Hypertonicity of the challenge solution may increase the diagnostic accuracy of histamine challenge

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Summary There is significant overlap in the responsiveness to direct airway challenges, such as the histamine challenge, between asthmatic and non-asthmatic subjects, which decreases their accuracy in the diagnosis of asthma. To minimise this overlap, a new test, hypertonic histamine challenge, was developed. Fifteen healthy subjects, 16 subjects with steroid-naive asthma, and 16 asthmatic subjects undergoing inhaled corticosteroid treatment underwent inhalation challenges with hypertonic saline, isotonic histamine, and hypertonic histamine, using an ultrasonic nebuliser and 2-min tidal breathing method. The increase in histamine solution tonicity decreased the histamine PC\textsubscript{20} values only in the steroid-naive asthmatic subjects (1.1 (0.5–2.7) vs. 0.5 (0.2–1.2) mg/ml, \(P = 0.047\)). Using 1 mg/ml as the cut-off value, the sensitivity, specificity, and accuracy of the hypertonic histamine challenge to detect steroid-naive asthma was 81\%, 100\%, and 90\%. The respective values for the isotonic histamine challenge were 56\%, 100\%, and 77\%. Furthermore, there was a statistically significant difference in the hypertonic histamine PC\textsubscript{20} between steroid-naive and steroid-treated asthmatic subjects, which could not be detected in the isotonic histamine PC\textsubscript{20}. The hypertonic histamine PC\textsubscript{20} was highly repeatable, with a single determination 95\% range of \( \pm 1.35 \) doubling concentrations. The hypertonic histamine challenge was safe but provoked more cough and throat irritation than the other two challenges. In conclusion, compared with a conventional, isotonic histamine challenge, hypertonic histamine challenge may be more accurate in the diagnosis of asthma and also, more capable to detect the effects of inhaled corticosteroid treatment.

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KEYWORDS
Asthma; Asthma diagnosis; Bronchial hyperresponsiveness; Histamine; Hypertonic saline

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Introduction

It is widely accepted that physicians need objective measurements to diagnose asthma,\(^1\,^2\) with tests to measure airway hyperresponsiveness (AHR) being among the most useful.\(^1\,^3\,^4\)

Most investigators assess AHR using histamine or methacholine as the provocative stimulus. These stimuli evoke airflow limitation predominantly via a direct effect on airway smooth muscle and the response is usually expressed as either the provocative concentration (PC\(_{20}\)) or dose (PD\(_{20}\)) producing a 20% fall in forced expiratory volume in 1 s (FEV\(_1\)).\(^5\) Airway responsiveness to histamine or methacholine is unimodally log-normally distributed within the population.\(^5\) This continuous distribution plus the 95% confidence interval (CI) of repeatability in the range of \(\pm 1.0\text{–}2.4\) doubling concentrations\(^6\,^7\) leads to a significant overlap in responses between asthmatic and non-asthmatic subjects, which decreases the accuracy of these challenges in the diagnosis of asthma. In the present study we describe a method to minimise this overlap, in order to increase the diagnostic accuracy of histamine challenge.

Indirect airway challenges, including hypertonic saline challenge, induce airflow limitation by acting on cells other than smooth muscle cells. They cause the airways to narrow indirectly by provoking a release of endogenous mediators from inflammatory cells, epithelial cells, and nerves. They are considered as less sensitive than the direct challenges in differentiating asthmatic subjects from normal subjects, but their strength is in their high specificity.\(^5\) Indeed, usually only asthmatic subjects develop airflow limitation in response to inhaled hypertonic saline whereas the changes in airflow parameters in healthy subjects are negligible.\(^9\) Therefore, we hypothesised that if the histamine challenge solution would be made hypertonic by adding saline in it, the bronchoconstriction in asthmatic subjects might appear at lower inhaled histamine concentrations than during a conventional isotonic histamine challenge. We also hypothesised that the increase in the histamine challenge solution tonicity would not alter the histamine responsiveness in healthy subjects. As a consequence, the difference in histamine responsiveness between asthmatic and healthy subjects would thus increase. The present study was planned to test these hypotheses. Since the asthmatic subjects’ response to hypertonic aerosols can often disappear after treatment with inhaled corticosteroids,\(^10\,^13\) we included an equal number of asthmatic subjects with and without this treatment in the present study, in addition to healthy controls.

