhalation. The subsequent inhaled solution was histamine diphosphate (Histamini phosphas; Ph.Eur., University Pharmacy; Helsinki, Finland) that was dissolved in the isotonic phosphate-buffered saline solution at doubling concentrations from 0.0075 to 8.0 mg/mL (osmolality, 291 to 341 mOsm/kg). The challenge continued until the FEV1 had fallen ≥20% from the baseline value, or up to the inhalation of the final solution.

During the hypertonic saline solution challenge, the inhaled solution was hypertonic phosphate-buffered saline solution (osmolality, 1,511 mOsm/kg) throughout the challenge. The hypertonicity was achieved by adding 45 g of saline solution per liter of solution instead of the standard 4.4 g/L. The challenge consisted of 2-min tidal breathing inhalations, which were repeated up to 12 times if the FEV1 had not fallen ≥20%. Again, the coughs were manually recorded during the 90-s period after each 2-min inhalation, and spirometry was performed in duplicate at 90 s from the end of each inhalation.

The hypertonic histamine challenge was identical to the iso-
tonic histamine challenge, but the first inhaled solution was the hypertonic phosphate-buffered saline solution and during subsequent inhalations it was histamine diphosphate dissolved in the hypertonic phosphate-buffered saline solution at doubling concentrations from 0.0075 to 8.0 mg/mL (osmolality, 1,522 to 1,577 mOsm/kg).

Statistical Analysis

An individual’s sensitivity to the cough-provoking effects of the challenges was expressed in several ways. Coughing frequency (Cf) was defined as the cumulative number of coughs divided by the cumulative duration of the observation periods (90 s after each nebulization, and a maximum of 12 nebulizations) and was expressed as the number of coughs per minute. To exclude the possible cough-provoking effect of bronchoconstriction, we also calculated the mean Cf at that stage of the challenge when the fall in FEV₁ was < 5% from the baseline value (Cfunder5). The cumulative number of coughs during the entire challenge was calculated. For the two histamine challenges, the cumulative number of coughs was divided by the final histamine concentration achieved (ie, coughs/concentration ratio [CCR]). The airway responsiveness to the two histamine challenges was expressed as the provocative concentration of a substance causing a 20% fall in FEV₁ (PC₂₀).

To find out whether the Cf during the two histamine challenges was mainly determined by the degree of induced bronchoconstriction or by the inhaled histamine concentration, a multiple regression analysis was used. In these analyses, the Cf after each histamine concentration was the dependent variable, whereas the percentage fall in FEV₁ from baseline and the inhaled histamine concentration (as doubling concentrations) were the independent variables. In order to account for the intrindividual dependence of the responses, subject-specific random effects were included. Kruskal-Wallis analysis of variance was used to compare the results among the three subgroups, with a post hoc Mann-Whitney test with Bonferroni correction to compare the results between two subgroups. The Spearman ρ (rs) was used to analyze correlations. Repeatability was determined by using the intraclass correlation coefficient. The results are expressed as means and 95% confidence intervals (CIs). The deviation of the distribution of the cough index from a normal distribution was studied by the one-sample Kolmogorov-Smirnov test. In case of a log-normal distribution, geometric means and 95% CIs are expressed. A p value of < 0.05 was accepted as the level of statistical significance. All analyses were performed using a statistical software package (SPSS for Windows, version 11.5.1; SPSS; Chicago, IL).

RESULTS

The hypertonic histamine challenge provoked more coughs than the other two challenges. The mean Cf values were 1.4 coughs per minute (95% CI, 0.8 to 1.9 coughs per minute) for the isotonic histamine challenge, 1.7 coughs per minute (95% CI, 1.0 to 2.4 coughs per minute) for the hypertonic saline solution challenge, and 2.8 coughs per minute (95% CI, 2.1 to 3.5 coughs per minute) for the hypertonic histamine challenge (p < 0.001).

Association of the Cf With the Degree of Induced Bronchoconstriction

The majority of the subjects developed bronchoconstriction (ie, a > 15% fall in FEV₁) during the isotonic histamine challenge (37 of 47 subjects) and hypertonic histamine challenge (38 of 47 subjects). On the contrary, only two subjects developed such a bronchoconstriction during the hypertonic saline solution challenge. Therefore, the association of the Cf with the degree of induced bronchoconstriction could be analyzed for the two histamine challenges but not for the hypertonic saline solution challenge. The coughing during the hypertonic saline solution challenge was analyzed with an assumption that bronchoconstriction did not markedly affect the coughing.

