EXPERIMENTAL STUDY

The effect of chemical stimulation of esophageal mucosa on citric acid induced cough and specific airway resistance in guinea pig

Kollarik M, Plevkova J, Brozmanova M, Revallo M, Varechova S, Bartos V, Plank L, Tatar M

Department of Pathophysiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia.**plevkova@jfmed.uniba.sk**

Abstract

Background: **Gastroesophageal reflux disease is one of the most common causes of chronic cough. The mechanism of the cough initiation in these patients remains unresolved.**
Objectives: **This study was designed to investigate the effect of intraesophageal (IE) administration of capsaicin on cough and specific airway resistance (Saw) in guinea pigs.**
Methods: **Male TRIK strain guinea pigs were used. In the first experiment 12 controls received IE saline, 9 animals (group 1) received IE capsaicin (400 μM, 0.2 ml) and 12 guinea pigs (group 2) received IE capsaicin (400 μM, 0.2 ml) 24 hours after IE administration of hydrochloric acid (3 M, 0.2 ml). Cough induced by inhalation of citric acid (CA) and Saw was determined after IE administration of saline in controls and capsaicin in groups 1 a 2. In the second experiment, CA induced cough was determined in guinea pigs (n=13) in the beginning of the study (control response), after NaOH (1 M, 0.2 ml) was administered IE. One week later in conditions of corrosive esophagitis CA induced cough was determined after IE administration of capsaicin (cough during esophageal stimulation).**
Results: **There was no difference in CA induced cough between controls, group 1 and 2 (p=0.98). Saw was not affected by IE capsaicin stimulation and CA inhalation in group1 and group 2. There was no difference found between control cough response and those induced after IE capsaicin in animals with corrosive esophagitis (p=0.75).**
Conclusion: **Esophageal stimulation with capsaicin did not trigger and/or modulate CA induced cough and Saw in guinea pigs models.** ***(Fig. 5, Ref. 22.)***
Key words: gastroesophageal reflux, cough, cough plasticity, guinea pigs.

Chronic cough, often persistent as an isolated symptom for years, substantially diminishes the quality of life and increases morbidity of affected patients (1). The most prevalent causes of chronic cough are asthma, rhinosinopathy and gastroesophageal reflux, which combined account for 30–90 % of chronic cough causes (1). The mechanism of reflux related chronic cough could be conceivably due to aspiration of refluxed gastric content. However, clinical observations suggest that this mechanism operates in minority of patients with most severe reflux. These conclusions of most clinical studies are consistent wit the notion that the reflux enhances cough via afferent nerve pathways from esophagus (2).

In addition to pathogenesis of chronic cough in patients with GORD, there may be at least one important mechanism involved. It is supposed that activity of central cough pattern generator (CPG) is under plasticity of numerous stimuli, conducted to the brainstem via afferent nerve connections of these afferents with neuronal circuits (network) responsible for cough. Afferent stimuli originated in the nasal mucosa in patients suffering from

Department of Pathophysiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia, Current affiliation: The Johns Hopkins Asthma and Allergy Center, Johns Hopkins University, Baltimore, USA, Institute of Applied Mathematics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia, and Institute of pathological anatomy, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia

Address for correspondence: J. Plevkova, Dept of Pathophysiology, Jessenius Faculty of Medicine Comenius University, Sklabinska 26, SK-037 53 Martin, Slovakia.
Phone: +421.43.4238 213, Fax: +421.43.4134 807

Acknowledgement: We acknowledge Mrs. K. Sotnikova and Mr. T. Zatko for outstanding technical assistance.

rhinitis and those originated in esophagus in patients with GORD and subsequent convergence of these afferent inputs in the nucleus of the solitary tract (nTs) could be responsible for cough in this group of patients, although the pathological process is localized outside the respiratory tract area, from which the cough could be clearly elicited (3).

