Increased cough reflex sensitivity is found in patients with allergic rhinitis and may contribute to cough caused by rhinitis. We have reported that cough to citric acid is enhanced in the guinea pig model of allergic rhinitis. Here we address the hypothesis that the cough reflex sensitivity is increased in this model. The data from our previous studies were analyzed for the cough reflex sensitivity. The allergic inflammation in the nose was induced by repeated intranasal instillations of ovalbumin in the ovalbumin-sensitized guinea pigs. Cough was induced by inhalation of doubling concentrations of citric acid (0.05-1.6 M). Cough threshold was defined as the lowest concentration of citric acid causing two coughs (C2, expressed as geometric mean [95% confidence interval]). We found that the cough threshold was reduced in animals with allergic rhinitis. C2 was 0.5 M [0.36-0.71 M] and 0.15 M [0.1-0.23 M] prior and after repeated intranasal instillations of ovalbumin, respectively, P<0.01, n=36). C2 was not affected in control animals (n=29). We have reported that the selective leukotriene cys-LT1 receptor antagonist montelukast inhibited cough enhancement in this model. We found that this was accompanied by inhibition of the changes in cough reflex sensitivity. C2 was reduced in animals with allergic rhinitis treated orally with vehicle (0.57 M [0.28-1.1] vs. 0.09 M [0.04-0.2 M], P<0.05, n=8), but not in animals treated with montelukast (0.57 M [0.22-1.4 M] vs. 0.52 M [0.17-1.6 M], NS, n=8). We conclude that the cough reflex sensitivity is increased in the guinea pig model of allergic rhinitis. Our results suggest that guinea pig is a suitable model for mechanistic studies of increased cough reflex sensitivity in rhinitis.

Key words: chronic cough, cough threshold, allergic rhinitis
INTRODUCTION

The cough reflex can be more readily induced by inhaled irritants in patients presenting with cough. This phenomenon, termed increased cough reflex sensitivity, is a consistent finding in patients with cough due to disparate causes (1-3). It is inferred that the increased cough reflex sensitivity contributes to the magnitude (severity) of coughing. Because of increased cough reflex sensitivity, the endogenous cough triggers, associated with the disease that causes cough, are predicted to be more effective in inducing cough. The mechanisms underlying changes in the cough reflex sensitivity are incompletely understood. Animal models of increased cough reflex sensitivity will likely allow for gaining more mechanistic insight into this phenomenon.

Rhinitis is a common cause of chronic cough (4, 5). The mechanisms that trigger cough in rhinitis are somehow controversial (5, 6). Among others, the proposed mechanisms include the penetration of inflammatory exudates from the nose into lower airways (referred to as a postnasal drip) and generalized airway inflammation. In some studies, the cough reflex sensitivity was increased in patients with chronic cough caused by rhinitis (3) and successful treatment of cough was accompanied by decrease in cough sensitivity (7). The results of other studies were less indicative of changes in cough sensitivity (1, 2). In separate studies, it was found that the cough reflex sensitivity is increased in patients with allergic rhinitis (8). Interestingly, in the pollen-sensitive patients with allergic rhinitis, the cough reflex sensitivity was further increased in the pollen season indicating a positive effect of the activity of nasal inflammation on the cough reflex sensitivity (8).

We have previously demonstrated that the cough to citric acid is enhanced in a guinea pig model of allergic rhinitis induced by repeated intranasal allergen challenge (9-12). It is not known if the cough reflex sensitivity is affected in this model. This information would be valuable in assessing the suitability of the guinea pig model for the study of the mechanisms of cough due to rhinitis. Here, we address the hypothesis that enhanced coughing in this model is accompanied by the increase in cough reflex sensitivity. We carried out additional analysis of the experiments from our previously published studies (9-12). We found that the cough reflex sensitivity is increased in the guinea pig model of allergic nasal inflammation. The similarity of the changes in the cough reflex sensitivity between patients with allergic rhinitis and the guinea pig model of allergic nasal inflammation support the utility of this model for the mechanistic studies of cough due to rhinitis.

MATERIAL AND METHODS

All experiments were approved by the Ethic Committee of the Jessenius School of Medicine in compliance with all applicable laws and policies. Male Trik guinea pigs (250-350g) were
obtained from the Department of Experimental Pharmacology, Slovak Academy of Science (Dobra Voda, Slovakia).

The data obtained in our previously published experiments in the guinea pig model of allergic rhinitis (9-12) and in some unpublished experiments were pooled and analyzed for the cough reflex sensitivity; the cough reflex sensitivity data were not presented in the referenced studies. All experiments carried out according to selected protocols (see below) were included in the analysis. In addition, 8 consecutive groups of animals in which the cough reflex sensitivity was determined prior any intervention (naive guinea pigs) were included in the separate analysis of the cough reflex sensitivity in naive guinea pigs (Fig. 1).