Multiple regression analysis revealed that the Cf during the isotonic histamine challenge was associated with the degree of induced bronchoconstriction in all subgroups (healthy subjects, p < 0.001; steroid-naive asthmatic subjects, p = 0.019; steroid-treated asthmatic subjects, p = 0.002). On the contrary, this cough was very weakly associated, if at all, with the inhaled histamine concentration (p = 0.17, p = 0.44, and p = 0.03, respectively).

Cf during the hypertonic histamine challenge was associated with the degree of induced bronchoconstriction in the healthy subjects (p < 0.001) and steroid-treated asthmatic subjects (p = 0.004), but not in the steroid-naive asthmatic subjects (p = 0.30). Again, this cough was not associated with the inhaled histamine concentration in the subgroups, the p value was between 0.26 and 0.68.

Between-Group Differences in the Cough Response

The Isotonic Histamine Challenge: Given the strong association between the cough and bronchoconstriction during the isotonic histamine challenge, the mean Cf values were plotted against the degree of induced bronchoconstriction to explore any between-group differences in the cough response. Figure 1 shows that in all groups the Cf increased similarly with the level of isotonic histamine-induced bronchoconstriction and that there were no differences between the groups at any level of induced bronchoconstriction. However, since the asthmatic subjects developed bronchoconstriction after considerably lower inhaled histamine concentrations than the healthy subjects, the geometric mean CCR values were largest in the asthmatic subjects; they were 1.2 coughs per mg/mL in healthy subjects (95% CI, 0.6 to 2.4 coughs per mg/mL), 9.4 coughs per mg/mL (95% CI, 2.9 to 31 coughs per mg/mL) for steroid-naive asthmatic subjects, and 3.8 coughs per mg/mL (95% CI, 1.5 to 9.8 coughs per mg/mL) for steroid-
treated asthmatic subjects (p = 0.038). Within the asthmatic subjects, the isotonic histamine CCR correlated closely with its respective index of airway hyperresponsiveness, the isotonic histamine PC_{20} (rs = −0.57; p = 0.001).

The Hypertonic Saline Solution Challenge: The steroid-naive asthmatic subjects coughed most frequently during the hypertonic saline solution challenge (Fig 2). They coughed most frequently also at that stage of the challenge when the bronchoconstriction had not yet developed: The Cfunder5 values were 0.7 coughs per minute (95% CI, 0.03 to 1.3 coughs per minute) for the healthy subjects, 2.7 coughs per minute (95% CI, 0.8 to 4.5 coughs per minute) for the steroid-naive asthmatic subjects; and 1.3 coughs per minute (95% CI, 0.6 to 1.9 coughs per minute) for the steroid-treated asthmatic subjects (p = 0.018).

The Hypertonic Histamine Challenge: By plotting the mean Cf values of the subgroups against the degree of hypertonic histamine-induced bronchoconstriction, it could be demonstrated that the steroid-naive asthmatic subjects coughed more frequently than the healthy subjects at that stage of the challenge when the bronchoconstriction had not yet developed. As the bronchoconstriction developed, the healthy subjects also started to cough, and the differences among the groups vanished (Fig 3). The Cfunder5 values were 0.8 coughs per minute (95% CI, 0.4 to 1.2 coughs per minute) for the healthy subjects, 3.6 coughs per minute (95% CI, 2.4 to 4.9 coughs per minute) for the steroid-naive asthmatic subjects, and 2.1 coughs per minute (95% CI, 1.0 to 3.1 coughs per minute) for the steroid-treated asth-
matic subjects ($p = 0.001$) [Fig 4]. Among the asthmatic subjects, the hypertonic histamine $C_{f \text{under}5}$ did not correlate with the hypertonic histamine $PC_{20}$ ($r_s = -0.06; p = 0.76$).

The hypertonic histamine CCR values separated the groups well, as follows: healthy subjects, 3.4 coughs per mg/mL (95% CI, 1.9 to 5.9 coughs per mg/mL); steroid-naïve asthmatic subjects, 53 coughs per mg/mL (95% CI, 23 to 121 coughs per mg/mL); and steroid-treated asthmatic subjects, 10 coughs per mg/mL (95% CI, 4.2 to 25 coughs per mg/mL; $p < 0.001$). Within the group of asthmatic subjects, the hypertonic histamine CCR correlated closely with its respective index of airway hyperresponsiveness, hypertonic histamine $PC_{20}$ ($r_s = -0.64; p < 0.001$).

