INTRODUCTION

Respiratory and airways reflexes are highly organized and well coordinated behaviors. They serve to precise function of respiratory system providing protection and defense of airways and lungs (1, 2). The high prevalence of respiratory-related reflexes, particularly cough, is associated with a number of acute and chronic diseases, multiple systemic and regulatory malfunctions (2), with hyper-responsiveness of respiratory tract, allergy and it is nowadays related to population diseases, lifestyle (2, 3), and environmental changes and influences (4, 5).

Reflex coughing is induced by multiple mechanical, chemical, and irritant stimuli directed toward laryngeal and/or tracheobronchial mucosa (1, 3). Individual cough represents complex inspiratory-expiratory pattern of enhanced inspiratory (I) activity immediately followed by powerful expiratory (E) expulsion (1, 6). Prolonged continuous stimulation elicits repetitive rhythmic coughs with orderly fashion during the stimulation and frequently also for a short period after it (7). The occurrence and excitability of cough can be modified by a number of afferent inputs, e.g., by chemoreceptive input (8, 9), by stimulation of afferents from the larynx, and from nasal mucosa (10, 11). These mechanisms supposedly participate in up- or down-regulation of cough during respiratory tract infections. However, the possible role of pharyngeal afferents in modulation of cough is mostly unknown. Mechanical stimuli to a nasopharyngeal mucosa induce aspiration reflexes (AspRs). They occur as an abrupt, short duration, powerful, spasmodic inspirations (SIs) that never arise repeatedly (non-rhythmic character) without immediate stimulus (1, 6). This reflex arises under a number of different conditions, providing multiple neuronal and neurological functional implications, such as an interruption of hypoxic apnea and acute cardio-respiratory failure, a support of respiratory and cardiac rhythmicity, etc. (6, 12-14). Repetitive AspRs decrease a subsequent respiration due to a hyperventilation and reduced respiratory drive (1). The goal of this study was to test the effects of nasopharyngeal stimuli and AspR on the excitability and periodic feature of repetitive coughing.

We hypothesized that: 1) the series of AspRs would reduce the excitability of tracheobronchial cough in terms of decreased number of coughs within the cough trial that instantly follows; 2) AspRs given to a period of persisting post-stimulation coughs would reduce the number of these coughs; 3) AspRs induced during the period of relative motor quiescence (between the cough efforts) within the cough trial could prolonged this period with consequently reduced number of coughs within the trial, and disrupt the rhythmicity of coughing; and 4) sub-threshold nasopharyngeal stimulation (failing to induce AspR) would not reduce coughing.

MATERIAL AND METHODS

General procedures and stimulation

All procedures were performed in accordance with the laws, rules, and regulation of Slovak Republic and EC. The Ethics Committee at Comenius University, Jessenius Faculty of Medicine in Martin approved the protocols.
Experiments were performed in 18 cats (3.6±0.3 kg; 10 females and 8 males) anesthetized with sodium pentobarbital (Vetbutal, Polfa; 40 mg/kg, i.p.). Supplementary anesthetic doses were administered (1-3 mg/kg, i.v.) as needed. Atropine (0.15 mg/kg, i.v.) was given at the beginning of the experiment to reduce secretions. The trachea, femoral artery and vein were canulated. The animals were allowed to spontaneously breathe a gas mixture of 25-40% oxygen, balance nitrogen. Arterial blood pressure (BP), end-tidal CO2 concentration (ETCO2), respiratory rate of quiet breathing and body temperature were monitored continuously. Body temperature was maintained at 37.5±0.5°C. Periodically, samples of arterial blood were removed for blood gas and pH analysis.

Bipolar fine wire hook electrodes were placed in the crural diaphragm (DIA) and the transversus abdominis or external oblique abdominal muscles (ABD) for a recording of electromyograms (EMGs). Proper function of electrodes was confirmed by an appropriate inspiratory (I) or E phase activity during breathing and the reflex responses. A soft balloon was inserted into the esophagus for a measurement of intrathoracic pressure changes (esophageal pressure - EP recording). Tracheobronchial cough was induced by mechanical stimulation of the intrathoracic airways with a soft polyethylene catheter or muscle loop fiber tool attached to the catheter. This aid was inserted into the trachea (and moved rostro-caudally and rotated) for periods of 10, possibly 15 or 20 s to elicit repetitive coughing. Cough was defined by a large augmenting burst of DIA EMG activity immediately followed (and partially overlapped) by a burst of E ABD EMG activity corresponding to the related I-E wave of EP. AspR was induced by a fiber or thin soft catheter loop or by air-puff pressure pulse delivered into the nasopharyngeal area via ventral medio-lateral pharyngostomy. This behavior was recognized as abrupt short burst of DIA EMG activity with corresponding sharp negative deflection of EP. Experiment was terminated by an overdose of pentobarbital i.v. followed by a saturated solution of KCl.

