In the present study we investigated the effects of nasal histamine on the intensity of coughing and the effects of intensified nasal breathing following nasal histamine on cough sensitivity (CS) in 14 subjects with seasonal allergic rhinitis. The study consisted of two parts performed one week apart. First, baseline CS to capsaicin was determined, followed by intranasal histamine challenge (4mg/ml, 0.1 ml) and the count of the number of coughs to inhaled capsaicin on the background of most intensive nasal symptoms (sneezing, itching, rhinorrhea, and nasal blockage) evoked by histamine. In the second part, CS was determined after intranasal histamine followed by 10 min of intensified nasal breathing through the nose or mouth in a randomized order at 2-day intervals. The number of coughs induced after intranasal histamine was significantly higher, compared with intranasal saline, [9 (7-12) vs. 4.5 (4-6), P<0.001]. CS also was significantly increased after nasal histamine, but nasal intensified breathing failed to cause any changes in CS. We conclude that stimulation of nasal mucosa with histamine enhanced the cough response in subjects with allergic rhinitis.

Key words: allergic rhinitis, cough, post-nasal drip, micro-aspiration

INTRODUCTION

Approximately 20% of adults and children have seasonal allergic rhinitis. Despite its prevalence, the condition is often treated inadequately and becomes chronic. A chronic state of nasal inflammation frequently leads to complications in both upper and lower airways (1).

A number of studies have been done to establish a link between allergic rhinitis and asthma (2). The mechanisms responsible for the relationship between the upper and lower airway disease is not completely clear, but several factors
could be involved. Firstly, the loss of nasal functions causes poor conditioning of air and possibly a reduction in natural bronchodilators (e.g. nitric oxide). Secondly, an upregulation of nasobronchial neuronal interactions (e.g. nasobronchial reflex) could occur. Finally, inflammation produced in the upper airways could be propagated through the airway or systemic pathways (3). Less attention has been given to another key problem occurring in patients with allergic rhinitis, which is the chronic cough.

A number of studies concerning the relationship between upper airway disorders and cough report that the postnasal drip syndrome (PNDS) is one of the most common causes of chronic cough that often also is underlain by gastroesophageal reflux and eosinophilic bronchitis (4). Diseases of the nose/sinuses and asthma are known to be other frequent causes of chronic cough. A heightened sensitivity of sensory afferent nerve endings in the airways is one mechanism responsible for coughing in patients suffering from rhinosinusitis. Pecova et al (5) have reported a heightened capsaicin cough sensitivity in patients suffering from allergic rhinitis in or out of pollen season. O’Connel et al (6) also have reported that cough sensitivity increases during upper respiratory tract infection and decreases following successful treatment of chronic cough.

An important mechanism of afferent nerve endings sensitization in the airways to produce cough might be micro-aspiration of nasal exudates. Curley et al (7) have shown that decongestant and antihistamine therapy in patients with rhinitis suffering from cough lead to a significant reduction of coughing in these subjects. The question arises what the role of the inflammatory process in the nose would be in the pathogenesis of cough, for it is known that it is impossible to induce cough directly from the nose (8). One plausible answer is that increased nasal secretion and obstruction affect the turbulence of inspiratory airflow through the nasal cavity (9), which is believed to support the normal function of the nose through warming, humidification, and filtration of inspired air (10). Nasal vascular congestion, mucosal edema, and overproduction of inflammatory fluids increase nasal airway resistance, so that there must be a stronger inspiratory effort to take the air in through the nose. That, in turn, could underlie the origin of polydisperse aerosol of which particles are breathed into the larynx and more peripheral airways, affecting the sensitivity of nerve endings that mediate cough (11, 12).

Our previous work has indicated that the process of intensified nasal breathing, which is a possible cause of micro-aspiration, does not influence cough sensitivity in healthy subjects (13). One explanation is that the duration of histamine-induced nasal symptoms was too short to induce any changes in the lower airways and this challenge did not induce an 'inflammatory' response in the nose, as allergic reaction with a high complexity of changes in patients suffering from allergic rhinitis would do. The aim of the present study was, therefore, to assess whether intensified nasal breathing during experimentally induced obstruction and hypersecretion in the nose could affect the capsaicin cough sensitivity in subjects.
with seasonal allergic rhinitis who were out of season, and to determine the effect of stimulation of nasal afferents with histamine on cough intensity.