### Repeatability of the Cough Response

Since the hypertonic histamine challenge was the only challenge that was repeated, only the hypertonic histamine $C_{f \text{under}5}$ and CCR intraclass correlation coefficient could be assessed (0.86 and 0.97, respectively).

## Discussion

To the best of our knowledge, this is the first study to compare the cough responses among direct, indirect, and combined airway challenges. The study showed that all challenges were able to provoke cough, but the significance of the cough response differs considerably among the challenges. The recording of the provoked coughs during the isotonic histamine challenge may not provide clinically useful information. On the contrary, the recording of the coughs during hypertonic saline solution and hypertonic histamine challenges may provide information that cannot be obtained by airflow measurements.

Coughing during isotonic histamine challenge seems to be a manifestation of bronchoconstriction. Since this can be more objectively measured with a spirometer, the recording of the coughs may be useless. The $C_f$ during isotonic histamine-induced bronchoconstriction did not differ between healthy and asthmatic subjects, and, therefore, this cough can be regarded as a physiologic phenomenon. The isotonic histamine CCR value, although capable of separating the subgroups, is actually an index of airway hyperresponsiveness. A high value indicates an appearance of the bronchoconstriction-associated cough in response to a low histamine concentration. The close correlation between the isotonic histamine CCR and $PC_{20}$ values supports this interpretation.

Some animal studies $^7,^8$ have suggested that smooth muscle contraction is not essential for the stimulant effects of histamine on cough receptors and that histamine might fire the receptors directly. The present results do not support this; the inhaled histamine concentration was weakly associated, if at all, with the $C_f$ when the degree of induced bronchoconstriction was included in the regression models. Thus, the present as well as the most recent animal studies $^{15-18}$ have consistently suggested that histamine provokes cough mainly indirectly, by inducing an airway smooth muscle contraction, which causes a mechanical stimulation of the airway rapidly adapting receptors. Methacholine is another widely used direct smooth muscle contractor, which probably has no direct effects on cough receptors either.$^6$ Although methacholine challenge was not included in the present study, the authors think that cough during that challenge can be interpreted in the same way as that during isotonic histamine challenge. If this is the case, the recording of the provoked coughs during methacholine challenge may also be useless.

Bronchoconstriction-associated cough probably exists during the hypertonic saline solution challenge and the hypertonic histamine challenge as well. However, there was also another type of cough that occurred during these challenges. When the bronchoconstriction was mild or absent, the asthmatic subjects, and the steroid-naïve asthmatic subjects especially, coughed statistically significantly more frequently than the healthy subjects. Therefore, this cough seems to be a pathologic phenomenon that is

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**Figure 4.** $C_f$ at the $C_{f \text{under}5}$ stage of the hypertonic histamine challenge.

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similar to mannitol-provoked cough.\textsuperscript{2} The pathophysiologic pathway from airway mucosal hyperresponsiveness to the cough response has not been fully defined but may be due to sensorineural hyperresponsive-ness, a well-established feature of allergic rhinitis,\textsuperscript{19,20} although much less studied in asthma patients.\textsuperscript{2,3}\ The results of the present as well as of the previous study with mannitol\textsuperscript{2} suggest that treatment with inhaled steroids diminishes the cough response to hypertonic stimuli in asthmatic subjects, indicating that the possible sensorineural hyperresponsive-ness in asthma patients may be associated with the degree of inflammation in the airways. The CF during the early stages of the hypertonic histamine chal-

lenge did not correlate with the degree of airway hyperresponsiveness, as documented by the hypertonic histamine PC\textsubscript{20}. This corroborates the view that hypertonicity-induced cough and bronchoconstriction have different pathways in asthma.\textsuperscript{3} Thus, the recording of both provoked coughs and induced bronchoconstriction during hypertonic challenges probably gives supplementary information about the patient’s disease.