The simplest hypothesis explaining the cough enhancement by reflux is that the intense activation of sensory nerves in esophagus either directly triggers cough and/or sensitizes the cough reflex. These changes would result in the ninappropriately active cough reflex leading to chronic coughing. Here we approached this hypothesis in the guinea pig cough models. We studied citric acid-induced cough during stimulation of the esophageal afferent nerves in the intact esophagus or esophagus with injured mucosa. We found that in either model the transient introduction of the nociceptive sensory nerve activator capsaicin into the esophagus did not trigger cough or affect cough induced by citric acid. Our results suggest that acute localized stimulation of esophageal mucosal nerves is not sufficient to trigger and/or enhance cough in guinea pigs.

Aim

The aim of this study was to test the hypothesis that afferent inputs from distal esophagus could enhance the cough response and specific airway resistance in naive animals, animals pretreated with hydrochloric acid and those pretreated with natrium hydroxide.

Methods

All experiments were approved by Jessenius Faculty of Medicine Ethical Committee and follow the criteria of well fare of experimental animals, as well.

Animals (guinea pigs) (body weight 350–450 g) were housed in an approved animal holding facility maintained at room temperature 21–22 °C, humidity 60–70 %, ventilation, 12-h light-dark cycle and free access to water and standard animal food. Male TRIK strain guinea pigs were obtained from the Department of Experimental Pharmacology, Slovak Academy of Science (Dobra Voda, Slovak Republic) and used after at least 1week adaptation period in the animal house. Guinea pigs were adapted to experimental conditions two times, by inhalation of nebulized saline in the plethysmographic box.

Intraesophageal administration of stimulating substances

Animals (n=46) were placed into a plastic cylinder a part of the body chamber of the plethysmographic box equipment, which is used in our department for induction of coughing. This allowed immobilization of animals.

After that, using a mouth opener device, thin portex catheter (external diameter 0.3 cm) with conducting wire was introduced into the esophagus of the animal. The catheter was tipped with cotton tampon and its end was positioned just behind the gastroesophageal junction (in the distal part of the esophagus).

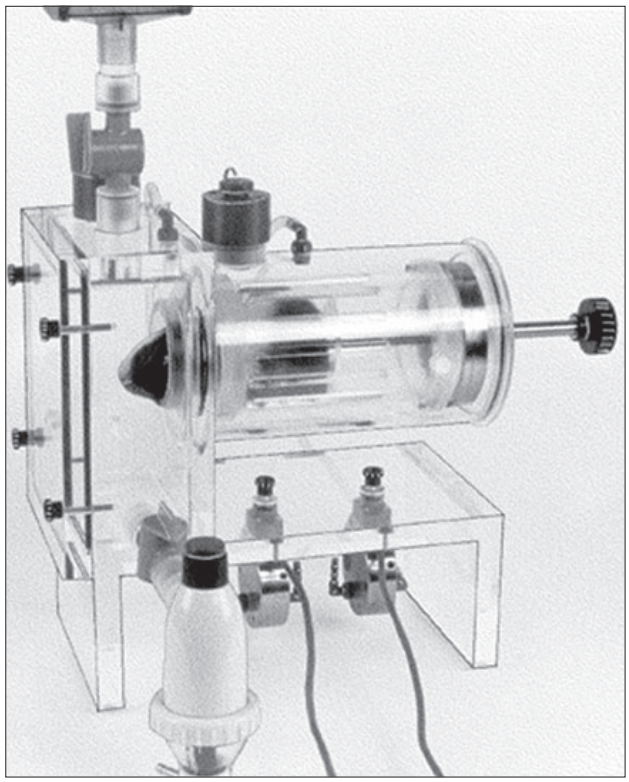

Fig. 1. Experimental setup developed for the investigation of bronchoactive substances on the conscious animals is used also for provocation of coughing by inhalation of tussive aerosols delivered into the head chamber of the plethysmograph.

Then conducting wire was extracted from the catheter, capsaicin, hydrochlorid acid or natrium hydroxide was applied into the catheter in a manner that allowed absorption of the solutions used into the cotton tampon. The solutions had been acting in the distal esophagus during defined time period (see below). Conducting wire was then introduced into the catheter again, catheter was passed into the stomach of the animals and cotton tampon was disengaged from its tip by means of conducting wire. This procedure was taken to prevent a microaspiration of the solutions absorbed in the tampon into the airways during the extraction of catheter from the esophagus.