Allergic nasal inflammation

Allergic nasal inflammation was induced by repeated (6-8 times) intranasal administration of allergen in sensitized guinea pigs as described in details before (10). Briefly 21 days following intraperitoneal injection of ovalbumin (10 µg in 100 mg aluminum hydroxide in 1 ml of saline, Sigma-Aldrich, St. Louis, MO) the guinea pigs were repeatedly intranasally challenged with ovalbumin (0.5%, 15 µl, into each nostril) at 7 days intervals. The cough reflex sensitivity was determined prior to the ovalbumin challenges (baseline) and 1 h following the ovalbumin challenge in week 6 in the animals pooled into a group designated as the rhinitis group (n=36). The animals in the control group (n=29) were manipulated in an identical manner, but received saline in place of ovalbumin. All experiments in which the citric acid-induced cough was evaluated prior to the ovalbumin challenges and 1 h following the ovalbumin challenge in week 6 were included in the analysis. In some animals, the citric acid cough was evaluated at an additional time point prior to the week 6. Separate analysis indicated that this did not affect the cough after the ovalbumin challenge in week 6 (data not shown). The effect of montelukast on the citric acid-induced cough was evaluated in a separate group of guinea pigs (n=8) (12). The cough reflex sensitivity was determined prior to the ovalbumin challenges, 30 min after the ovalbumin challenge in week 6, and 30 min after the ovalbumin challenge in week 8. The animals were pretreated with montelukast (10 mg/kg, Singulair, Merck, USA, once a day) for 14 days prior to the ovalbumin challenge in week.
A parallel group of animals (n=8) underwent the same protocol, but received saline instead of montelukast.

**Cough reflex sensitivity**

The protocol of the citric acid inhalation is described in details in the above referenced studies. Briefly, cough was induced by inhalation of aerosols with doubling concentrations of citric acid (saline, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mol/l). Each concentration of citric acid was inhaled for 30 s and cough was counted during 30 s of inhalation and the subsequent 60 s. The individual inhalations were separated by ~90 s. Cough detection was aided by the analysis of the sound power spectra allowing for improved discrimination between cough and sneezing.

Three indexes of the cough reflex sensitivity were defined based on the protocols for a study of the cough reflex sensitivity in humans (13, 14). In the citric acid inhalation protocol described above, the threshold for 1 cough (C1) was defined as the lowest concentration of citric acid at which at least one cough was recorded, the threshold for 2 coughs (C2) was defined as the lowest concentration of citric acid at which at least 2 coughs were recorded, and the threshold for 2 cumulative coughs (C2cum) was defined as the lowest concentration of citric acid at which at least two cumulative coughs were recorded. The cumulative number of coughs for a given concentration of citric acid was calculated as the sum of coughs induced by that concentration and all prior concentrations. If a threshold was not attained even at the largest concentration of citric acid used (1.6 mol/l), it was assigned a double value of the largest concentration (i.e., 3.2 mol/l). Conversely, if a threshold was already attained at the inhalation of the first solution (saline), it was assigned a half value of the lowest concentration used (i.e., 0.025 mol/l).

**Statistical analysis**

Cough thresholds were log transformed, expressed as mean (95 %CI) and compared by using a non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison test. P<0.05 was considered significant. Cumulative numbers of coughs are presented as means ±SE.

**RESULTS**

The cough reflex sensitivity to citric acid was analyzed in 8 consecutive groups of naive guinea pigs (total 211 animals). The groups were obtained from the same commercial source in the period of 2002-2006. Fig. 1 illustrates the variability of the cough threshold among the groups. This observation underscores the importance of a study design in which the cough reflex sensitivity is evaluated in parallel control and intervention groups. All experiments analyzed in this study followed parallel controlled design.

We have previously shown that the cough to citric acid is enhanced in the guinea pigs with allergic nasal inflammation 30-60 min following the intranasal instillation of the allergen (9-12). In these studies, only the overall cough response (i.e., the total number of coughs) was analyzed and reported. Here, we addressed the hypothesis that the cough enhancement by allergic nasal inflammation is accompanied by increased cough reflex sensitivity. We analyzed pooled data from the experiments in which cough was evaluated prior
to inducing nasal inflammation and following repeated (6 times in 7 days intervals) nasal instillation of allergen (cough to citric acid was tested 60 min after the last instillation of ovalbumin). The cough thresholds to citric acid (C1, C2 and C2cum) were reduced approximately 3-fold, showing increased cough reflex sensitivity (Fig. 2A). The cough reflex sensitivity was not affected in naive guinea pigs which received intranasal saline (control group, Fig. 2B).

