INTRODUCTION

Bronchial asthma (BA) is one of the most frequent chronic respiratory diseases which can be seen in daily pediatric practice. Besides the typical clinical presentation commonly observed in adults (night/morning cough, dyspnoea, wheezing, and chest tightness), the most prevalent clinical sign of BA in children is cough of various types. Although cough is a major symptom in some children with asthma, the relationship between cough and the severity of asthma is defined insufficiently. There is only limited data on the frequency, severity, and prevalence of cough in children with classical asthma (1, 2). However, the presence of cough alone in children with asthma can be interpreted as a symptom of inadequately treated asthma. It was also shown that children with recurrent cough differ from those with asthma with or without cough.

One of the main barriers to understanding the importance of cough in asthma has been the lack of well validated measures. The measurement of cough in both clinical practice and in clinical trials has been generally restricted to the use of subjective reports or scoring systems (3). As cough represents common problem of pediatrics, several objective methods aimed in objective assessment of various characteristics of cough were developed. Recently, several research groups aimed to establish well-standardized clinical method suitable for clinical assessment of cough (4). Most of the studies were performed in the populations consisting mainly from adults, but there were only a few clinical trials with children and adolescents (1, 2, 5, 6). These methods can serve for the evaluation of physical aspects of cough, such as its amplitude or frequency. Cough reflex sensitivity (CRS) test with capsaicin is one of the most important tools for studying cough. In the present study, we aimed to study the CRS in various phenotypes of childhood asthma. We found that, in general, CRS was increased in asthmatic children compared with controls. The most evident increase of CRS was observed during acute asthma exacerbation, in children suffering from asthma with concomitant allergic rhinitis, and in atopic asthmatics. Interestingly, we noted a significant decline in lung function after capsaicin CRS. Various laboratory and clinical characteristics of asthmatic children influence cough sensitivity to a different extent. Cough reflex sensitivity measurement can add valuable information beside the commonly used spirometric and inflammometric methods in the management of asthmatic children.

Key words: bronchial asthma, capsaicin challenge, children, phenotype, cough reflex sensitivity

COUGH REFLEX SENSITIVITY IN VARIOUS PHENOTYPES OF CHILDHOOD ASTHMA

Cough is a major symptom in some children with asthma, but the relationship between cough and the severity of asthma is defined insufficiently. As cough represents common problem of pediatrics, several objective methods for its assessment were developed. Cough reflex sensitivity (CRS) test with capsaicin is one of the most important tools for studying cough. In the present study, we aimed to study the CRS in various phenotypes of childhood asthma. We found that, in general, CRS was increased in asthmatic children compared with controls. The most evident increase of CRS was observed during acute asthma exacerbation, in children suffering from asthma with concomitant allergic rhinitis, and in atopic asthmatics. Interestingly, we noted a significant decline in lung function after capsaicin CRS. Various laboratory and clinical characteristics of asthmatic children influence cough sensitivity to a different extent. Cough reflex sensitivity measurement can add valuable information beside the commonly used spirometric and inflammometric methods in the management of asthmatic children.

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COUGH REFLEX SENSITIVITY IN VARIOUS PHENOTYPES OF CHILDHOOD ASTHMA

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MATERIAL AND METHODS

The study was approved by the Ethics Committee of Jessenius School of Medicine in Martin, Slovakia. Informed written consent was obtained from the parents of all tested children.

We studied a population of 101 children (53% boys and 48% girls): 86 asthmatic children (mean age 13 ±3 yr) and 15 healthy age-matched subjects (mean age 14 ±3 yr). None of the subjects was smoking and none of the healthy controls was atopic. Asthmatic children involved in the study were characterized by recurrent airways obstruction manifested by wheezing and dyspnea relieved spontaneously or by bronchodilator therapy (as defined in GINA). 15 children (17%) were tested during the acute exacerbation of bronchial asthma (episode of progressive worsening of asthma symptoms such as shortness of breath, wheezing, chest tightness, and/or coughing) and 71 children (83%) were examined when their disease was fully clinically controlled (steady-state of chronic asthma). 33 children (38%) of our asthmatics suffered from the isolated form of asthma without any allergic co-morbidity and 53 subjects (62%) had concomitant allergic asthma (defined clinically in the ARIA).

