Early and Late Allergic Phase Related Cough Response in Sensitized Guinea Pigs with Experimental Allergic Rhinitis

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Summary

Cough is a common and important symptom of asthma and allergic rhinitis. Previous experimental evidence has shown enhanced cough sensitivity during early phase of experimental allergic rhinitis in guinea pigs. We hypothesized that airway inflammation during the late phase response after repeated nasal antigen challenge may affect the afferent sensory nerve endings in the larynx and tracheobronchial tree and may also modulate cough response. In the present study we evaluated the cough sensitivity during a period of early and late allergic response in sensitized guinea pigs after repeated nasal antigen challenges. Forty-five guinea pigs were sensitized with ovalbumin (OVA). Four weeks later 0.015 ml of 0.5 % OVA was intranasally instilled to develop a model of allergic rhinitis that was evaluated from the occurrence of typical clinical symptoms. Animals were repeatedly intranasally challenged either by OVA (experimental group) or by saline (controls) in 7-day intervals for nine weeks. Cough was elicited by inhalation of citric acid aerosols. Cough was evaluated at 1 or 3 h after the 6th nasal challenge and 17 or 24 h after the 9th nasal challenge. The cough reflex was significantly increased at 1 and 3 h after repeated nasal challenge in contrast to cough responses evoked at 17 and 24 h after repeated nasal challenge. In conclusion, enhanced cough sensitivity only corresponds to an early allergic response after repeated nasal challenges.

Key words

Introduction

Asthma and allergic rhinitis are the most frequent causes of the chronic cough (Morice and Kastelik 2003). Both conditions have similar imunological mechanisms and underlying pathogenesis (Nutku *et al.* 2001). The pathophysiological events following allergen exposure are described as biphasic, composed of an early (mediated by mast cells-derived mediators) and a late phase response (occurs from 4 h to 12 h after exposure and persists to 24 h) and involves increased recruitment and activation of inflammatory cells such as T cells, neutrophils, macrophages and eosinophils. Several factors could be suggested to explain the relationship between the upper and lower airways. Increased levels of inflammatory factors in the blood and

propagation of inflammation through the airway or systemic pathways, can be possible mechanisms for lower airway dysfunction among patients with upper airway disease such as rhinitis (Lipworth and White 2000). Nevertheless, the underlying pathology of both asthma and rhinitis is inflammation in which eosinophilia is a characteristic feature (Lipworth and White 2000, Nutku et al. 2001).

According to the association between rhinitis and asthma several pathophysiological mechanisms could explain the enhanced cough sensitivity during rhinopathies: nasal obstruction leading to mouth breathing, an increased deposition of inhaled allergen in lower airways, a nasal-bronchial reflex, microaspiration of nasal secretion, postnasal drip syndrome and an increased bronchial hyperresponsiveness in subjects with allergic rhinitis (Lalloo et al. 1996, Plaschke et al. 2000).

In case of allergic rhinitis, the cough reflex may be sensitized through an action of inflammatory mediators from the nasal mucosa or reflex sensitization of airway sensory nerves or facilitation of the central cough generator from nasal reflex input (Lalloo et al. 1996, Mazzone and Canning 2002, Plevková et al. 2004).

Previous experimental evidence has demonstrated significantly enhanced cough sensitivity during exudative allergic rhinitis in awake sensitized guinea pigs immediately after nasal challenge (Tatár et al. 2002). Our hypothesis is that cough sensitivity could be increased by the airway allergic reaction characterized by airway eosinophilic inflammation. We supposed that airway inflammation during the late phase response after repeated nasal antigen challenges through recruitment and activation inflammatory cells might affect afferent sensory nerve endings in the lower airways and this could modulate the cough response. Therefore, the goal of our study was to elucidate the hypothesis and clarify the characteristics of the increased cough sensitivity in the early as well as in the late allergic response in sensitized awake guinea pigs after repeated nasal antigen challenges.

Methods

Animals

Male Trik strain guinea pigs (n=45) weighing 250-350 g were used and divided into two separate experimental groups according to a cough protocol. During an adaptation phase animals were housed in an air-conditioned room and were fed a standard laboratory diet and given drinking water *ad libitum*. All experiments complied with the national guidelines and were approved by Jessenius Faculty of Medicine Ethics Committee of Martin, Slovakia.