Induction of coughing

Awake animals were individually placed in a bodypletysmograph box (Hugo Sachs Electronic) HSE type 855 (Fig. 1). To expose an animal to the aerosol, the head chamber was connected to a nebulizer (Pari Provokation Test I, Menzel, Germany, manufacturer's specification: output 5 l/min, particle mass median aerodynamic diameter 1.2 μ). A suction device adjusted to the same input (5 l/min) was connected to the head chamber to maintain constant airflow through the chamber during aerosol administration. Respiratory changes in the airflow were measured using a pneumotachograph (Godart, Germany) with Fleisch head connected to the head chamber and recorded directly with the moving pen recorder (Multiscriptor Hellige, Germany). Respi-

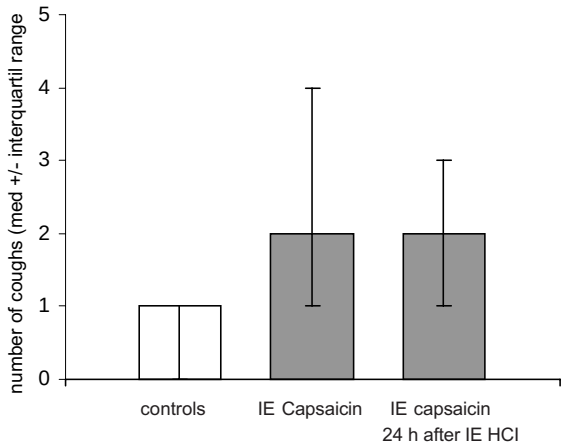

Fig. 2. The effect of stimulation of esophageal afferent nerve endings with capsaicin on citric acid induced cough in awake guinea pigs. Esophageal stimulation with capsaicin did not affect citric acid induced cough in awake guinea pigs (IE capsaicin, dark column). The effect on cough was the same in the group of animals pretreated with hydrochloric acid (IE capsaicin 24 hours after IE HCl, dark column; empty column represents the cough response of controls, which received intraesophageally saline).

ratory sounds including cough and sneezing were recorded with a microphone placed in the roof of the head chamber and connected to a preamplifier and loudspeaker. Pneumotachograph and microphone output were simultaneously recorded with a PC for off-line analysis.

Cough challenge was performed by inhalation of 0.3 M citric acid for 2 min. Cough was detected from the expiratory change of airflow interrupting basic respiratory pattern accompanied by a cough sound. Cough sound was detected from sound power spectra using fast Fourier transformation computer implementation Cough2 (Prof. Lorand A. Debreczeni, St. Emeric Teaching Hospital, Budapest, Hungary). Preliminary studies showed that in guinea pig the maximal intensity of cough sound is in the 1.0–2.5 kHz frequency range while that of sneezing is in 0.4-0.6 kHz range. Cough was analyzed by an investigator blind to the animal treatment. The number of coughs was counted during the 2 min of the citric acid inhalation and subsequent 1 min period.

Measurement of specific airway resistance in awake guinea pigs

Specific airway resistance (Saw) was measured by a simple non-invasive plethysmographic technique, Pennock's method (4). Awake animals were placed in a double chamber body plethysmograph composed of head chamber and body chamber (type 855, Hugo Sachs Electronic, Germany). The nasal airflow was measured in the head chamber, the thoracic respiratory airflow in the body chamber. Both measurements were made with differential pressure transducers and recorded to the PC (Simsoft, Martin, Slovakia). In this system, the specific airway resistance is proportional to phase difference between nasal airflow and thoracic respiratory flow (4). Specific airway resistance was calculated from the phase difference between the thoracic respiratory flow and nasal airflow based on lissajous loop presentation of the thoracic respiratory flow and nasal airflow on the x and y axis. This method is commonly used to study effects of bronchoactive substances (5, 6). Specific airway resistance was measured baseline, after intraesophageal capsaicin and after tussive challenge with citric acid.