The information provided by the cough response to hypertonic challenges can probably be used to investigate the mechanisms of cough in asthma and asthma-like disorders.\textsuperscript{2} At present, it may be too early to suggest any direct clinical applications, but the possibilities are many. First, the diagnostic sensi-
tivity of hypertonic challenges is generally low if the airflow indexes are used solely to express the response.\textsuperscript{21,22} If the cough response is also utilized, the number of pathologic responses may increase among asthmatic subjects, and the diagnostic sensitivity of these challenges would thus increase. This possibility is supported by the fact that in the present study a pathologic cough response to hypertonic saline solution was far more prevalent than a pathologic bronchoconstrictive response among the asthmatic sub-

jects. Accordingly, several asthmatic subjects who did not develop bronchoconstriction in response to inhaled mannitol coughed pathologically frequently during the challenge.\textsuperscript{2} Second, airway hyperresponsive-

ness is a relatively insensitive tool with which to demonstrate the effects of inhaled corticosteroids in asthma patients,\textsuperscript{23} and the hypertonic challenges are not necessarily better than the so-called direct chal-

lenges in this respect.\textsuperscript{24} The results of the present cross-sectional study as well as the previous longitudi-

nal study\textsuperscript{2} consistently suggest that the cough response to hypertonic challenges attenuates during treatment with inhaled corticosteroids. Thus, utilizing the cough response might increase the sensitivity of the hypertonic challenges to demonstrate the effects of the antiinflammatory treatment in asthma patients. Third, the recording of the cough response does not require cooperation on the patient’s behalf. Hypertonic challenges with CF as the primary end point could be used for the clinical management of small children as well as for adults with poor cooperation. Fourth, although the present study included only subjects with “classic” asthma,\textsuperscript{10} it is well-known that there are also several patients with asthma-like symptoms and eosinophilic airway inflammation but without airway hyperresponsiveness.\textsuperscript{25,26} Hypertonic challenge cough response may provide a simple, cheap, and minimally invasive tool for the clinical management of such patients. A clinical utilization of the cough response is also supported by the good repeatability of the hypertonic histamine and man-

Nitol\textsuperscript{3} cough indexes.

The present study can be criticized in that neither the hypertonic saline solution nor the isotonic histamine challenges were carried out in a standard way. This was due to the authors’ intention to use identical challenge procedures and the same nebulizer in all challenges, ensuring that all differences in the end point variables (ie, CF and airway responsiveness) between the challenges were due to differences in the challenge solutions. In previous studies with hypertonic saline solution, the saline solution has usually been inhaled in doubling durations, and the output of the ultrasonic nebulizers has been at least 1.2 mL/min,\textsuperscript{13} which is more than twice the output of the nebulizer used in the present study (0.44 to 0.48 mL/min). That is probably why only few subjects developed significant bronchoconstriction during the present hypertonic saline solution challenge. On the other hand, the output of the nebulizer used in the present study was still considerably higher than that of the jet nebulizer used by Cockcroft et al\textsuperscript{27} in their standardized tidal breathing histamine challenge method. Since nebulizer volume output is a crucial factor in determining the airway responses,\textsuperscript{28–30} this difference in outputs may have resulted in lower PC\textsubscript{20} values compared with those obtained by the standard method of Cockcroft et al.\textsuperscript{27} However, the authors think that these methodological variations have not markedly affected the main findings of the present study (the association of cough and bronchoconstriction during the isotonic histamine challenge, and the association of cough and asthma during the two hypertonic challenges).

One could also criticize the study population of the present study. In the real-life clinical setting, the problem is not how to differentiate coughing asthmatic subjects from healthy subjects. The problem is how to differentiate asthmatic subjects from subjects with chronic cough due to causes other than asthma (eg, postnasal drip syndrome or gastroesophageal reflux).\textsuperscript{31} Such subjects were not included in the present study, and their sensitivity to the cough-
provoking effect of hypertonic challenges should be assessed before any clinical utilization of this response. In conclusion, coughing during an airway challenge with a direct smooth muscle contracting agent such as histamine is a physiologic response to induced bronchoconstriction. As bronchoconstriction can be more objectively measured with a spirometer, the recording of the provoked coughs may not be useful. On the contrary, frequent coughing during hypertonic saline solution and hypertonic histamine challenges in the absence of bronchoconstriction is a pathologic phenomenon. Sensitivity to the cough-provoking effect of hypertonic challenges seems to be enhanced in asthma patients. The results of the present study as well as those of the previous study on mannitol\(^3\) suggest that this sensitivity and airway hyperresponsiveness have different pathophysiologic pathways. Therefore, the recording of coughs during hypertonic challenges may provide information that cannot be obtained by airflow measurements.

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