MATERIAL AND METHODS

Subjects

The study was approved by the Ethics Committee of the Jessenius Medical School of Comenius University and informed consent was obtained from all subjects after the protocol of the study had been explained. A group of 15 subjects (F/M - 7/8, mean age 22 yr) was recruited from the student and staff population of the Jessenius Faculty of Medicine. They were non-smokers with normal spirometry and anterior rhinoscopy, and with no acute respiratory infection during the last 4 wk. All subjects were suffering from allergic rhinitis of a seasonal type with the symptoms being present in the period April-July each year. They were tested from September to December 2004 and during the study they were free of allergic symptoms and they did not take any antiallergic or anti-inflammatory drugs. Based on a structured, interviewer-led questionnaire, each subject was asked about respiratory symptoms and a past and family history of bronchial asthma, gastroesophageal reflux, cardiovascular diseases, metabolic diseases and ACE inhibitor treatment.

Assessment of cough sensitivity (CS)

CS examination took approximately 30-40 min and revealed the cough threshold of the airways. The subject, with a nose clip in place, breathed through a nebulizer device. He inhaled an aerosol of saline, as control, followed by progressively increasing concentrations of the capsaicin solution (0.49, 0.98, 3.9, 7.8, 15.62, 31.25, 65.5, 125, 250, 500, and 1000 µmol/L) prepared by jet nebulizer (Provo Jet, Ganzhorm Medizin Elektronik, Germany). Penetration of aerosol particles into the airways during 400 ms of inspiration was ascertained by computer-driven nebulization. The number of capsaicin-induced coughs was counted manually. Capsaicin cough threshold was set as the lowest concentration of capsaicin eliciting two or more coughs and was taken as an index of the airway cough sensitivity.

Assessment of cough intensity (CI)

Examination of the cough intensity, which refers to intensive stimulation of nasal afferents and therefore lasts incomparably shorter than the CS examination, was restricted to a defined time point (14). The CI was determined from the number of coughs induced by 4 successive inhalations of the histamine concentration that was sufficient to induce 2 or more coughs (C2) during baseline CS examination. The cough response was determined in this manner after intranasal histamine and compared with that after intranasal saline challenge.

Histamine nasal challenge

Histamine (histamine hydrochloride, Biosynth, Riedel de Haen AG, Germany) was dissolved in 0.9% NaCl and diluted to the concentration of 8 mg/ml ex tempore. The subject’s head was lying on a side and 100 µl of the histamine solution was instilled into the nostril of the other side, ~1.5-2.0 cm from the external orifice. The external surface of the nose was gently massaged for a few seconds to enhanced penetration of the instilled volume in the nasal mucosa.
Histamine symptom scores

Subjective symptoms were assessed by use of a scoring system (Table 1), according to Label et al (11), every 5 min through the whole of 30-min intranasal histamine challenge.

Table 1. Histamine symptom scoring system.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4 sneezes per provocation</td>
<td>1</td>
</tr>
<tr>
<td>&gt;5 sneezes per provocation</td>
<td>3</td>
</tr>
<tr>
<td>anterior rhinorrhea</td>
<td>1</td>
</tr>
<tr>
<td>posterior rhinorrhea</td>
<td>1</td>
</tr>
<tr>
<td>difficult nasal breathing</td>
<td>1</td>
</tr>
<tr>
<td>one nostril blocked</td>
<td>2</td>
</tr>
<tr>
<td>both nostrils blocked</td>
<td>3</td>
</tr>
<tr>
<td>pruritus of the nose</td>
<td>1</td>
</tr>
<tr>
<td>pruritus of the palate or ear</td>
<td>1</td>
</tr>
<tr>
<td>conjunctivitis</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td><strong>0-11</strong></td>
</tr>
</tbody>
</table>

Measurement of nasal airway resistance

Total nasal airway resistance (NAR) was measured by the method described by Taylor et al (15) at the time of the subjective evaluation of nasal symptoms. Nasal resistance to airflow was calculated from the following equation: \( R = \frac{\Delta P}{V'} \), where \( R \) is resistance to airflow (kPa/L/s), \( \Delta P \) is transnasal pressure, and \( V' \) is nasal airflow. The method of testing consisted of posterior rhinomanometry in which oropharyngeal (transnasal) pressure is measured using a tube placed between the tongue and the hard palate. The measurement requires the subject to position the tongue and the soft palate in such a way that both the oropharynx and nasopharynx remain open. The subjects breathed gently through the nose and simultaneous measurements of transnasal pressure and airflow were performed with an electromanometer (Electromanometer HSE, Hugo Sachs Elektronik, Germany). The airflow was measured over the nose by means of a face mask equipped with a pneumotachographic head device (Gould Godart Statam BV 18518, Godart, Germany). The data were stored in a PC for off-line analyses. In addition to NAR measurement, the active blow of secretion was collected and measured during the period of provocation by use of a pre-weighted handkerchief.