Experimental protocols

Experiment 1 (esophageal pretreatment with hydrochloric acid)

Animals were divided into 3 groups:

1) control group of the animals (n=12) received intraesophageally saline (naive animals),

2) (group 1) (n=9) received intraesophageally capsaicin (400 μM, 0.2 ml), cotton tampon absorbed with capsaicin had been acting in esophagus for 1 minute, then was passed into the stomach,

3) (group 2) (n=12) received intraesophageally hydrochloric acid (3 M, 0.2 ml), cotton tampon absorbed with hydrochloric acid had been acting in esophagus for 30 seconds, then was passed into the stomach. Twenty four hours after that the same animals received intraesophageally capsaicin (400 μM, 0.2 ml) (acting in esophagus 1 minute).

Specific airway resistance was measured both in the group 1 and 2 before intraesophageal administration of irritants, then after administration of capsaicin and finally after citric acid induced cough.

The cough challenges with citric acid aerosol were performed immediately after intraesophageal administration of saline (naive animals) and intraesophageal administration of capsaicin (groups 1 and 2).

Experiment 2 (esophageal pretreatment with natrium hydroxide)

1) Citric acid induced cough was determined in awake guinea pigs (n=13) just in the beginning of the study and was taken as a control cough response.

2) All animals received intraesophageally NaOH (1 M, 0.2 ml) to cause an injury of the esophageal mucosa, macroscopically described as a corrosive esophagitis. Hydroxide absorbed in the cotton tampon had been kept in the esophagus for 30 seconds and then it was passed into the stomach.

3) One week after that (in conditions of corrosive esophagitis) citric acid induced cough was determined after IE administration of capsaicin (400 μM, 0.2 ml), cotton tampon absorbed with capsaicin had been acting in esophagus for 1 minute and then it was passed into the stomach. Cough response was taken as a cough during esophageal stimulation. In this group of animals specific airway resistance was not determined.

Results

In the first experiment we have found that there was no difference in number of citric acid induced coughs between controls, group 1and group 2 (number of coughs (median±interquartil range) (1 (0–1) vs 2 (1–4) vs 2 (1–3), p=0.98) (Fig. 2). Specific

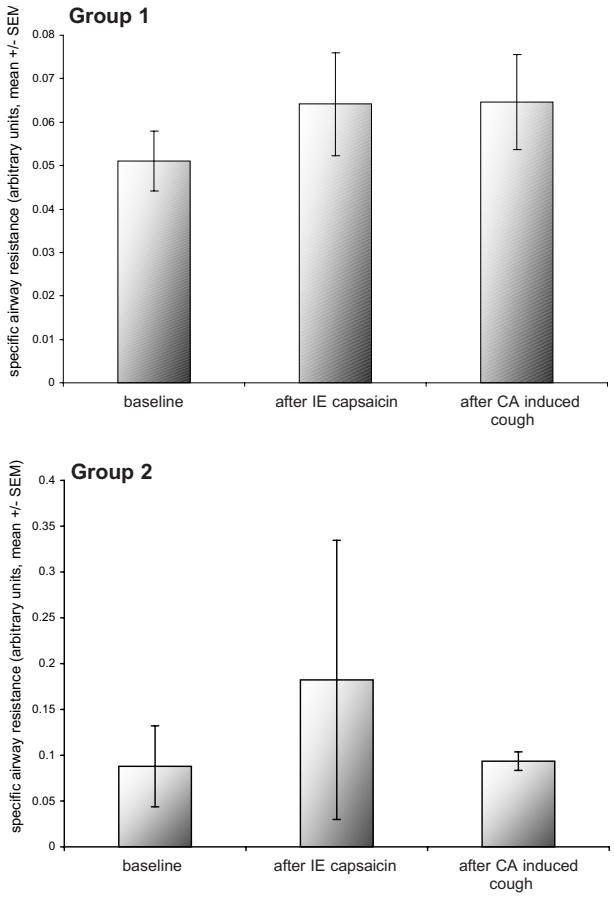

Fig. 3. Specific airway resistance measured by Pennock,s method in animals of group 1 (received IE capsaicin) and 2 (received IE capsaicin after pretreatment with HCl). There is no significant difference between Saw measured baseline and that one obtained after intraesophageal administration of capsaicin (after IE capsaicin), as well as after cough challenge (after CA-induced cough) in both group of animals.

airway resistance was not changed significantly form the baseline to the values obtained after intraesophageal administration of capsaicin and after citric acid cough challenge in the group 1 (0.051±0.007 vs 0.064±0.012 vs 0.065±0.011, p=0.12) and in the group 2, as well (0.088±0.004 vs 0.182±0.015 vs 0.094±0.010, p=0.36) (Fig. 3). But there is about two times higher specific airway resistance in the group of animals pretreated with hydrochloric acid in all three measurements than that in naive animals.