Ovalbumin sensitization

After acclimatization to laboratory conditions, in the first phase of our experiments, the sensitization of animals was performed. All animals were sensitized with ovalbumin (OVA, 10 μg, Sigma), intra-peritoneally administered together with aluminium hydroxide (100 mg) in saline (1 ml i.p.), using the modified method described by Underwood et al. (1995). Twenty-one days later, successful sensitization was confirmed by the intradermal injection of ovalbumin solution (25 μl of 200 μg.ml^{-1}) into the dorsal back surface. Sensitized animals were used 7 days later for experiments.
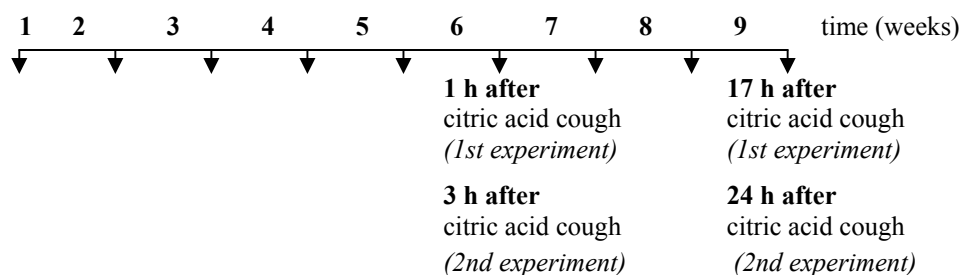
Model of allergic rhinitis

In the second phase of our experiments sensitized experimental animals (n=24) were used for developing a model of allergic rhinitis by repeated intranasal instillation of 0.015 ml of 0.5 % ovalbumin separately for each nostril using a thin catheter. Animals were tested at 7-day intervals, totally 9 times. Control animals (n=21) were repeatedly intranasally challenged with saline in the same dose as the experimental animals. The immediate reaction of animals after nasal challenge was examined according to symptoms of allergic rhinitis.

Evaluation of clinical symptoms

After nasal provocation with ovalbumin, the allergic rhinitis was evaluated from the occurrence of typical clinical symptoms with respect to nose and eyes irritation, like sneezing, conjunctival and nasal secretion, and nasal acoustic phenomenon reflected the degree of nose obstruction. Having standard method the personal monitoring of these symptoms during a period of one hour after nasal challenge was done in each animal.

The frequency of sneezes and other nasal symptoms such as the nasal acoustic phenomenon and lacrimation were evaluated using a scoring system. Symptom scores were graded on a four-point scale. Each grade was assigned a numerical score (0-3), and data were analyzed both as separate symptoms and as a total symptom score. Nasal acoustic symptom scores were graded in points as follows: 0 – none; 1 – impaired inspiration, alar breathing; 2 – nasal crackles; 3 – intensive nasal crackles and severe breathing impairment.

Fig. 1. Time course of repeated nasal ovalbumin/saline challenges (weeks) and cough provocation

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  1    2      3      4      5      6      7      8      9      time (weeks)
  ↓    ↓      ↓      ↓      ↓      ↓      ↓      ↓      ↓
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1 h after
citric acid cough
(1st experiment)

17 h after
citric acid cough
(1st experiment)

3 h after
citric acid cough
(2nd experiment)

24 h after
citric acid cough
(2nd experiment)

Lacrimation scoring was done in the following points: 0 – none; 1 – hazy eyes; 2 – intensive lacrimation; 3 – manifested conjunctivitis. Both phenomenon scores were summed to one value. The maximum total score might be six points.

Chemically-induced cough and evaluation

Unanesthetized animals were individually placed in a two-chambered body plethysmograph box (type 855, Hugo Sachs Electronic, Germany) and were exposed to citric acid aerosol (Lachema) in double gradually increased concentrations (from 0.05 to 1.6 M) for 30 s. The interval between some exposures was 1 min. Physiological saline was used as the first challenge. The tussive agent, citric acid aerosol was generated *via* a jet nebuliser (Pariprovocation test I, Pari Starneberg, Germany) delivered to the head part of a body plethysmograph box. The particle size produced had an aerodynamic mass with a median diameter of 1.2 μm and the output of the nebuliser was 5 l/min. Respiratory changes in the airflow were measured using a pneumotachograph (Godart, Germany) with a Fleish head connected to the head chamber and recorded with a pen recorder (Multiscriptor Hellige 21, Germany). The appearance of the cough was detected by means of a microphone placed in the roof of the head chamber and connected to a tape recorder. Pneumotachograph changes and the cough sounds were simultaneously recorded in PC for off-line analysis. The number of elicited cough efforts was concurrently counted by an uninformed observer.