Data processing and their analysis

All EMGs were amplified, filtered (200-5000 Hz; Iso DAMS, WPI), digitalized (12-bit multi-function plug-in ISA card, Dataq Instruments; sampling frequency of 10,000 Hz), and recorded (WinDaq, Dataq Instruments, Akron, OH) along with the waveforms of BP and EP (in some cases when air pulse pressure stimulation was used also airway pressure within the nasal cavity was recorded). The EMG signals were then rectified and integrated (time constant 200 ms; Advanced CODAS, Dataq Instruments, Akron, OH).

The number of coughs in response to mechanical stimulation of the trachea (average number of coughs per 10 s stimulation - CN), amplitudes of DIA and ABD EMG moving averages, the peak I and E EP during cough were analyzed. The number and other parameters of persisting cough efforts that occurred after the stimulation of tracheobronchial area had been terminated (post-stimulation coughs) were also analyzed. Under this protocol a few AspRs were induced within the period of 2-4 s just after the termination of the cough stimulation. The delay from the beginning of tracheal-bronchial stimulation to the beginning of cough-related DIA activity and to the first maximum of cough ABD activity was measured when a series of SIs was induced just before the tracheal-bronchial stimulation. The temporal analysis was performed on coughs with AspRs induced in the quiescent periods between individual cough efforts. The duration of cough-related DIA and ABD bursts, their augmenting and decrementing parts, the time relation between DIA and ABD activation e.g. the delay between the ABD and DIA moving average maxima, the duration of quiescent period between coughs, cough inspiratory (CTi), expiratory (CTe), and total cycle durations (CTtot) were compared. CTi was defined as the period from the onset of DIA EMG activity until its maximum during the cough. CTe was defined as the interval from the maximum of DIA activity to the onset of the next cough or respiration-related DIA EMG burst (15). Sub-threshold air pulses stimulation that had induced only very weak or no signs of AspR (ABD EMG and EP trace) was also tested to quiescent periods between individual cough efforts within the trial.

Magnitudes of the moving averages during coughing were normalized relative to the mean intensities of coughs induced during first 2 control cough trials. The characteristics of coughing with particular intervention (induced AspRs) were compared to those in control trials just before and immediately after the intervention trial. Then they were averaged over all groups of controls vs. intervention trials. Results are expressed as a mean values ±SE. For statistical analysis a paired t-test, Wilcoxon’s matched pairs test, and repeated measures ANOVA with Student-Newman-Keuls post test were applied as appropriate (GraphPad Instat). The differences of variables were considered significant if P<0.05.

RESULTS

Mechanical tracheal-bronchial stimulation regularly produced repetitive coughing during control trials as well as during trials combined with nasopharyngeal stimulation. No difference in AspRs induced by tactile vs. air pressure pulses stimulation was seen. The data obtained with both types of nasopharyngeal stimuli were pooled and analyzed together.

Within the period of 5-10 s just before the beginning of tracheal-bronchial stimulation 17.2±2.4 SIs were induced in 7 cats. The CN was reduced from 5.5±1.45 in control to 4.17±1.02 in the trials with preceding AspRs (P<0.05; Fig. 1A). The periods from the beginning of cough-related stimulation to the beginning of cough DIA activity (from 0.6±0.2 s to 2.1±0.5 s; P<0.02) and to the first maximum of cough ABD activity (from 2.1±0.4 s to 3.9±0.6 s; P<0.01) were prolonged. Other cough parameters were not significantly altered by this intervention (Fig. 1A). The nasopharyngeal stimulation was applied within the first 2-4 s after a completion of tracheal-bronchial stimulation during persisting post-stimulation coughs in 6 cats; 5.9±1.0 AspRs were induced within this period (Fig. 1B). The CN (9.29±0.70 vs. control 9.86±0.69; P>0.05) and number of post-stimulation coughs (2.52±0.52 vs. control 3.17±0.32; P>0.05; Fig. 1B) were not significantly reduced. SIs induced during the post-stimulation coughing enhanced I component of persisting cough efforts expressed as a higher amplitude of DIA EMG moving average (84±11% vs. control 52±5%; P<0.01) and the maximum of I cough-related EP (0.60±0.10 kPa vs. control 0.34±0.06 kPa; P<0.01), leading to higher I EP amplitude within all cough trial (0.75±0.13 kPa vs. control 0.65±0.11 kPa; P<0.05). Other cough characteristics were not significantly altered.