Materials and methods

Subjects

Forty-nine subjects were recruited for the study. The asthmatic subjects were recruited from the outpatient clinic of the authors’ hospital. All asthmatic subjects were originally referred to the authors’ hospital due to diagnostic uncertainty at the primary health care level. They were included if their symptoms suggested asthma\(^14\) and if they showed variable airway obstruction in ambulatory peak flow (PEF) monitoring, according to previously described criteria.\(^15\) The diagnosis of asthma was not based on any kind of bronchial provocation test to avoid selection of subjects. The exclusion criteria were a respiratory tract infection within four weeks, and FEV\(_1\), less than 65% of predicted.\(^16\) The patients in whom the staff physician considered COPD as the most probable diagnosis were excluded, even if the variable airway obstruction criteria in the ambulatory PEF monitoring were fulfilled. There were 17 newly diagnosed asthmatic subjects who had never used any corticosteroid preparations and 17 asthmatic subjects who used inhaled corticosteroids. However, one steroid-naive subject discontinued the study due to an acute respiratory tract infection and one steroid-treated subject due to severe coughing and headache during the hypertonic histamine challenge. The steroid-naive asthmatic subjects had to be currently symptomatic, i.e., suffering of asthmatic symptoms at least once per week. The 15 healthy volunteers had no chronic respiratory diseases or symptoms and were life-long non-smokers. Table 1 shows the basic characteristics of the 47 subjects who completed the study. The seven current smokers among the asthmatic subjects had smoked median 5.5 (range 0.2–18) pack years. 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.

Protocol

Skin prick tests were performed against common aeroallergens (Soluprick SQ\(^16\), ALK-Abelló, Hörsholm, Denmark) and atopy was defined as at least a 3 mm wheal reaction to any of the allergens.\(^17\) The asthmatic subjects completed a symptom questionnaire and their asthma severity was determined using the Global Initiative for Asthma classification.\(^18\) All subjects underwent three airway challenges in a random order, with at least two
nights between the challenges, approximately at the same time of the day, within 3 weeks. The challenge solutions were hypertonic saline, isotonic histamine and hypertonic histamine. The asthmatic subjects also underwent a second hypertonic histamine challenge, to study its repeatability. For safety reasons, all healthy subjects were studied first, between April and August, followed by the asthmatic subjects between September and April, i.e., outside the pollen season. The study was not blinded.

The challenges

Subjects had refrained from taking aerosol short-acting beta₂ agonists for 6 h, aerosol long-acting beta₂ agonists for 48 h, and antihistamine and leucotriene receptor antagonists for 3 days before the challenges. Inhaled corticosteroids were not taken on the study days.

First, current symptoms were assessed using 120 mm visual analogy scale lines, one line for each of the following symptoms: Headache, skin flushing, nausea, throat irritation, and dyspnoea. Blood pressure (Omron 711, Omron Ltd., Tokyo, Japan) and heart rate were measured. After that spirometry (Model M9449, Medikro Ltd, Kuopio, Finland) and PEF measurements (The Mini Wright, Clement Clarke International Ltd., Harlow, UK) were carried out at least three times, but not more than eight times, according to the American Thoracic Society guidelines and the largest FEV₁ values were used as baseline values.

Every challenge was performed in a similar way, only the challenge solutions changed. Each solution was inhaled for 2 min using tidal volume breathing. Spirometry was performed in duplicate at 90 s from the end of the inhalation and the larger of the two FEV₁ values was used for analysis. The inhalation of the next solution began 4 min after the start of the previous inhalation and the challenge continued until the FEV₁ had fallen 20% or more from the baseline, or up to the inhalation of the final solution.