To quantify intensity of the cough reaction, the cough response was expressed as the total number of coughs during all citric acid challenges. The cough response was evaluated on the basis of a sudden enhancement of expiratory airflow accompanied by typical cough sound. The cough sound was analyzed from power spectra using fast Fourier transformation computer implementation Cough2 (Prof. Lorand A. Debreczeni, St. Emeric Teaching Hospital, Budapest, Hungary). This method can differentiate a cough from sneezing.

Cough challenge protocol

Animals were divided into two separate experiments because the purpose of our investigation was to examine the cough response at different time points (1 h, 3 h, 17 h and 24 h) after nasal challenge and it was not suitable to carry out more cough challenges at short time interval in one experimental group of animals to avoid tachyphylaxis. Experimental animals (n=24) were sensitized with intraperitoneal OVA. Animals of the experimental group (n=15 in the first experiment, n=9 in the second experiment) were repeatedly intranasally challenged with OVA at 7-day intervals for nine weeks. Control animals were intranasally challenged by saline (n=11 in the first experiment, n=10 in the second experiment) under the same conditions as an experimental group. The cough reflex was elicited at 1 h after the sixth nasal challenge and at 17 h after the ninth NCh in the first experiment composed of 15 experimental and 11 control animals and at 3 h after the sixth nasal challenge and at 24 h after the ninth nasal challenge in the second experiment composed of 9 experimental and 10 control animals. The cough challenge protocol is illustrated in Figure 1.

Histological preparation

At the end of the experiment, animals were killed by an overdose of anesthesia (Urethane, Riedel-de Haen AG) and samples of nose, larynx, trachea, bronchi and lungs were removed to assess the histomorphological findings. All tissues were fixed in 10 % formalin solution, dehydrated in graded alcohols and embedded in paraffin. A transverse section was cut and stained with haematoxylin and eosin. The histopathological assessment was performed by light microscopy.
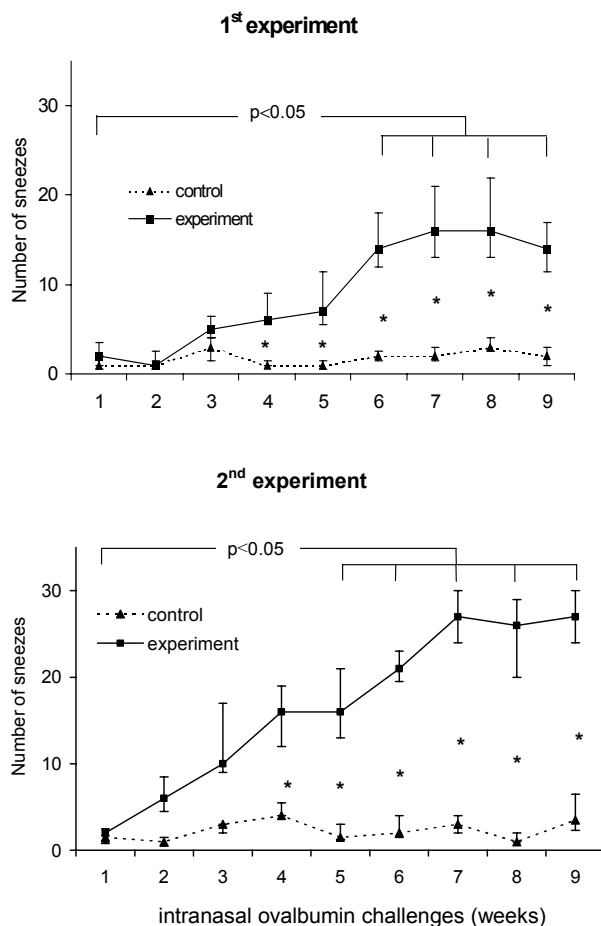
1st experiment

2nd experiment

intranasal ovalbumin challenges (weeks)

Fig. 2. The number of sneezes in sensitized animals in time course from the 1st to 9th nasal ovalbumin challenge compared to those in the control group challenged with saline. Sneezing was monitored one hour after nasal challenge in both experimental groups. Data are expressed as median and intequartile range. ∗ – significant difference (p<0.05) between experimental and control animals at the same time point of challenges.