Data analysis

The capsaicin cough threshold was expressed as geometric mean value with 95% confidence intervals of the C2 capsaicin concentration. The number of coughs was presented as median and interquartile range. Cough counts after intranasal saline and histamine challenges were compared by Wilcoxon's signed rank test. Cough sensitivity changes in volunteers were analyzed by multiplicative comparison. Analysis of variance and correlation analysis were used to evaluate changes in NAR after intranasal histamine challenges. The interpretation of the nasal score was correlated with NAR. Other data were presented as means ±SD. A value of P<0.05 was considered to indicate significant differences.

Study protocol

The study consisted of two parts completed by all subjects.
First part:
- CS to capsaicin and C2 concentration were determined;
- 3 days later, intranasal histamine challenges were performed to assess the nasal effects of histamine;
- The intranasal histamine or saline challenges were blindly and randomly conducted 3 days apart.
- Coughing was induced by 4 successive inhalations of the C2 concentration of capsaicin during the time of most intensive nasal symptoms after histamine challenge (or at the corresponding time points after saline challenge).

Second part:
- The effect of intranasal histamine/saline challenge was determined (weight of nasal secretion, subjective feelings and nasal airway resistance);
- CS and C2 were determined;
- Nasal histamine challenges were carried. After the appearance of difficulties in nasal breathing or increased nasal secretion, the subjects were instructed to perform a maneuver of ‘intensified nasal breathing’ (5-6 sniff-like aspirations or inspirations through the nose near the total lung capacity in 10 min, repeated ~10 times). CS was again examined after this maneuver.
- Nasal histamine challenges were repeated. When difficulties in nasal breathing arose, the subjects were instructed to perform a maneuver of intensive inspirations through the mouth similar to that used before for the nose. CS was then examined after 10 min. Baseline CS was compared with CS after nasal provocations, followed by breathing through the nose or mouth.

In the second part of the study, all subjects were examined several times; the examinations being separated by at least 3 days’ intervals.

RESULTS

Nasal symptoms induced by administration of histamine

The application of histamine into the human nasal mucosa was immediately followed by unpleasant sensations (Fig. 1) described by all subjects as itching. A variable number of sneezes (1-5) occurred in the majority of the subjects 5 min after the challenge; three subjects did not sneeze. These symptoms were accompanied by seromucous nasal secretion and nasal breathing difficulties. Some of the subjects reported itching of the palate or feeling something dripping down into the pharynx with an urge to clean throat or swallow. NAR increased significantly over the baseline value (ANOVA, $P=0.042$) (Fig. 2). The increased occurred at different time points after histamine challenge and receded to the baseline after ~30 min. Average weight of the nasal fluids amounted to $1.2 \pm 0.35$ g. No coughing was observed during intranasal histamine challenge. With respect to the symptoms induced by intranasal histamine, the subjects were divided into runners (with predominantly secretory response, $n=5$) and blockers (elevation of NAR without intensive production of nasal fluids, $n=9$).

![Fig. 1. Scoring of unpleasant sensations evoked by intranasal histamine (instilled at the arrow) in subjects with allergic rhinitis. B - basal prehistamine score. The horizontal bar on the x axis shows the time of most intense nasal symptoms during which cough challenges during which cough challenges were performed.](image-url)
accompanied by seromucous nasal secretion and nasal breathing difficulties. Some of the subjects reported itching of the palate or feeling something dripping down into the pharynx with an urge to clean throat or swallow. NAR increased significantly over the baseline value (ANOVA, \( P=0.042 \)) (Fig. 2). The increased occurred at different time points after histamine challenge and receded to the baseline after \(~30 \text{ min.} \) Average weight of the nasal fluids amounted to \(1.2 \pm 0.35 \text{ g.} \) No coughing was observed during intranasal histamine challenge. With respect to the symptoms induced by intranasal histamine, the subjects were divided into runners (with predominantly secretory response, \(n=5\)) and blockers (elevation of NAR without intensive production of nasal fluids, \(n=9\)).