The airway responsiveness to isotonic and hypertonic histamine challenges was expressed as PC₂₀ by linear interpolation of the relationship between the percent decrease in FEV₁ and the concentration of histamine solution required to provoke the decrease. If the fall in FEV₁ was smaller than 20% after the final histamine concentration, an arbitrary value of 16 mg/ml (twice the maximal concentration administered) was used as the PC₂₀. Coughs were manually recorded throughout the challenges.

Immediately after the challenge, the symptoms were assessed again, and the blood pressure and

<table>
<thead>
<tr>
<th>Table 1 The basic clinical characteristics of the subjects.</th>
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<tbody>
<tr>
<td>Healthy subjects, n = 15</td>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Gender distribution (F = females, M = males)</td>
</tr>
<tr>
<td>Number of atopic subjects</td>
</tr>
<tr>
<td>Number of current smokers</td>
</tr>
<tr>
<td>GINA* classification of asthma severity</td>
</tr>
<tr>
<td>Duration of the inhaled corticosteroid treatment (months)*1</td>
</tr>
<tr>
<td>Daily inhaled corticosteroid dose (μg)*1</td>
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<tr>
<td>FEV₁ (% of predicted1)</td>
</tr>
<tr>
<td>Duration of asthmatic symptoms (months)2</td>
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<tr>
<td>Duration of the inhaled corticosteroid treatment (months)3</td>
</tr>
<tr>
<td>The data is expressed as means, with the minimum and the maximum values in parenthesis.</td>
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<tr>
<td>The reference values are those of Viljanen et al.16</td>
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<tr>
<td>The amounts indicate directly the dose of beclomethasone and budesonide. The fluticasone dose is multiplied by two.</td>
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the heart rate were measured. If the FEV₁ had decreased by 20% or more, the subject inhaled 0.2 mg of salbutamol (Buventol Easyhaler, Orion Ltd., Espoo, Finland) and the spirometry was performed in duplicate at 15 min thereafter. After the latter of the two hypertonic histamine challenges, 10 asthmatic subjects did not inhale salbutamol but their FEV₁ values were monitored in duplicate every 10 min after the challenge up to 1h, to follow their spontaneous recovery. After all the challenges the subjects were asked to record their PEF values in triplicate every second hour until bedtime, and also on the next morning. They were also encouraged to write down in a symptom diary up to the next morning every possible symptom which might be related to the challenges.

The nebuliser

A hand-held ultrasonic nebuliser was used (Omron U1, Omron Ltd., Tokyo, Japan). Before starting the study the volume output of the nebuliser was measured by nebulizing various solutions for 2 min with the mouthpiece attached and weighting the nebuliser plus the mouthpiece before and after nebulisation by a calibrated, high-quality weighing machine, as previously described. The output was steadily 0.44–0.48 ml/min irrespective whether the solution was water, isotonic saline, hypertonic saline, isotonic histamine, or hypertonic histamine within a concentration range of 0.0075–16 mg/ml. The authors did not measure the droplet size distribution of the nebuliser with the various solutions. According to the manufacturer the mass median diameter of the nebuliser is 6 μm. The same nebuliser was used throughout the study.