According to the results of skin prick tests with common inhalant allergens, 51 children (69%) had so-called atopic asthma and 23 (31%) had non-atopic asthma. All the children with at least one positive skin prick test to common aeroallergens were considered as atopic. The children or their parents filled a questionnaire aimed on personal and family history of allergic and respiratory diseases, frequency of upper or lower respiratory infections, presence of pets at home, actual clinical complaints, signs of respiratory disease, and provided therapy. All the children underwent blood sampling for blood cell count and basic biochemical analysis (C-reactive protein and total IgE in serum). The children also underwent the measurement of fractional exhaled nitric oxide (FeNO) with NIOX Mino® (Aerocrine, Sweden). The FeNO values were estimated prior to the assessment of cough reflex sensitivity.

Assessment of cough reflex sensitivity

Cough reflex sensitivity was assessed according to the guidelines of European Respiratory Society on assessment of cough (4). All the testing was performed with compressed air-driven nebuliser (model 646; DeVilbiss Health Care, Somerset, PA) controlled by a standardized dosimeter (KoKo DigiDoser-Spirometer; nSpire Health, Louisville, CO) with an inspiratory flow regulator valve added (RIFR; nSpire Health, Louisville, CO) to provide an identical inspiratory flow rate during the inhalation of capsaicin aerosol in all the tested subjects. Each subject inhaled saline randomly interspersed with 12 incremental capsaicin aerosol concentrations (from 0.61 to 1250 µmol/l). At the beginning of the testing, each child inhaled the aerosol of a negative control solution (0.9% saline), followed by the inhalation of 12 capsaicin aerosol concentrations. Each administration of saline and capsaicin aerosol was performed at 1 min interval with the inhalation time set at 400 ms. During the inhalation of each concentration of capsaicin and control solution, we counted the number of coughs during 30 s after actuation of the dosimeter. The testing was stopped when 5 coughs were counted or when the maximum concentration of capsaicin (1250 µmol/l) was reached (C5 parameter). Cough reflex sensitivity (CRS) was defined as the lowest capsaicin concentration required to provoke two or more coughs (C2 parameter) and five or more coughs (C5 parameter) (4). We performed also basic spirometry before and after capsaicin challenge (KoKo DigiDoser-Spirometer; nSpire Health, Louisville, CO).

Skin prick tests

Skin prick tests (SPT) were done in a predefined area on the volar side of the left forearm, with the following inhalant allergens panel: Dermatophagoides pteronyssinus, Dermatophagoides farinae, mixed 5 grasses (Avena sativa, Hordeum vulgare, Secale cereale, Triticum sativum), cat dander, dog dander, feather mix, Artemisia vulgaris, Artemisia folia, Betula alba, mixed 4 cereals and mixed moulds (Cladosporium herbarum, Aspergillus mix, Alternaria alternata) (ALK-ABELLO, Horsholm, Denmark). We also performed a prick with histamine ditydrochloride (10 mg/dl). 50% glycine in saline was applied as a negative control on the right forearm. We used tip metallic lancets (ALK-ABELLO, Horsholm, Denmark). The lancet was pricked vertically into the skin through each drop for 2 s with firm pressure. A new lancet was used for each prick test. 15 minutes after the procedure ended, the wheals were outlined with a thin felt-tip pen. The contours were transferred to a record sheet with a translucent tape. The size of each wheal was measured as the mean of the longest diameter and the diameter perpendicular to it at its mid point. A Skin prick test with allergens was defined as positive if the wheal was ≥3 mm in its longest dimension. All the tests were performed by the same well-trained operator.

Statistical methods

Data are expressed as means ±SE unless otherwise indicated. Data were analyzed with the software package SPSS version 9.0 (SPSS Inc. Chicago, IL). Student’s two-tailed t test, Mann-Whitney U non-parametric test, chi square (χ²) test, and Fisher’s exact test were used for statistical comparison. Spearman’s correlation (rS) was used for correlation studies. For paired data we used a paired-samples t-test. P values ≤0.05 were considered to indicate statistical significance.