1st experiment

2nd experiment

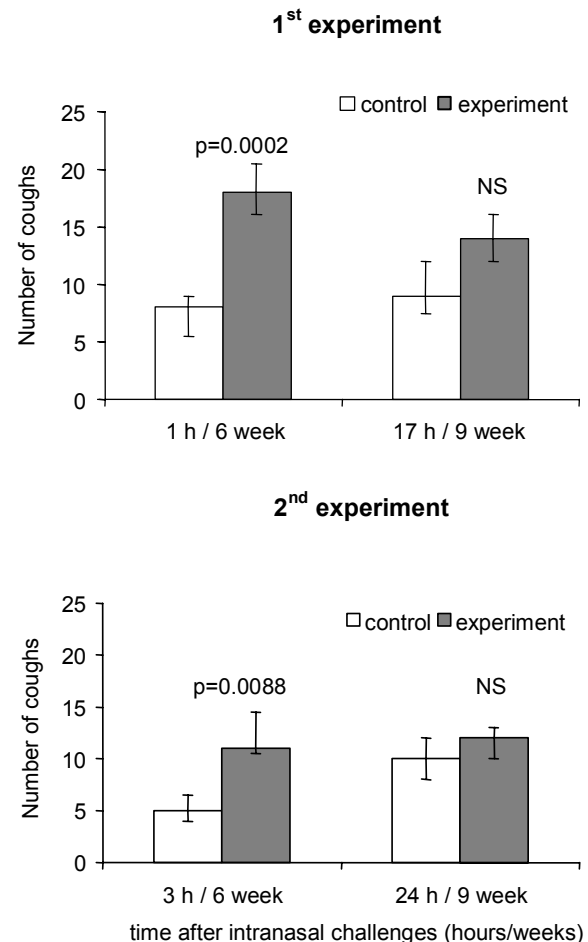time after intranasal challenges (hours/weeks)

Fig. 3. Citric acid-induced cough intensity changes in sensitized groups of animals at 1 and 3 h after the 6th nasal ovalbumin challenge (1 h / 6 week, 3 h / 6 week) and 17 and 24 h after the 9th nasal ovalbumin challenge (17 h / 9 week, 24 h / 9 week) compared to control animals challenged with saline in the first and the second experiments. Data are expressed as median and interquartile range, NS – not significant.

Statistical analysis

The data are expressed as median and interquartile range. Statistical analysis for the number of coughs was performed using the Mann-Whitney U test for non-parametric data. Symptoms scores and sneezing were analyzed using Kruskal-Wallis one-way analysis and the Friedman test. If a significant difference was detected, the Duncan multiple range test was done using Statgraphics version 5.0 program. Significance was accepted at the p<0.05 level.

Results

The effect of repeated nasal challenges on clinical symptoms of allergic rhinitis

Repeated nasal OVA challenges in sensitized animals lead to a significant increase in the number of sneezes starting from the fifth (2nd experimental group) and from the sixth challenge (1st experimental group) continuing to the end of the experimental procedure. Significant differences in sneezing frequency were present between experimental and control animals from the 4th week (Fig. 2).

The intensity of nasal acoustic phenomenon and lacrimation expressed as the symptom score was significantly enhanced in the experimental group starting from the 6th and continuing onto the last challenge (Table 1). Significant differences in symptom score between experimental and control groups occurred from the 3rd week of nasal challenge and they persisted till the 9th nasal provocation. In addition, there were no symptoms in control animals. Taken together, these results clearly indicate that the 6th nasal challenge is the

Table 1. Symptoms score (nasal acoustic phenomenon and lacrimation) in sensitized animals during the 1st to 9th nasal ovalbumin challenge compared to those in the control group challenged with saline monitored one hour after nasal challenge in the first and the second experiment.

	Time course of nasal antigen challenge (weeks) - 1st experiment								
	1	2	3	4	5	6	7	8	9
Experiment (n=15)	0(1)	0(2)	1(3)*	1(3)*	1(5)*	2(2)*#	3(3)*#	2(4)*#	2(3)*#
Control (n=11)	0	0	0	0	0	0	0	0	0

	Time course of nasal antigen challenge (weeks) - 2nd experiment								
	1	2	3	4	5	6	7	8	9
Experiment (n=9)	1(2)*	1(1)*	3(2)*	3(2)*	3(1)*	3(3)*	3(1)*	4(0)*#	4(3)*#
Control (n=10)	0	0	0	0	0	0	0	0	0

Data are expressed as median and interquartile range. ∗ – significant difference (p<0.05) between experimental and control animals at the same time point of challenges; # – significant difference (p<0.05) compared to initial values. Control groups were without any symptoms.

key time point of enhanced nasal responsiveness.