The challenge solutions

The histamine solutions were prepared and packaged by the Pharmacy Department of Kuopio University Hospital. Briefly, the histamine diphosphate powder (Histamini phosphas, Ph.Eur., University Pharmacy, Helsinki, Finland) was dissolved in phosphate-buffered saline (PBS) solution or hypertonic phosphate-buffered saline (HPBS) solution 8 mg/ml. The PBS was prepared according to an international recommendation and its osmolality was 292 mOsm/kg (measured by freezing-point depression, using The Advanced Micro Osmometer 3300-BR, Advanced Instruments, Inc., Norwood, MA, USA). The formula for the preparation of HPBS is expressed in Table 2 and differs from the PBS formula in that 45 g of NaCl was added instead of 4.4 g, to achieve a high osmolality (1511 mOsm/kg). Other inhalation solutions were made by diluting the isotonic or hypertonic histamine diphosphate 8 mg/ml solution with PBS or HPBS, respectively. The solutions were sterilised by filtration using a 0.22 μm polymer filter (Millex-GV 0.22 μm, Millipore corp., Bedford, MA, USA). Procedures were completed using aseptic techniques under a laminar flow hood. The final product was a unit-dose syringe containing 3 ml of one of the following concentrations of isotonic or hypertonic histamine diphosphate solution: 0.0075, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/ml. The osmolality of the isotonic histamine dilutions was 291–341 mOsm/kg and that of the hypertonic histamine dilutions 1522–1577 mOsm/kg. The syringes were stored in dark location at 4 °C. Before the challenges, they were allowed to equilibrate to room temperature for 30 min and were shaken well.

In the isotonic histamine challenge, the first nebulised solution was pure PBS and in the

<table>
<thead>
<tr>
<th>Matter</th>
<th>Weight (g)</th>
<th>Equivalent weight (g)</th>
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<tr>
<td>Hypertonic phosphate-buffered saline (HPBS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.808</td>
<td>Na₂HPO₄ × 2H₂O</td>
</tr>
<tr>
<td>Na₃HPO₄</td>
<td>7.576</td>
<td>Na₃HPO₄ × 12H₂O</td>
</tr>
<tr>
<td>NaCl</td>
<td>45.000</td>
<td></td>
</tr>
<tr>
<td>H₂O (pH 7.40)</td>
<td>1.000</td>
<td>ad 1000 ml</td>
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</tbody>
</table>

Hypertonic histamine diphosphate 8 mg/ml (26 mmol/l) solution

Histamine diphosphate 8 = HDP × 1H₂O
HPBS ad 1000 ml

Other dilutions
Made by diluting the hypertonic histamine diphosphate 8 mg/ml (26 mmol/l) solution with HPBS

Table 2  Preparation of the hypertonic histamine challenge solutions.
hypertonic histamine challenge it was pure HPBS. The subsequent solutions were the isotonic or hypertonic histamine diphosphate dilutions at doubling concentrations, respectively. In the hypertonic saline challenge the subjects inhaled the HPBS all the time, up to twelve 2 min inhalations. The lung function measurements and the intervals between the inhalations were identical to the histamine challenges.

**Statistical analysis**

The results are expressed as means and 95% CIs. Due to the significant deviation of the distribution from a normal distribution (one-sample Kolmogorov–Smirnov test), the PC20 values are expressed as geometric means and 95% CIs. Wilcoxon signed-rank test was used for paired comparisons, Kruskal–Wallis ANOVA was used to compare the results between the three subgroups, with a post-hoc Mann–Whitney test with Bonferroni correction to compare the results between two subgroups. Spearman’s rho ($r_s$) was used to analyse correlations. To compare the diagnostic accuracy of the histamine and hypertonic histamine challenge as well as to detect the best cut-off value in separating the steroid-naive asthmatic patients and healthy subjects, receiver operator characteristic (ROC) curves were graphically constructed by plotting sensitivity against false-positive rate (1−specificity) for several cut-off values of PC20. Repeatability of the responses was determined by using the single determination 95% range and the intraclass correlation coefficient. All analyses were carried out using SPSS for Windows 9.0.