RESULTS

We studied cough reflex sensitivity measured by the inhalation of capsaicin aerosol in increasing concentration in the asthmatic children with various clinical phenotypes and expressions of asthma. The children were divided during the comparative studies into various subgroups: asthmatics vs. healthy controls, stable vs. acute asthma, isolated asthma vs. asthma associated with allergic rhinitis, and atopic vs. non-atopic asthma. 11% (9/86) of the children were treated with inhalant corticosteroid (ICS), 38% (33/86) with leucotrien receptor antagonists, 69% (59/86) had fixed combined inhalant preparation in their therapy (inhalant corticosteroid + long-acting β₂-agonist) and 90% (77/86) also were taking antihistamines. Compliance with our recommended therapy was noticed in 92% of all children (79/86) and the rest of the children did not take their medicaments regularly or ever (8%, 7/86). 76% of children (65/86) confirmed the presence of recurrent respiratory diseases (predominantly of lower airways with bronchoobstruction). 24% of all subjects (14/59) had a pet in their house (cat, dog, or bird). We observed 44% positive reactions in skin prick testing to Dermatophagoides pteronyssinus, 41% to Dermatophagoides farinae, 17% to mixed feather allergens, 21% to cat dander, and 15% to dog dander. The frequencies of positive SPT to other allergens were 18% to Artemisia folia, 21% for Artemisia vulgaris, 23% for Betula alba, 43% for mixed grasses, 22% for mixed cereals and 19% for moulds. According to the results of SPT, 69% (51/74) children were atopic (at least one positive skin reaction).

The values of the C2 parameter were somewhat lower in girls compared with boys, and also were lower in younger...
compared with older children, both of which point to a tendency
to decreased cough reflex sensitivity. These differences,
however, did not reach statistical significance.

Cough reflex sensitivity expressed by the C2 parameter
widely differed in various subgroups of asthmatic children in
comparison with healthy subjects. Comparing CRS between
asthmatic population of 86 children and 15 healthy subjects, we
observed significantly lower C2 values in asthmatics (C2:
53.6±19.0 µmol/l vs. 116.0±58.1 µmol/l, respectively, P=0.017;
Fig. 1A). Asthmatic children during acute exacerbation showed
an evident increase in capsaicin hypersensitivity compared with
stable asthmatics in clinically-controlled phase of BA (C2:
5.1±2.7 µmol/l vs. 63.3±22.6 µmol/l, respectively, P=0.002) and
compared with healthy controls (C2: 5.1±2.7 µmol/l vs.
116.0±58.1 µmol/l, respectively, P<0.001; Fig. 1B). Children
with isolated form of BA were characterized by nearly the same
values of the C2 parameter as healthy controls (C2:
93.9±45.9 µmol/l vs. 116.0±58.1 µmol/l, respectively, P=0.066;
Fig. 1C), but conversely, children with asthma and concomitant
allergic rhinitis or rhinoconjunctivitis revealed increased
capsaicin sensitivity expressed by lower values of C2 (C2:
28.7±11.9 µmol/l vs. 116.0±58.1 µmol/l, respectively, P=0.014;
Fig. 1C). Atopic bronchial asthma was associated with enhanced
cough reflex sensitivity compared with healthy control subjects
(C2: 17.2±3.5 µmol/l vs. 116.0±58.1 µmol/l, respectively,
P=0.005; Fig. 1D). Capsaicin hypersensitivity was also increased
in non-atopic asthmatics compared with healthy subjects (C2:
34.3±23.7 µmol/l vs. 116.0±58.1 µmol/l, respectively, P=0.019;
Fig. 1D), but this difference was smaller than the difference in
CRS between atopic asthmatics and healthy controls. We did not
observed a significant difference between atopic and non-atopic
asthmatic children (C: 17.2±3.5 µmol/l vs. 34.3±23.7 µmol/l,
P=0.86; Fig. 1D). No significant difference in C2 was found
between the children with or without recurrent upper or lower
respiratory infections and with or without the exposure to pets
(data not shown). Analyzing the changes in the C5 parameter, we
were not able to show any differences among various asthmatic
subgroups and healthy subjects.

Spirometric indices (forced expiratory volume in 1 second,
FEV1; forced vital capacity, FVC; forced expiratory flow, FEF25-
75%; and peak expiratory flow rate, PEFR25-75%) did not show any
significant changes between the studied subgroups of asthmatic
children and healthy controls, with the exception of children
with isolated BA and asthmatics during acute exacerbation who
had significantly lower FEV1, FEF25-75%, and PEFR than those in
healthy subjects. Comparing these parameters before and after
capsaicin CRS challenge, we noted a significant decline in FVC
(101±1.3% vs. 100±1.4% predicted, P=0.021) and PEFR25-75%
(74±1.7% vs. 70±1.8% predicted, P=0.005), while FEV1 and
FEF25-75% did not change significantly.