In the second experiment in animals with injured esophagi with alkaline challenges (natrium hydroxide) was found no difference between control cough response and cough response induced after intraesophageal administration of capsaicin in animals with corrosive esophagitis (Fig. 4) (4 (2–7) vs 4 (3–6), p=0.75) (Fig. 5).

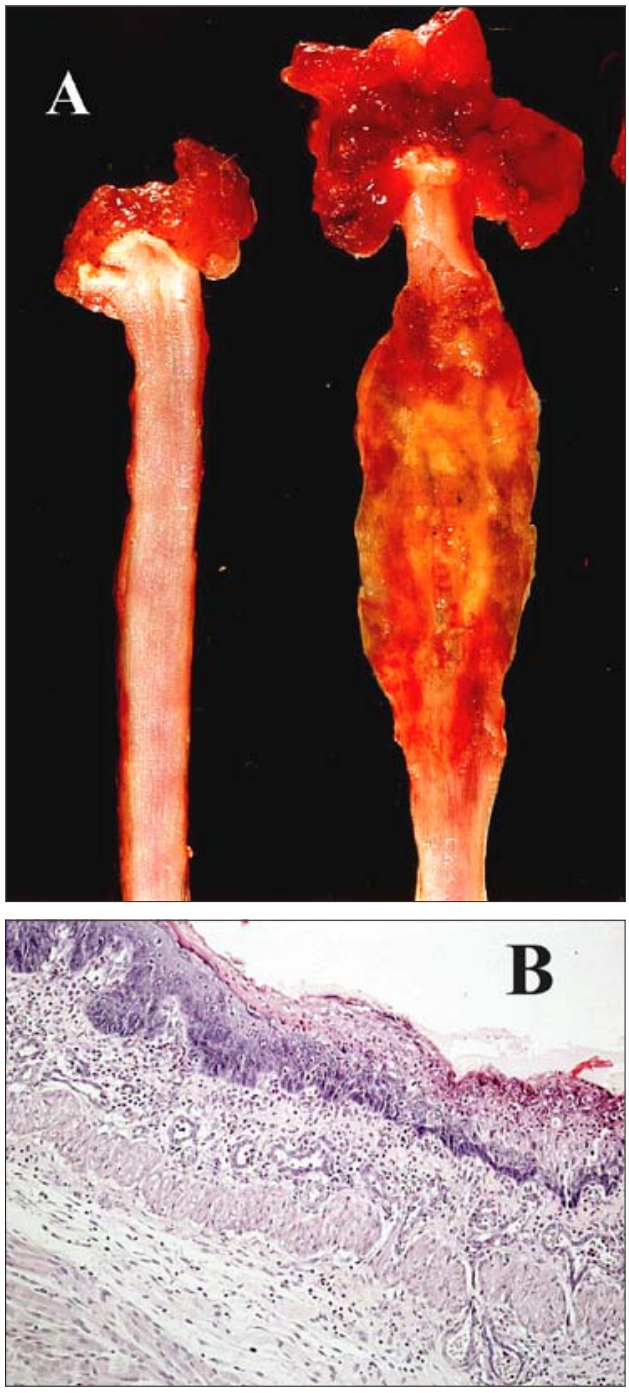

Fig. 4. a) Macroscopical changes in the distal part of the esophagus in animals with corrosive esophagitis induced by administration of natrium hydroxide analyzed post mortem on the left side of the figure, on the right side of the figure there is a esophagus after intraesophageal administration of hydrochloric acid and capsaicin – without any macroscopical differences from the intact one. b) Microscopical changes in the esophagus exposed to natrium hydroxide – severe alteration of the esophagus: the mucosa is atrophic and shows focal superficial exulceration with formation of purulent pseudomembranous inflammatory response, in the submucosal layer signs of hyperaemia and leucocytic – lymphocytic reaction are developed.