**Results**

**Airway responsiveness to the challenges**

The increase in histamine challenge solution tonicity significantly decreased the histamine PC20 values in the steroid-naive asthmatic subjects but was without effect on those values in the healthy subjects and in the steroid-treated asthmatic subjects (Table 3). As a consequence, the hypertonic histamine challenge differentiated the steroid-naive asthmatic subjects from the healthy subjects better than the isotonic histamine challenge, which could be demonstrated by the left-upward shift of the ROC curve (Fig. 1). The ROC curve suggested 1 mg/ml as the best cut-off value for both hypertonic and isotonic histamine PC20. Using this cut-off value, the sensitivity, specificity and diagnostic accuracy (steroid-naive asthmatics with a positive test result plus healthy subjects with a negative test result divided by the sum of the two groups) of the hypertonic histamine challenge were 81%, 100%, and 90%. The respective values for the isotonic histamine challenge were 56%, 100%, and 77% (Fig. 2a and b). Also, there was

<table>
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<th>Table 3</th>
<th>Airway responsiveness to the challenges.</th>
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<tr>
<td>Group</td>
<td>Hypertonic saline (%)</td>
</tr>
<tr>
<td>1: Healthy subjects $n = 15$</td>
<td>$-0.3 (-2.8$ to $2.2)$</td>
</tr>
<tr>
<td>2: Steroid-naive asthmatic subjects $n = 16$</td>
<td>$-5.7 (-9.7$ to $-1.7)$</td>
</tr>
<tr>
<td>3: Steroid-treated asthmatic subjects $n = 16$</td>
<td>$-2.4 (-6.4$ to $1.6)$</td>
</tr>
<tr>
<td>$P$ value $^+$ between the three groups</td>
<td>0.039</td>
</tr>
<tr>
<td>Paired comparisons between the groups, $P$-values $^+$</td>
<td>1 vs. 2: 0.040</td>
</tr>
<tr>
<td>1 vs. 3: 0.73</td>
<td>1 vs. 3: 0.024</td>
</tr>
<tr>
<td>2 vs. 3: 0.13</td>
<td>2 vs. 3: 0.42</td>
</tr>
</tbody>
</table>

*Since the great majority of the subjects did not show a 20% fall in FEV₁ during the hypertonic saline challenge, the response is expressed as the maximal percentage fall in FEV₁.

$^+$Comparison between histamine and hypertonic histamine, Wilcoxon signed-rank test.

$^+$Kruskal–Wallis ANOVA.

$^+$Mann–Whitney test with Bonferroni correction.
a statistically significant difference in the responsiveness to hypertonic histamine between steroid-naive and steroid-treated asthmatic subjects (Table 3) whereas the responsiveness to isotonic histamine did not differ significantly between these subgroups. The responses to hypertonic saline were mild. It induced a statistically significant ($P = 0.008$) change in FEV$_1$ in steroid-naive asthmatic subjects only. A 20% or greater fall in FEV$_1$ was only demonstrated by one steroid naive and one steroid-treated asthmatic subject and the responses to saline are therefore expressed as the percentage change in FEV$_1$ after the final saline inhalation (Table 3). The PC$_{20}$ of the hypertonic histamine challenge was highly repeatable, with a single determination 95% range of $\pm 1.35$ doubling concentrations and with an intraclass correlation coefficient of 0.965.

**Safety issues**

None of the challenges induced any statistically significant changes in the systolic blood pressure but all induced slight changes in heart rate. Hypertonic saline decreased it by 4 (2–6) beats/min ($P<0.0001$), isotonic histamine increased it by 3 (1–5) beats/min ($P = 0.006$), and hypertonic histamine increased it by 5 (2–8) beats/min ($P = 0.001$). The hypertonic histamine-induced bronchoconstriction was rapidly reversed by 0.2 mg of salbutamol in the asthmatic subjects. Without the bronchodilating treatment, the recovery was almost complete within 1 h after the challenge (Fig 3). The late PEF recordings showed no signs of late asthmatic responses after any of the challenges (data not shown).