**Cough intensity changes after intranasal histamine challenge**

The intensity of the cough response induced by the C2 capsaicin concentration was enhanced significantly during the intranasal histamine compared with saline challenge - [9 (7-12) vs. 4.5 (4-6), \( P<0.001 \)] (median ± interquartile range) (Fig. 3). Since we could not show any difference between the runners and blockers in this response, the whole study group data are presented.
possibility of rapidly acting protective and defensive mechanisms of the airways, which could clean up the lower respiratory tract from the aspirated material with subsequent deglutition during 24 h. Another possibility is that the duration of the induced symptoms of rhinitis in healthy subjects was too short to induce any changes in the threshold level and irritability of nerve fibers mediating cough, or the experimentally induced hypersecretion and blockage of administration into a maxillary sinus (including night seep period), but the aspirate appeared in the stomach and upper gastrointestinal tract. However, the authors did not consider the authors failed to show the presence of the sinus aspirate in the respiratory tract 24 h after its aspiration of the sinus content into the lower airways via radio labeled scintigraphy. The authors have not caused 'micro-aspiration' of nasal inflammatory aerosols and thus nerve endings in the airways mediating cough were not stimulated. Bardin et al (17) have previously tested symptoms after topical nasal histamine (13). This change in capsaicin cough sensitivity in contrast to the healthy ones studied previously (14). The present finding is in accord with our other data that show heightened capsaicin cough sensitivity in the presence of nasal hypersensitive, as they display cough in response to natural allergens. In the

Cough sensitivity after intranasal histamine challenges followed by intensified breathing

CS was significantly increased after nasal histamine [1.24 (0.9-1.57) vs. 0.58 (0.25-0.91) vs. 0.57 (0.27-0.87), P1=0.004; P2=0.122] (geometric mean of C2 ± 95% CI), but nasal intensified breathing did not induce any changes in CS (Fig. 4). As in the case of cough intensity, we did not find any difference between the runners and blockers.

DISCUSSION

In a previous study we have shown that the intranasal histamine challenge followed by a maneuver of nasal intensified breathing did not affect capsaicin cough sensitivity in healthy subjects (13). The issue, however, has remained unresolved in seasonal rhinitis patients who, expectedly, might be hypersensitive, as they display cough in response to natural allergens. In the
nucleus of the solitary tract enhance cough in guinea pigs exposed to cigarette smoke. The induced effects. Inputs from afferent bronchopulmonary C fibers endings of the vagus into the Central cough plasticity could also play into the enhanced CI on the background of histamine-induced coughing during intensive itching of the nasal mucosa was significantly higher than that during nasal saline challenge. Similar results were obtained for healthy volunteers after topical nasal capsaicin and histamine (13, 14). This result is in line with the enhancement of mucosa.

The nose does not provide as complex a reaction as allergic rhinitis does due to exposure to antigen. Broffeldt et al (18) have shown that intranasal histamine challenge leads to and tryptase (marker of mast cells degranulation) is linked to late phase response of the nasal mucosa. But in the early phase of response (which was only considered in our study) the fluids contain proteins markers of plasma transudation (albumin, immunoglobulines), the surface goblet cells, and a mixture of serous and mucous glycoproteins. The mediator assays have showed that presence of eosinophilic cationic protein overproduction of secretion. That, along with increased total nasal airways resistance, makes this model suitable for the subsequent study of capsaicin cough sensitivity.

In the present study we set out to investigate the effects of potential micro-aspiration of nasal 'inflammatory' aerosols on the capsaicin cough sensitivity and the effects of intensive stimulation of nasal afferents on the cough intensity in the rhinitis subjects.

Histamine, a biogenic amine synthesized and stored mainly in mast cells and basophiles, plays a prominent role in the pathogenesis of allergic rhinitis. Nasal effects of histamine are mediated via interaction with both H1 and H2 receptor subtypes. These effects include: sneezing, itching in the nose, nasal discharge and a decrease of nasal patency (16). Histamine is capable of stimulation sensory nerve endings and receptors in the nasal mucosa, acting also indirectly via plasma leakage or pro-inflammatory changes.