Discussion

We show that the transient introduction of capsaicin into intact or injured esophagus did not trigger cough or affect the citric acid induced cough in the awake guinea pigs models. This

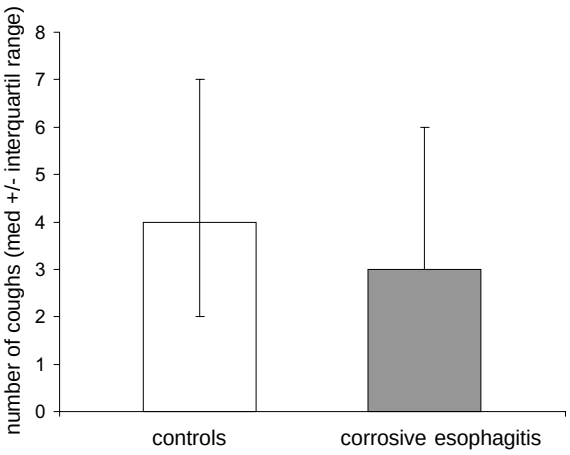

Fig. 5. The effect of intraesophageal administration of capsaicin on citric acid induced cough in animals with corrosive esophagitis. There is no difference between cough response induced in the beginning of the study (control) and that obtained after intraesophageal administration of capsaicin after injury of esophageal mucosa by alkaline pretreatment.

esophageal stimulation does not influence either the specific airway resistance measured by Pennock's method. Although the cough and bronchoconstriction are separate processes they may influence one another (7).

These data indicate that acute localized stimulation of esophageal mucosal nerves is not sufficient to trigger and/or enhance cough in naive animals.

The vaniloid receptor 1 (VR1, recently renamed to TRPV1) agonist capsaicin was chosen for esophageal nerves. This choice was based on the nearly uniform sensitivity of the guinea pigs nociceptive sensory nerves (i.e. the subclass of sensory nerves responsive to noxious stimuli) to capsaicin and its high efficacy (8). Capsaicin is more efficient on vagal sensory nerves than acid and activates most of acid-sensitive afferents. However the intact esophageal mucosa is a resistant barrier for diffusion of chemicals from the lumen into the esophageal wall.

Morphological and neurophysiological studies indicate that only a proportion of the sensory nerve terminals in healthy esophageal mucosa can be accessed by diffusion form the esophageal lumen (9). To offset the barrier effect we introduced large concentration of lipofilic capsaicin (1000 μM, 1000 times the maximal effective concentration on the guinea pig TRPV1 receptor) for prolonged periods of time. Based on the published data we believe that this stimulus activated majority of mucosal afferent nerves in the area of stimulation.

On the other hand, functional and morphological changes in esophagus of patients with reflux may lead to direct activation of sensory nerves located in deeper layers such as esophageal muscle and serosa. It is unlikely that in our guinea pig model capsaicin penetrated from the lumen into the deeper layers. We also attempted to impair barrier function with noxious insult to esophageal mucosa. Hydrohloric acid (3 M) did not induce any visible changes in mucosa (examined post mortem immediately after the cough challenge) (Fig. 4) thus the efficacy of this manipulation remain unconfirmed. In contrast, sodium hydroxide resulted in extensive mucosal ulcerations (Fig. 4). While the esophageal barrier was undoubtedly compromised, this insult might also have destroyed the esophageal sensory nerves. This caveat could be used for the interpretation of the negative results from these studies.

Stimulation of esophageal sensory nerves triggers cough and/or sensitizes the cough reflex in patients with reflux-related chronic cough (10). In other words, infusion of acid into the esophagus evokes cough and lowers the threshold for cough induced by inhalation of a defined tussigen (11). Thus, these studies show the additive interactions between esophageal sensory nerves and cough reflex in patients with reflux-related chronic cough.