Our data suggest quite a large individual variability in sneezing and other clinical symptoms. Clinical symptoms of allergic rhinitis manifested in sneezing, rhinorrea, nasal cracles arose at 5-10 min after nasal ovalbumin challenge and individually persisted. In many animals ovalbumin-induced allergic rhinitis with clinical symptoms lasted 20-40 min, in some cases even for an hour. A few animals showed extremely long period of exudative allergic rhinitis-induced clinical symptoms up to 3 h. In addition, there were no anaphylactic reactions examined after ovalbumin nasal challenges at all.

The effect of repeated nasal challenges on citric acid-induced cough

Our findings have shown that the citric acid cough response was significantly increased one hour after the sixth nasal OVA challenge in the first experiment [18(14-23) vs 8(3-10); p=0.0002] and at 3 h after the sixth nasal OVA challenge in the second experiment [11(10-18) vs 5(3-8); p=0.0088] compared to control values (Fig. 3).

Intensity of the cough reflex elicited 17 h after the sixth nasal challenge was not significantly different between experimental and control group of animals although there is a mild tendency to an increase [14(10-18) vs 9(6-15); p=0.124]. A similar but not significant effect was found at 24 h after the ninth nasal challenge [12(8-14) vs 10(6-14); p=0.712] (Fig. 3).

Histological assessment

Histological examination of the nasal, laryngeal and tracheal mucosa revealed excessive vascular dilatation, congestion and edema in OVA-challenged animals. There was widespread eosinophilia throughout the airways. Within the mucosa, diffuse infiltration by an increased number of eosinophils was present especially in nasal mucosa, sometimes accomplished by the presence of lymphocytes. In addition, diffuse mild hyperplasia of serous glands and hyperplasia of epithelium were observed. In the lungs there were only few mostly dispersed eosinophils around small bronchi. In contrast, the nasal, laryngeal and tracheal mucosa of control animals was covered by „normal" columnar epithelium without significant pathological changes although only focal mild dilatation of lymphatic and blood vessels in mucosa and few eosinophils was seen.

Discussion

Recently, many researchers have extensively focused on the early and late allergic response especially in relation to pulmonary hyperresponsiveness and eosinophil accumulation. The guinea pig has been widely used to investigate allergen-provoked changes in pulmonary function and inflammatory cell influx because of the similarities that exist with the human asthmatic response, the mediators involved and the reactivity of the airways to bronchoactive agents (Underwood et al. 1995, Lawrence et al. 1997, Nabe et al. 1997).

In sensitized animals, two phases of the response to antigen challenge can be observed (Underwood et al. 1995, Lawrence et al. 1997, Nabe et al. 1997). Despite some studies, Nabe et al. (1997) indicated that the first, second or third challenge hardly produce a late allergic response and have since reported that repeated inhalations of antigen for long intervals of time can develop highly reproducible pulmonary dysfunction with early and late airway responses to every repeated antigen challenge in sensitized guinea pigs.

Our studies were focused not only on the cough response in the early airway response to nasal antigen challenge but they were expanded to include studies of the cough reflex in the late phase response following nine nasal antigen challenges. The present findings have shown significant enhanced cough sensitivity to citric acid in guinea pigs at 1 h and 3 h after nasal challenge that corresponds to an early allergic response after repeated nasal challenge when clinical symptoms were most conspicuous. The previous studies have demonstrated significant enhancement in the airway resistance to histamine immediately after antigen provocation (Underwood et al. 1995, Nabe et al. 1997) and the number of mast cells and histamine in nasal mucosa increased 30 min after an intranasal antigen challenge (Nakamoto et al. 1997). In our study, we concurrently investigated the clinical symptoms in an animal model with allergic rhinitis after repeated nasal challenge that gradually increased from week to week with maximum sneezing from the 5th to 6th nasal challenge.

With respect to the cough, airways inflammation with an increased number of inflammatory mediators including histamine, neuropeptides, leukotrienes, etc., is associated with increased responsiveness to capsaicin and other tussigenic stimuli, suggesting afferent nerve fiber and central sensitization of the cough reflex (Mazzone and Canning 2002).