We found that intranasal administration of histamine in subjects with seasonal allergic rhinitis, but remaining out of pollen exposure and having no nasal symptoms, resulted in nasal itching, sneezing, and an urge to clear the nose from overproduction of secretion. That, along with increased total nasal airways resistance, makes this model suitable for the subsequent study of capsaicin cough sensitivity. We further found that this sensitivity is increased in such subjects in
contrast to the healthy ones studied previously (14). The present finding is in accord with our other data that show a heightened capsaicin cough sensitivity in the presence of nasal symptoms after topical nasal histamine (13). This change in capsaicin cough sensitivity depended neither on the subject being referred to as a runner (mostly with secretory response) nor blocker (with a decrease of nasal patency with minimal secretion) nor on the breathing maneuvers performed (breathing through the nose or mouth).

We may propose several plausible explanations for the difference in CS between rhinitis and healthy subjects. In the latter subjects, the process of intensified nasal breathing might have not caused 'micro-aspiration' of nasal inflammatory aerosols and thus nerve endings in the airways mediating cough were not stimulated. Bardin et al (17) have previously tested aspiration of the sinus content into the lower airways via radio labeled scintigraphy. The authors failed to show the presence of the sinus aspirate in the respiratory tract 24 h after its administration into a maxillary sinus (including night seep period), but the aspirate appeared in the stomach and upper gastrointestinal tract. However, the authors did not consider the possibility of rapidly acting protective and defensive mechanisms of the airways, which could clean up the lower respiratory tract from the aspirated material with subsequent deglutition during 24 h. Another possibility is that the duration of the induced symptoms of rhinitis in healthy subjects was too short to induce any changes in the threshold level and irritability of nerve fibers mediating cough, or the experimentally induced hypersecretion and blockage of the nose does not provide as complex a reaction as allergic rhinitis does due to exposure to antigen. Broffeldt et al (18) have shown that intranasal histamine challenge leads to production of measurable amount of nasal fluids. But in the early phase of response (which was only considered in our study) the fluids contain proteins markers of plasma transudation (albumin, immunoglobulines), the surface goblet cells, and a mixture of serous and mucous glycoproteins. The mediator assays have showed that presence of eosinophilic cationic protein and tryptase (marker of mast cells degranulation) is linked to the late phase of response of the nasal mucosa.

Another important finding in the present study was that the intensity of histamine-induced coughing during intensive itching of the nasal mucosa was significantly higher than that during nasal saline challenge. Similar results were obtained for healthy volunteers after topical nasal capsaicin and histamine (13, 14). This result is in line with the enhancement of the cough response in animals with experimentally induced rhinitis (12) and during chemical and mechanical stimulation of nasal mucosa (19).

Irritation of the nasal mucosa expressed as a burning or itching sensation is liable to be a nociceptive stimulus that carried into the brainstem could increase arousal of its structures. Central cough plasticity could also play into the enhanced CI on the background of histamine-induced effects. Inputs from afferent bronchopulmonary C fibers endings of the vagus into the nucleus of the
solitary tract enhance cough in guinea pigs exposed to cigarette smoke. The nucleus is the first relay in the central circuitry where afferent vagal signals may be subject to modulation (20). Due to signal conditioning at these first central synapses, sensory information may be further transmitted or modulated e.g.: amplified, blinded or extinguished.

Our results may be explained by an interaction between the nasal afferent input and cough pathways. Little information is available on the central integration and regulation of cough. It is possible that sensory information originating in the nasal mucosa influences the activity of neurons in afferent cough pathways. For instance, trigeminal nasal afferent input has been shown to influence the activity of neurons in the NTS, whereas primary trigeminal sensory afferents project to the nucleus spinalis n. trigemini (21). Since NTS is the site of central projections of vagal afferents (including putative cough-mediating afferents), nasal afferents might increase NTS second order neurons' output and affect the activity of cough pathways neurons. Indeed, an interaction between disparate afferent inputs (termed convergence) has been proposed in the regulation of bronchial tone and other C fiber-mediated reflexes (22). Although central convergence would provide a simple explanation, the interaction between nasal afferent input and cough pathways may also take place at other central levels. Eccles (23) has reported a powerful effect of placebo treatment in children with cough related to upper airway disorders.

In conclusion, our data suggest that the stimulation of afferent nerves in the nasal mucosa can lead to a neuron-mediated potentiation of cough. This mechanism may contribute to chronic cough in patients with allergic rhinitis.

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