Multiple reflex SIs (8.4±1.6 AspRs per 10 s of the cough stimulation trial) were placed in the period of relative motor quiescence between individual cough efforts in 8 cats (8.0±1.6 SIs within these “inter-cough” periods; success rate 87-100%). This intervention caused irregular occurrence of cough efforts within the trials with reduced CN and unevenly prolonged quiescent periods between coughs and consequently prolonged and variable CTi and CTe (Table 1; Fig. 1C). The standard deviation of CTi increased from 1.7 s to 2.6 s (range 2.9-11.7 s vs. control 1.6-6.9 s). The occurrence of AspRs within “inter-cough” periods also moderately shortened the duration of ABD activation and the time from the maximum of DIA activity to the
end of ABD cough-related activity (Table 1). During cough trials with SIs induced within inter-cough periods we detected short lasting abrupt expirations (3.2±0.8 event per 10 s tracheobronchial stimulation and per 8 reflex SIs within these "inter-cough" periods; Fig. 1C) that occurred with a short delay after preceding AspR (110±6 ms; P<0.001 compared to overlapping of DIA and ABD activity in cough 430±70 ms). The duration of these post-AspR expulsions (activation of ABD lasted 277±27 ms; P<0.01) similarly as the amplitudes of EP (0.70±0.15 kPa; P<0.05) and ABD EMG moving averages (45±9%; P< 0.01) during post-AspR responses were significantly lower than these characteristics during cough (Table 1).

The subthreshold nasopharyngeal stimulation (failing to evoke any motor sign of AspR) applied within the periods of

### Table 1. Effects of repetitive aspiration reflexes induced within the periods of relative motor quiescence between individual coughs (inter-cough intervals) on the parameters of these coughs.

<table>
<thead>
<tr>
<th></th>
<th>Control coughs</th>
<th>Coughs with AspR</th>
<th>P</th>
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<tbody>
<tr>
<td>CN</td>
<td>4.90±0.81</td>
<td>2.45±0.49</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cough peak inspiratory EP (kPa)</td>
<td>0.76±0.13</td>
<td>0.85±0.17</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>DIA EMG moving average amplitude (%)</td>
<td>80±7</td>
<td>109±21</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Cough peak expiratory EP (kPa)</td>
<td>1.27±0.39</td>
<td>1.52±0.46</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>ABD EMG moving average amplitude (%)</td>
<td>96±17</td>
<td>96±15</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Total cough cycle duration (s)</td>
<td>3.9±0.6</td>
<td>6.3±0.9</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>T inter-cough (s)</td>
<td>1.56±0.51</td>
<td>4.42±0.90</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>T DIA max - ABD end (s)</td>
<td>1.21±0.31</td>
<td>0.88±0.09</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>T DIA max - next DIA start (CT₁) (s)</td>
<td>2.77±0.47</td>
<td>5.29±0.90</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>T ABD (s)</td>
<td>1.48±0.37</td>
<td>1.02±0.09</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

CN, number of cough efforts per 10 s tracheobronchial stimulation; EP, esophageal pressure; DIA, diaphragm; ABD, abdominal muscles; T inter-cough, the duration of periods of relative motor quiescence between cough efforts; T DIA max - ABD end, the time interval from the maximum of DIA EMG moving average to the termination of related ABD EMG activity; T DIA max - next DIA start (CT₁), cough expiratory phase = the time interval from the maximum of DIA EMG moving average to the beginning of following respiratory or cough related DIA EMG activity; T ABD, the duration of cough-related ABD EMG activity.