**Side effects**

There were the following statistically significant ($P<0.001$) increases in visual analogy scale symptom readings: Saline challenge induced throat irritation (17 (11–24) mm). Isotonic histamine induced dyspnea (38 (28–47) mm), throat irritation (24 (16–32) mm), and skin flushing (8 (3–13 mm). Hypertonic histamine induced dyspnea (39 (29–49) mm), throat irritation (40 (30–51) mm), and skin flushing (17 (8–26) mm). Though not included in the visual analogy scale lines, nasal running and sputum production were reported by some asthmatic patients during all challenges. The total number of coughs was higher during the
hypertonic histamine challenge than during the isotonic histamine and the hypertonic saline challenge (40 (31–50) coughs, 20 (13–28) coughs, and 27 (15–39) coughs, respectively, \( P < 0.0001 \)). In general, the subjects experienced the hypertonic histamine challenge as more unpleasant than the hypertonic saline and the isotonic histamine challenge (49 (39–59) mm, 33 (25–42) mm, and 38 (29–48) mm, respectively, \( P = 0.001 \)). In the symptom diary after the challenges up to the following morning there were the following infrequent reports about side effects after hypertonic and isotonic histamine challenges: headache (five and two out of the 47 subjects, respectively), hoarseness (eight and four subjects), and cough (four and five subjects).

**Discussion**

The present study demonstrated that it is possible to increase the diagnostic accuracy of histamine challenge by using a hypertonic challenge solution. The rise in tonicity of the challenge solution decreased the histamine PC_{20} values in steroid-naive asthmatic subjects but was without effect on those values in healthy and steroid-treated asthmatic subjects. As a consequence, the difference in histamine responsiveness between healthy and steroid-naive asthmatic subjects increased and the diagnostic accuracy of the histamine challenge therefore improved. This feature of the hypertonic histamine challenge may be especially advantageous in a situation where there is a high degree of diagnostic uncertainty, like in case of the asthmatic patients who participated in the present study. The authors stress that the patients in the present study were not pre-selected on a basis of responsiveness to any kind of airway challenge but probably well represent an asthmatic population referred to a tertiary hospital due to diagnostic difficulties at the primary health care level. The present study also demonstrated that hypertonic histamine challenge is safe and gives well repeatable results. However, it induced more side-effects than the conventional, isotonic histamine challenge.

The decrease in the histamine PC_{20} values by the rise in challenge solution tonicity in steroid-naive asthmatic subjects is probably related to the increased sensitivity of asthmatic airways to various osmotic stimuli.\(^{23}\) Indeed, inhalation of hypertonic saline in the present study induced statistically significant bronchoconstriction only in the steroid-naive asthmatic subjects. On the basis of the present knowledge about hypertonic aerosols,\(^{24}\) the hypertonic-histamine induced bronchoconstriction in steroid-naive asthmatic subjects was probably a sum effect of exogenous histamine plus the various endogenous bronchoconstrictive mediators released by the airway mast cell. The airway mast cell is capable to release smooth muscle-active mediators like prostaglandin D_{2}, cysteinyl-leukotrienes, as well as histamine, in response to a rise in ambient tonicity.\(^{24,25}\)

Theoretically, there might also be technical factors, which could have explained the difference in histamine PC_{20} values between isotonic and hypertonic histamine challenges in the steroid-naive asthmatic subjects. Thought the volume output of our nebuliser was not affected by the change in challenge solution tonicity, we do not know the retained doses of the produced aerosols in each region of the airways. Particle size is the prime factor governing the fraction of inhaled particles that penetrates past the oropharynx and enters the lungs, and the particle fraction that deposits in each region of the airways.\(^{26}\) Therefore, if the change in challenge solution tonicity had affected the droplet size distribution in the present study, it could have caused a change in the lower airway histamine dose. For example, hypertonic droplets gain water and grow within the airways during inhalation whereas isotonic droplets maintain their size.\(^{26}\) This rise in droplet size probably increases the airway deposition of the initially very small droplets, but may also decrease the lower airway deposition of the initially 2–8 μm droplets, since fewer than half particles larger than 8 μm get past the larynx during tidal breathing.\(^{26}\)
Hypertonicity of the challenge solution may increase the diagnostic accuracy of histamine challenge

study an experimental airway model with a relative humidity of 99% should have been used. Such a device was not available for the authors and the droplet size distribution was therefore not measured. However, since the change in histamine challenge solution tonicity did not affect the histamine PC_{20} values in the healthy and steroid-treated asthmatic subjects, the authors assume that technical factors associated with the change in the challenge solution tonicity had not changed the retained dose of histamine in the lower airways significantly, and can therefore not explain the finding in steroid-naive asthmatic subjects.