As mentioned above, sensitivity of the cough reflex was increased during ovalbumin-induced exudative rhinitis in guinea pigs (Tatár et al. 2002). Riccio et al. (1996) have shown that the acute allergen challenge lowers the mechanical threshold for activation of rapidly adapting receptors in guinea pig trachea in vivo. The hyperreactive cough reflex, which demonstrates the correlation between the cough response and chronic airway inflammation was observed in multiple-immunochallenged guinea pigs, with significant increase of eosinophils in the airway epithelium and submucosa and bronchoalveolar lavage compared with normal or passively sensitized animals (Xiang et al. 1998). Similar results have been obtained when cough reflex sensitivity to capsaicin challenge was studied in pollen-sensitive patients with seasonal allergic rhinitis. Their cough sensitivity to capsaicin was significantly increased not only in the pollen season but also out of season compared with healthy volunteers (Fujimura et al. 2000, Pecová et al. 2001). As histomorphological assessment in our study has shown, repeated intranasal ovalbumin challenge resulted in the marked degree of eosinophilia within nasal mucosa. Eosinophils gradually decreased along airways from larynx to the lungs. In the lungs, there were only a few mostly dispersed eosinophils around small bronchi.

Recently, based on the observation of a high frequency of asthma coexisting with allergic rhinitis, Grossman (1997) introduced a concept of "one airway, one disease". Thus, upper and lower airway diseases are described as a continuing of inflammation involving one airway that may have a common origin for underlying pathological process.

Many authors observed airway hyperreactivity and a large influx of eosinophils into the epithelium of the trachea and bronchi from 18 h to 24 h after OVA challenge that is characteristic for the late phase inflammatory response in guinea pigs (Underwood et al. 1995, Lawrence et al. 1997). Our data revealed that the cough response provoked at 17 h and 24 h after repeated nasal challenge was not significantly different, although there is a mild tendency to increase. On the other hand, our findings support clinical observation of Minoguchi et al. (2003) who reported no correlation between cough reflex sensitivity and airway inflammation at 24 h after an allergen challenge in asthmatic patients. Therefore, the relationship between cough receptor sensitivity and eosinophilic inflammation of the airway in patients with asthma or allergic rhinitis remains still unclear.

The time course of cough provocation in our study during the late allergic response was chosen in accordance with the protocol of Nabe *et al.* (1997). They observed an increased bronchial reactivity during an early and late airway responses in guinea pigs challenged repeatedly with inhaled antigen on a long-term basis (10 times). Because we did not find the enhanced cough response after the 9th nasal challenge in the phase of a late allergic response, the question is addressed whether citric acid cough is enhanced or not during the early allergic response after the 9th nasal challenge. Our assumption could be probably positive because our recent study pointed to an enhanced cough response in guinea pigs after the 8th nasal challenge during the early phase response related to apparent symptoms of allergic rhinitis (Brozmanová *et al.* 2005).

Our results have shown a significant increase in the cough response only during the early allergic response after the 6th nasal challenge, when exudative rhinitis was evident and associated with rhinorea, mucus hypersecretion, and sneezing. In this case cough reflex sensitivity could be due to either an aspirated inflammatory secretion that sensitize cough mediating sensory nerves in the lower airways (Tatár *et al.* 2002) or central sensitization of cough reflex during the stimulation of afferent nasal mucosal nerve-endings (Plevková *et al.* 2004). On the other hand, the airway inflammation was also present in our study, but the cough response was not enhanced during the late allergic response after the 9th nasal challenge. Indeed, the airway inflammation was confirmed as well as by histomorphological examination that revealed an excessive vascular dilatation, congestion and edema in nasal, laryngeal and tracheobronchial mucosa with diffused infiltration of eosinophils and other inflammatory cells into the epithelium. In addition, diffused mild hyperplasia of serous glands and hyperplasia of epithelium was observed. Our results indicate that in contrast to the late allergic phase, an acute exudative phase of airway inflammation plays an important role in the enhanced cough sensitivity, which was evident in our previous study (Tatár *et al.* 2002). From common tussive agents we used citric acid to evoke cough. Citric acid and capsaicin stimulate C-fibers and rapidly adapting receptors (RARs) in airways. Citric acid confers the advantage of allowing repeated cough measurements without the occurrence of tachyphylaxis whereas repeated exposure to capsaicin is known to result in tachyphylaxis thus preventing the production of a reproducible cough response in the same animal (Morice *et al.* 2001, Tatár *et al.* 2002).

We can conclude that repeated nasal challenges enhance cough sensitivity to citric acid that only corresponds with an early allergic response. At the present stage there are still many uncertain points in the understanding of the mechanisms of allergic rhinitis and asthma-related cough. Understanding these mechanisms leading to enhanced cough sensitivity is essential for future therapeutic strategies and the successful treatment of the cough disorder.

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Reprint requests

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