Our results indicate that the acute guinea pig model is not suitable for studying the mechanisms of reflux-related chronic cough. The acute model probably cannot adequately mimic the changes occurring in esophagus and or neural pathways in patients with reflux-related chronic cough. The chronic exposure of esophagus to noxious components in refluxed material leads to functional and morphological changes in the esophageal sensory nerves. Indeed the nerve density and as well as neuropeptide and nociceptive receptors expression by neural tissue is increased in the esophagi of patients with GER (12). In addition, chronic stimulation of esophageal sensory nerves may lead to changes in the properties and connectivity of central components of esophageal sensory pathways. For example, the expression of neurotransmitter substance P is regularly increased in sensory nerves supplying the injured tissues. Recent papers suggest that substance P acts as a volume neurotransmitter in the sites of central projections of vagal sensory nerves (i.e. nucleus of the solitary tract) and this effect may substantiate the interactions between esophageal and bronchopulmonary (including cough or bronchoconstriction) pathways (13). In any event, these plastic changes typically develop over long periods of time and are therefore inherently absent in the acute models.

It was also demonstrated that direct stimulation of the afferent nerve endings in the nose/esophagus did not trigger cough (14, 15).

In the relation to the hypothesis of different stimuli acting on the central cough pattern generator (CPG) (3) it is interesting that stimulation of C-fibers endings in the abdominal cavity or cardiac nerve endings (16, 17) as well as stimulation of the nasal nerve afferents with capsaicin (16) could interfere with cough reflex in a manner that reduce or enhance cough response. Probably these afferent inputs could interfere with activity of CPG.

NTS is the site of central projections of vagal afferents (including putative cough-mediating afferents) it is possible that esophageal afferents affects the activity of cough pathways neurons (3). Indeed, a mechanism of interaction between disparate afferent inputs (termed convergence) has been proposed in regulation of the bronchial tone (3, 14) and other C-fibres mediated reflexes. However, although convergence would provide the simplest mechanism, the interaction between esophageal afferent input and cough pathways can be at other central levels.

It was also found that in the dog, the esophagus appears to have scant vagal inervation, with a preponderance of SAR-s hav-

ing a possible role in the mechanisms of deglutition. Moreover, there is a poor response to acid solutions and other irritants. These receptors have different properties than do vagal airway receptors a do not appear to sustain an important role of the esophagus in response to chemical irritants (19).

In this relation is very important finding that whereas the majority of cough associated with esophageal diseases is due to reflux (20), there is some recent experience suggesting that other esophageal diseases such as dysmotility and esophageal spasm may be equally important in the aethiology of chronic cough rising from the upper gastrointestinal tract (21). It was suggested that in these patients, cough may arise directly from receptors in muscle or in the submucsa stimulated by muscle contraction.

In conclusion we could resume that esophageal stimulation with capsaicin had no effect on the citric acid induced cough as well as on the specific airway resistance measured by Pennock's method in awake guinea pigs. These parameters were no affected by acid pretreatment and the cough response sustained unchanged also in conditions of corrosive esophagitis, as well. There is also suggestion that stimulation of afferent nerve endings in injured esophageal mucosa is able to change the cough sensitivity only in patients with some subclinical pathological changes in the airways (12).

The clear evidences for mechanisms responsible for coughing in patients with GORD are still not completely understood, but the close proximity of the trachea and the esophagus with the afferent nerve fibers of the vagus running between the two organs give the opportunity for multiple mechanisms to be relevant in the production of cough from esophageal disease (22).

This study is an initial part of our effort to develop a relevant animal model for reflux-related cough. As a first step we chose the simplest approach: pharmacological stimulation of sensory nerves in intact esophagus and in esophagus pre-exposed to presumably intense noxious insults. To further explore this question a chronic model of esophageal injury is needed. Our experiments also stimulated preliminary study in humans. Unlike in patients with reflux-related chronic cough, stimulation of esophageal sensory nerves with acid infusion did not affect the sensitivity of cough reflex in healthy volunteers. This observation is in agreement with the results of this study.

References

1. Irwin RS, Boulet LP, Cloutier MM et al. Managing cough as a defense mechanism and as a symptom. A consensus panel report of the American College of chest physicians. Chest 1998; 114: 133—181.