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**Fig. 1.** Effects of the repetitive aspiration reflexes (AspRs) on cough. The first cough effort occurred later with consequently lower number of cough efforts within the cough trial when tracheobronchial stimulation (TB stim) followed the series of AspRs (AspRs - cough; Panel A). The number of persisting post-stimulation cough efforts was not reduced significantly by AspRs (cough - AspRs; Panel B). Spasmodic inspirations of AspR within inter-cough intervals during the cough trial (cough with AspRs) disrupted periodic occurrence of cough efforts, prolonged inter-cough distances and produced post-AspR expirations (Panel C). Note that the cough effort consists of prolonged deep inspiration followed by expulsion. On the right hand side of panel C there is quiet breath followed by 2 coughs, weak AspR, another cough and then by 5 powerful AspRs each with post-AspR expiration and another cough (a last ABD maximum). BP, arterial blood pressure; EP, esophageal pressure; DIA, ABD, moving averages of diaphragm and abdominal muscles EMGs; and AspRs (underneath the panels), nasopharyngeal stimuli inducing AspRs.
The main finding of our study is that reflex SIs induced by mechanical stimulation of nasopharyngeal mucosa either immediately before mechanical tracheal-bronchial stimulation or during inter-cough intervals between individual cough efforts within the cough series were able to postpone the occurrence of the following cough and reduce the number of the cough efforts. Mechanical tactile as well as pressure pulse stimulation of nasopharyngeal mucosa regularly evokes the reflex SIs of AspR (1, 16). In order to avoid an interaction of individual cough and AspRs motor patterns, which is a topic of another study (17), we targeted the nasopharyngeal stimuli before the cough stimulation or between individual cough efforts within the cough trial.

SIs induced under these patterns of stimulation had reduced the CN and prolonged the latency period of the next cough. It is very unlikely that any peripheral afferent input induced by SIs may account for such changes of coughing. Subthreshold nasopharyngeal stimulation that did not produce AspRs had no effect on tracheobronchial coughing in our animals, providing evidence that the observed modulation of coughing is related to behavioral expression of AspRs. SIs could vigorously stimulate mechanoreceptors of airways and lungs particularly subpopulations of the rapidly adapting receptors, which are possibly involved in an initiation of cough and apparently enhance cough (6, 7, 18). Multiple SIs induce a hyperventilation with consequent reduction of respiratory drive. There is no report indicating that short lasting temporary changes of chemoreceptive drive alter the excitability or other parameters of coughing (and AspR). Repetitive cough efforts may produce hyperventilatory condition as well, but cough similarly as AspR expresses very stable motor pattern for prolonged period of stimulations (no reduction of excitability, rhythmicity, and intensity) and it is inducible during hyperventilation apnea (1, 6, 7). The central or peripheral chemoreceptors were certainly not involved in any effect of the reflex SIs on cough in our experiments. We monitored the respiratory rate, depth of I, ETCO₂, BP, and periodically PO₂, PCO₂, pH, base excess and other blood parameters and kept them within physiological range for anesthetized cat, thus eliminating their long-lasting alterations and possible effect on respiration and/or reflexes induced (2, 8, 9).

Our present data support the concept of relative temporal stability of cough motor pattern (15, 19-21), as no significant changes in motor pattern of the coughs that follow SI were found. However, AspRs placed within an interval of relative motor quiescence of ongoing cough effort prolonged this “quiescent” period till the occurrence of the next cough and consequently CTex and CTg. The inter-cough intervals were prolonged unevenly resulting in unpredictable and non-rhythmic occurrence of individual cough efforts during the stimulation (Fig. 1C). It has been reported that few AspRs do not disrupt rhythmicity and do not alter phase durations of breathing (1). The SI efforts including AspRs provide powerful inhibition of E neuronal activity as well as E motor output (6, 22). This temporary but general inhibition of E neuronal activity may affect the processing (a “summation”) of cough-related afferent input possibly resulting in a late trigger of the cough effort. It is unlikely that interference at level of motor outputs is involved, because SIs were induced when virtually no cough motor activity had arisen. Moreover, interaction of motor patterns has no effect on cycling feature of coughing (17). The E inhibitory effect of AspRs, which have occurred early after the cough expulsion, certainly could terminate remaining ABD discharge during the late decrementing part of the cough-related ABD activity resulting in its moderate shortening. Consequently, related interval from the maximum of cough DIA activation to the termination of cough ABD activity became compressed.

Rhythmic coughing that replaces eupneic breathing due to cough-related stimulation is a complex reflex motor behavior produced by polysynaptic brainstem neuronal network (6, 18, 21, 22). This network probably splits into functionally separated neuronal circuits of cough central pattern generator - CPG (18) and behavioral control elements controlling the occurrence (excitability) of cough (7, 21, 23). The cough CPG requires tonic input from the 2nd order cough interneurons processing afferent stimuli from cough-related receptors (18). Modulation of such tonic input (e.g., by SIs) would alter most (if not all) spatial-temporal characteristics of produced cough efforts (7, 18, 21, 23). Various interventions at the brainstem level, such is application of central antitussives (15), stimulation of cough suppressor (19), or even stimulation of some neuronal populations most likely involved in the generation of cough motor pattern (20) typically alter the CN and amplitudes of E cough component (24). Vice versa, the timing of cough motor pattern and cough I component represent very stable characteristic of coughing. It has been proposed that coughing results from parallel afferent inputs from cough-related receptors and behavioral control elements directed into the CPG and into respiratory motor output (6, 15, 18, 23). We presume that modulation of coughing by SIs given outside the active cough phases occurs at behavioral control neuronal elements driving the cough CPG. Neuronal substrate of such interaction is to date unknown.