Capsaicin cough reflex sensitivity expressed by C2 and C5
values did not correlate with spirometric indices, peripheral blood
eosinophilia, exhaled nitric oxide fraction, or asthma control test
scores. During the testing of cough reflex sensitivity, we did not
observe any serious adverse effects, although only a few patients
complained about throat itching. Bronchoconstriction with
wheezing developed in one boy during the testing, but was
resolved with the inhalation of short-acting β2-agonist.

**DISCUSSION**

In the present study we assessed changes in cough reflex
sensitivity by capsaicin challenge in various phenotypes of

![Fig. 1](image-url). Comparison of cough reflex sensitivity among the groups of children studied: Panel A - between asthmatic children and healthy control subjects; Panel B - among children with acutely exacerbated, stable asthma, and healthy subjects; Panel C - among children with asthma associated with other allergic respiratory co-morbidity (allergic rhinitis), with isolated asthma, and healthy subjects; Panel D - among atopic asthmatics, non-atopic asthmatics, and healthy subjects.)
childhood asthma according to different laboratory and clinical characteristics of the studied subjects. We found that asthmatics showed, in general, a lower cough threshold than healthy subjects and cough reflex sensitivity also was higher during acute exacerbation of asthma. In the literature, there are conflicting results regarding the CRS in asthmatics. The heterogeneity of the studied populations and methodological differences in CRS challenges could likely contribute to the discordant data. Several studies have shown that in asthmatics in whom cough is the sole or predominant symptom, CRS is significantly enhanced (7), whereas in those with wheezing and dyspnea predominating, CRS is not altered (8).

In the present study, during severe exacerbations of asthma, CRS was significantly increased. CRS of the group who coughed was significantly higher than those who did not cough during acute asthma. CRS was, however, not different between the groups studied in relation the presence of viral infection. Altered sensitivity of cough receptors during an acute exacerbation may be a reason why some children with asthma cough (1). In another study of the same group, asthma exacerbation was characterized by increased cough symptoms and eosinophilic inflammation. Cough scores and CRS did not reflect eosinophilic airway inflammation. Both CRS outcomes (C2 and C5) did not correlate to any marker of clinical severity (asthma score, cough score, quality of life score), pulmonary function indices (FEV1, or its variability, FVC), or inflammatory markers (IL-8, serum and sputum eosinophilic cationic protein, serum and sputum eosinophils and neutrophils) of asthma during any of the test days (9). The results of the challenge with acetic acid also were compared among healthy children, children reporting cough and asthmatic children (10). Differences in cough threshold between the groups were non-significant, although a subgroup of subjects with asthma showed a reduction in cough threshold. The authors suggested that the hyperresponsiveness of individual cough receptors without the stimulation of irritant receptors is present in patients with asthma. Increased CRS in asthmatics has been confirmed by other studies (11). It is probable that increased cough susceptibility in asthmatics is produced either by very non-specific means or involves an entirely different pharmacological pathway from the mechanisms which determine the severity of airways reactivity or resting airway caliber (11). In contrast, several authors did not observe the differences in CRS between asthmatics and healthy subjects (9,12-14). Fujimura et al. (14) supposed that cough sensitivity is entirely independent of bronchial responsiveness or bronchomotor tone, and it is within normal limits in typical asthma. All these studies do not show differences in CRS expressed usually by the C5 parameter, which is in agreement with the present results. However, we did observe increased cough sensitivity when C2, the smallest concentration of a tussinogen inducing two coughs, was employed in cough assessment. In contrast, Blanc et al. (15) showed differences in C5 and not in C2 in asthmatic cough assessment. A recently published study analyzed the relationship between objective and subjective measures of cough in subjects with typical asthma, not selected for the symptom of cough. The authors showed that the correlations between objective cough frequency and subjective measures of cough in asthma were at best moderate. The best relationship with objective cough rates was seen for cough-related quality of life assessed by a standardized questionnaire. The authors also found that C2 rather than C5 relates to cough in asthma, which was unexpected. These different cough reflex thresholds may be of different importance depending on the kind of disease. Perhaps, a lower intensity of the cough eliciting stimulus suffices in asthma compared with other conditions in which C5 is more closely related to objective cough frequency (3). Although C5 appears the preferred end-point of cough challenge testing by a majority of investigators, and has a higher reproducibility in some (16), but not all studies (17), other previous publications have provided only C2 data. According to our experience, C2 parameter seems more suitable for the characterization of CRS in asthmatic children.