2. Irwin RS, Richter JE. Gastroesophageal reflux and chronic cough. Amer J Gastroenterol 2000; 95 (Suppl 8): 9—14.

3. Ing AJ, Ngu MC, Breslin ABX. Chronic persistent cough and clearance of esophageal acid. Chest 1992; 102: 1668—71.

4. Mazzone SB, Canning BJ, Widdicombe JG. Sensory pathways for the cough reflex. 161—172. In: Chung F, Widdicombe JG, Boushey HA (Eds). Cough: Causes, mechanisms and therapy. Oxford: Blackwell Publishing, 2003.

5. Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. A noninvasive technigue for measurement of changes in specific airway resistance. J Appl Physiol 1979; 46: 399—406.

6. Tohda Y, Muraki M, Iwanaga T, Haraguchi R, Fukuoka M, Nakajima S. Dual-phase response model for bronchial asthma. Allergy Asthma Proc 2000; 21: 79—84.

7. Chong BT, Agrawal DK, Romero FA, Townley RG. Measurement of bronchoconstriction using whole-body plethysmograph: comparison of freely moving versus restrained guinea pigs. J Pharmacol Toxicol Methods 1998; 39: 163—168.

8. Tatár M: Kašeľ a bronchálna hyperreaktivita. Stud Pneumol Phtiseol 1997; 6: 247—250.

9. Undem BJ, Chuaychoo B, Lee MG, Wenreigch D, Mayers AC, Kollarik M. Subtypes of vagal afferent C-fibers in the guinea pig lung. J Physiol 2004; 556: 905—917.

10. Blackshaw LA, Page AJ, Partosoedarso ER. Acute effects of capsaicin on gastrointestinal vagal afferents. Neuroscience 2000; 96: 407—416.

11. Ing AJ, Ngu MC, Breslin ABX. Pathogenesis of chronic persistent cough associated with gastroesophageal reflux. Amer J Resp Crit Care Med 1994; 149: 160—167.

12. Tatar M, Javorkova N, Demeter M, Dilova Z, Biringer K, Pecova R, Balaz D, Hyrdel R. Effect of acidification of distal esophagus on cough sensitivity in patient with gastroesophageal reflux disease. Europ Resp J 2003; 22 (Suppl 45): 172.

13. Mazzone SB, Canning BJ. Synergistic interactions between airway afferent nerve subtypes mediating reflex bronchospasm in guinea pigs. Amer J Physiol Regul Integr Comp Physiol 2002; 283: 86—98.

14. Irwin RS, French CL, Curley JJ, Zawacki JK, Bennet FM. Chronic cough due top gastroesophageal reflux: clinical, diagnostic and pathogenetic aspects. Chest 1993; 104: 1511—1517.

15. Ing AJ, Ngu MC, Breslin ABK. Pathogenesis of chronic persistent cough associated with gastroesophageal reflux. Amer J Resp Crit Care Med 1994; 149: 160—167.

16. Plevkova J, Kollarik M, Brozmanova M, Revallo M, Varechova S, Tatar M. Modulation of experimentally-induced cough by stimulation of nasal mucosa in cats and guinea pigs. Resp Physiol Neurobiol 2004; 142: 225—235.

17. Tatar M, Nagyova B. Abdominal capsaicin sensitive receptor activation and mechanical induced cough in anaesthetized guinea cats. Prague, International Union of Physiological science, 1991; 98.

18. Tatar M, Nagyova B, Widdicombe JG. Veratrine induced reflexes and cough. Resp Med 1990; 85 (Suppl A): 51—55.

19. Sekizawa S, Ishikawa T, Sant Ambrogio SB, Sant Ambrogio G. Vagal esophageal receptors in anaesthetized dogs: mechanical and chemical responsiveness. J Appl Physiol 1999; 86: 1234—1235.

20. Irwin RS, Curley FJ, French CL. Chronic cough. The spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of specific therapy. Amer Rev Resp Dis 1990; 141: 640—647.

21. Kastelik JA, Azis I, Thomsopson R. Gastroesophageal dysmotility as a cause of chronic persistant cough. Amer J Resp Crit Care Med 2001; 163: A60.

22. Morice AH. Epidemiology of cough. Pulmonary Pharmacology and Therapeutics 2002; 15: 253—259.

Received December 14, 2004.
Accepted February 20, 2005.