Sensory afferents from nasal and laryngeal (10, 11) mucosa may enhance coughing. Contrary, we found that specific type of abrupt and rapid mechanical stimulation of nasopharyngeal mechanoreceptors inducing SI inhibited an expression of cough for a period of several seconds (without any pronounced effect on cough motor pattern). We also concluded that this suppression is associated with the occurrence of AspRs (no effect of subthreshold stimulation). However, our findings do not rule out the possibility that other types of nasopharyngeal stimulation, which may not induce SIs, might be efficient in modifying the cough response. Effects of chemical agents, neuromediators, and inflammation within the pharynx on coughing remain unclear. Multiple effects of nasopharyngeal stimulation associated with AspR on the respiratory, cardiovascular, and neural systems were documented in animal experiments (6, 12-14). Most of them were observed, tested, and confirmed in human studies although AspR is much less pronounced and inducible in humans comparing with a number of animal species (1). We propose the usefulness of testing the nasopharyngeal stimulation for prevention, suppression, and management of chronic cough. Aspiration represents a significant component in the development of chronic cough associated with the gastro-esophageal reflux and postnasal drip syndrome (25). It is thought that an enhancement of I efforts under this condition may increase the severity of symptoms or accelerate the development of the disease. However, we speculate that a concomitant stimulation of both pharyngeal and laryngeal areas may improve the protective and defensive mechanisms of airways. Enhanced airways protection can prevent the development or reduce the severity of chronic complications. Our present data suggest that reflex SIs (and pharyngeal stimulation) may reduce CN; however, the intensity and supposedly the efficacy of coughing are preserved. Moreover, AspRs when induced during the cough I phase enhance both I and E components of cough efforts (17); thus possibly increasing their protective and defensive efficiency.

We report a higher CN in trials with persisting post-stimulation cough compared to CN under other protocols. Only animals presenting vigorous coughing and a high number of post-stimulation coughs within the trials, which did not diminish
quickly, were analyzed. A non-significant reduction in the number of post-stimulation cough efforts with AspRs suggests limited impact of SIs on the excitability of ongoing coughs. Enhanced I component of these coughs is likely caused by AspRs placed within their I period (17), as during the post-stimulation the AspRs were not targeted exclusively into inter-cough periods. Some "additional” enhanced coughs were sparsely seen after the period of AspRs interacting with post-stimulation coughs (Fig. 1B).

Weak expiratory activation following the reflex SI was sometimes observed before. It was explained by neuronal inhibitory-excitatory rebound phenomenon at the central level (1, 26). We observed a higher number of short lasting E responses that followed the AspR after approximately 0.1 s delay. Cough motor pattern is characterized by activation of E motor output before I related DIA activity is terminated - an overlapping of DIA and ABD activity (see results). Such overlapping of post-AspR expulsion ABD activity with preceding AspR-related DIA activity was never detected in our data. In addition, cough I component, representing prolonged and enhanced inspiration, was reflected by augmented pattern of DIA activation. Post-AspR expulsions occurred almost exclusively when the SI induced within inter-cough intervals during tracheal-bronchial stimulation. Cough related stimulation may generally enhance E motor drive resulting in more pronounced occurrence of expirations (induced here by preceding powerful AspR). Stretch receptor activation that arises during SIs may participate in the generation of these responses.

Our results indicate that the excitability and rhythmicity of mechanically induced tracheobronchial cough can be reduced by reflex SIs of AspRs, but not by subthreshold nasopharyngeal stimulation that fails to evoke motor signs of AspR in anesthetized cats. SI efforts induced by nasopharyngeal stimulation that fails to evoke motor signs of AspR in reflex SIs of AspRs, but not by subthreshold nasopharyngeal stimulation. Cough reflex after mechanical stimulation can be reduced by severe hypoxia in respiratory defence reflexes in anesthetized cats. Respir Physiol 1986; 49: 114-121.


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