We also evaluated the data from separate experiments in which cough was induced 30 min after the nasal instillation of allergen in week 6. The cough reflex sensitivity was increased already at 30 min after the nasal instillation of ovalbumin. In these animals the cough threshold C2 was reduced from 0.57 M [0.34-0.94 M] to 0.12 M [0.06-0.26 M] (P<0.05, n=16). C2 was not changed in parallel naive control animals receiving intranasal saline (NS, n=10). Similar changes were observed in C2cum (data not shown). The magnitude of the change in cough reflex sensitivity was compared 30 and 60 min after the nasal instillation of ovalbumin. We found that the increase in cough reflex sensitivity was marginally higher at 30 min compared to 60 min. There was approximately 4-fold reduction in C2 at 30 min compared with approximately 3-fold reduction in C2 at 60 min (P<0.05, data not shown). Thus, the cough reflex sensitivity is increased already 30 min after intranasal ovalbumin, and
We reported preliminary data suggesting that the oral treatment with montelukast inhibits the enhancement of cough in this guinea pig model (12). We hypothesized that this effect is accompanied by a change (decrease) in the cough reflex sensitivity. In these experiments cough to citric acid was induced 30 min after the nasal instillation of ovalbumin in week 8. Analysis of the cough reflex sensitivity showed that the cough threshold was reduced in the animals receiving vehicle, but not in the animals receiving montelukast (Fig. 3). C2 was reduced in animals with allergic rhinitis treated orally with vehicle (0.57 M [0.28-1.1] vs. 0.09 M [0.04-0.2 M], P<0.05, n=8), but not in animals treated with montelukast (0.57 M [0.22-1.4 M] vs. 0.52 M [0.17-1.6M], NS, n=8). Similar changes were observed in C2cum (data not shown).

**Fig. 3.** Treatment with montelukast prevented the increase in cough reflex sensitivity in guinea pigs with allergic nasal inflammation. Ovalbumin-sensitized animals received repeatedly intranasal ovalbumin at 7 days intervals for 8 weeks. In week 8, the cough reflex sensitivity was increased in vehicle-treated animals (n=8) (shown in panel A), but not in animals treated with montelukast (shown in panel B). C- Changes in the cough threshold from panels A and B. The cough threshold C2 is expressed as log C2 (mean [95% CI]). *P<0.05, NS- not significant.
DISCUSSION

We found that the cough reflex sensitivity is increased in the guinea pig model of allergic rhinitis. This is similar to observations made in human studies suggesting that guinea pig is a suitable model for studies of increased cough reflex sensitivity in rhinitis (8).

In human studies, the cough reflex sensitivity is typically evaluated by using successive inhalations of increasing concentrations of a standard tussigenic chemical (most often capsaicin or acidic solutions) (13, 14). The cough reflex sensitivity is inferred from the cough threshold defined as the lowest concentration of the tussigen that evokes a defined number of coughs (usually two coughs, C2). Based on this approach we defined several indexes of cough threshold C1, C2, and C2cum in the guinea pig (see methods). Similar to human studies we noted that the thresholds defined on 2 coughs (C2 and C2cum) showed better reproducibility and were sufficiently sensitive to changes in cough.

Cough reflex sensitivity is increased in patients with allergic rhinitis (8). The magnitude of this increase is comparable to that demonstrated in patients with chronic cough from various causes. Yet, patients with allergic rhinitis rarely complain of coughing. Similarly, increased cough reflex sensitivity has been demonstrated in certain other diseases which are not accompanied by cough (15, 16). These observations indicate that the increased cough reflex sensitivity, by itself, is not sufficient to cause clinically relevant coughing. It is predicted, however, that the increased cough reflex sensitivity exaggerates cough once endogenous cough triggers are present. Mechanistic insight from animal models will likely aid the understanding of the role of cough reflex sensitivity in the pathogenesis of cough. Incidentally, we noted that although the cough reflex sensitivity was increased in the guinea pig model of allergic rhinitis after intranasal allergen instillation, spontaneous coughing (i.e., coughing not induced by the aerosol inhalation) was rarely observed.

Mechanistic understanding of increased cough reflex sensitivity in this model requires further studies. In theory, two major mechanisms may operate: inflammation spreading into the lower airways and central sensitization of the cough reflex by afferent input from the nose. The former mechanism cannot be excluded in the whole animal. However, a dramatic increase in tissue eosinophilia in the nose, but not in the lungs, argues against the role of inflammation spreading beyond upper airways in our model (9). The sensitization of the cough reflex by afferent input from the nose has been demonstrated in humans and in animal models. We have shown that the activators of nasal afferent nerves applied to nasal mucosa positively regulate (enhance) the cough reflex in humans (17-19) and animal models (20). The sensitization of cough by afferent input from the nose is attributed to central sensitization of the cough reflex, which also underlies the sensitization of cough by afferent inputs from other organs (i.e., lungs and esophagus) (21).
In our previous preliminary study oral pretreatment with the cys-LT<sub>1</sub> receptor selective antagonist montelukast prevented the enhancement of cough (12). The analysis of the data from this study shows that this treatment also prevented the increase in the cough reflex sensitivity (Fig. 3). The site of action of montelukast which causes the inhibition of changes in cough reflex sensitivity in this model is unknown. Multiple cell types in the nose (and elsewhere in respiratory system) express the cys-LT<sub>1</sub> receptor. Interestingly, a recent study demonstrated sensitization of nasal nociceptive afferent neurons by activation of the cys-LT<sub>1</sub> receptor (22). Validation of the role of the cys-LT<sub>1</sub> receptor in cough sensitization and identification of its site of action requires further controlled pharmacological analysis.

Conflicts of interest: No conflicts of interest were declared in relation to this article.

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Received: June 16, 2008
Accepted: August 22, 2008

Author’s address: M. Kollarik, JHANC RM3A18, 5501 Hopkins Bay view Circle, Baltimore, MD 21224; e-mail: kollarik@jhmi.edu