Direct airway challenges such as the histamine challenge have often been accused of being insensitive in demonstrating the effect of inhaled corticosteroid treatment in asthma. According to a meta-analysis, a long-term treatment with high doses of inhaled corticosteroids causes on average of just 1.16 doubling doses or concentrations change in the airway responsiveness to direct stimuli. Our results, though obtained from a cross-sectional study, are in line with this observation. Indeed, there was just a 0.9 doubling concentration difference in the isotonic histamine PC_{20} between steroid-naive and steroid-treated asthmatic subjects, which was not statistically significant in our rather small study group. On the contrary, the difference in the hypertonic histamine PC_{20} between the steroid-naive and steroid-treated asthmatic subjects was 2.2 doubling concentrations and this was statistically significant. Therefore, hypertonic histamine challenge may be more sensitive than isotonic histamine challenge in demonstrating the effect of inhaled corticosteroid treatment in asthma. This is probably based on the fact that airway responsiveness to hypertonic aerosols is very sensitive to the effects of inhaled steroids. However, a follow-up study would be needed to confirm the findings of the present study.

Comparison of our results with those of previous studies is difficult due to technical and methodological differences. The present histamine challenge method is developed from that described by Cockcroft et al., who used a jet nebuliser with a volume output of 0.13 ml/min. However, we had to use an ultrasonic nebuliser, which is recommended for non-isotonic challenges because it produces more dense aerosols. We intended to use a relatively low-volume output ultrasonic nebuliser but its output (0.44–0.48) was still considerably higher than that used by Cockcroft et al. Since nebuliser volume output is a crucial factor determining the airway response, direct comparison of our results with those obtained using the Cockcroft method is not valid. Similarly, comparison of our hypertonic saline responses with those in previous studies is difficult since in previous studies the output of the ultrasonic nebulisers has been at least 1.2 ml/min, more than twice to that of the Omron nebuliser. This and the decision to challenge the asthmatic patients outside the pollen season probably mainly explain why so few asthmatic patients responded to hypertonic saline in the present study.

Hypertonic histamine challenge induced more side effects than the other two challenges, mainly cough and throat irritation. However, it produced well repeatable responses and was safe. It did not induce any clinically significant changes in heart rate or blood pressure. There were no significant adverse effects up to the following morning and the late PEF recordings showed no evidence of any late asthmatic reaction. The induced bronchoconstric-

tion was rapidly reversible with a conventional dose of inhaled salbutamol, and without treatment it almost completely vanished within 1 h. Due to the fact that the steroid-naive asthmatic patients usually responded to less than 1 mg/ml hypertonic histamine concentration, in future this test could probably be terminated after the administration of 2 or 4 mg/ml solution. This practice would decrease the side effects and therefore increase the acceptability of the hypertonic histamine challenge.

In conclusion, it seems that the diagnostic accuracy of histamine challenge can be improved by using a hypertonic challenge solution. Hypertonic histamine challenge may also be more capable to detect the effects of inhaled corticosteroid treatment than the conventional, isotonic histamine challenge. Further studies with a larger number of patients are needed to confirm these findings and also, to explore whether these benefits of hypertonic histamine challenge outweigh the slightly greater side effects associated with this challenge.

Acknowledgements

This work was performed at Kuopio University Hospital, 70211 Kuopio, Finland. This work was financially supported by Kuopio University Hospital. The authors thank Raija Tukiainen, RN, for her assistance.

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