Children suffering from bronchial asthma together with allergic rhinitis revealed a higher cough reflex sensitivity than healthy subjects; the latter did not differ from the children with isolated asthma. An association between allergic rhinitis and bronchial asthma is well known and sufficiently documented in the literature, although the exact pathophysiological interaction between upper and lower airways is not completely understood. CRS is significantly increased in pollen-sensitive subjects suffering from seasonal allergic rhinoconjunctivitis during both pollen season and out of it. The severity of asthma symptoms correlates with the symptoms of allergic rhinitis. Treating the symptoms of allergic rhinitis improves those of asthma and vice versa inducing allergic inflammation in the nose worsens asthma symptoms (18). It has been shown that pathological processes in the nose of any ethiology could cause a sensitization of the cough reflex, with decreased cough threshold during asymptomatic period of allergic rhinitis (19). CRS is markedly increased in children with acute upper respiratory infection (20).

In the present study, atopy contributed to the enhancement of CRS in asthmatic children. Fujimura et al. (14) showed that the patients with atopic cough (defined as persistent non-productive cough with the presence of one or more findings confirming atopy) had higher CRS in comparison with so-called cough variant asthma. In their another study, atopy indicated by specific IgE to mite-related antigens, but not to pollen antigens, was associated with non-specific bronchial responsiveness to methacholine in asthmatics, while it was not a determinant of airway cough sensitivity (expressed by C5 parameter) in healthy non-asthmatics. Capsaicin cough threshold was not associated with atopy indicated by specific IgE to common aeroallergens (13, 21). Also, in the study of Riordan et al. (22), the cough threshold (assessed by citric acid challenge) was unrelated to respiratory symptoms, bronchial responsiveness, parental smoking, and atopic status. Capsaicin CRS in allergic atopic asthmatics increases during the birch season, which is likely a consequence of prolonged and intensive allergen exposure (23). Increased CRS in association with allergen sensitization has been confirmed in animal models. Active sensitization with the protein fraction of Aspergillus restrictus, even without subsequent allergen challenge, enhances the excitability of cough receptors to tussigenic stimuli (24).

The relation between CRS and respiratory function has been studied, but the results are contentious. The present findings show that capsaicin challenge influenced to pulmonary function, as it caused decreases in forced vital capacity and peak expiratory flow rate. In the study of Chang et al. (6), FEV1 (% predicted) also correlated with cough sensitivity measures. Inhalation of large doses of capsaicin can probably influence airway hyperresponsiveness. Measuring airway conductance, a more sensitive method than spirometry, to assess the airway caliber, a temporal and short-lasting (maximal within 20 s and lasting less than 60 s) bronchoconstriction has been reported after capsaicin inhalation (25). Inhaled capsaicin significantly decreases FEV1 at the C5 threshold dose in patients with asthma or chronic bronchitis in the study of Fujimura et al. (26). In contrast inhaled procaterol increases FEV1 in similar patients. Furthermore, procaterol inhibits the capsaicin-induced fall in FEV1 (25). In a study of Doherty et al. (11), CRS was independent of the degree of airway obstruction and in asthmatics it was not related to PEF variability. Other authors have not been able to confirm the relationship between CRS
threshold and respiratory function, as measure by spirometry (12, 21). Therefore, the mechanisms producing cough are likely to be different from those causing airways obstruction (11).

In conclusion, capsaicin cough challenge, assessing the C2 parameter, is a simple and reproducible laboratory method for the assessment of cough susceptibility, severity, and variability in a wide range of diseases. Since the mechanisms producing cough in asthma are different to those provoking airways obstruction, the CRS measurement can add valuable information besides commonly used spirometric and inflammometric methods in clinical praxis in management of asthmatic children.

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