INTRODUCTION

Aspiration reflex (AspR) represents a reflex spasmodic inspiration (SI) characterized by sudden and short but very intense inspiratory (I) effort followed by passive expiration (E). AspR is induced by mechanical stimuli within the nasopharynx providing a slide of foreign bodies from nasopharynx to oropharynx to support their elimination by subsequent reflex coughing and/or swallowing (1, 2). AspR can be regularly induced within any phase of respiratory cycle with a frequency of several responses per second. Each nasopharyngeal stimulation results in short-latency excitation of virtually all I-related neurons including I motor output accompanied by inhibition of expiratory (E) activity (1-4). The occurrence of AspR within the I phase of respiratory cycle may enhance the I-related activity of the actual inspiration (2). Higher excitability of AspR during late inspiration (I) and very early E phase was detected by electrical stimulation of nasopharyngeal mucosa (5).

The mechanism of hyperventilatory repetitive AspRs and/or massive I-related neuronal activation by AspR within the respiratory neuronal circuits were suggested to participate in arousal, normalizing and resuscitation phenomena including the restoration of the respiration during hypoxic apnea, acute cardiorespiratory failure, and comatose states (6-10).

The aim of our study was to examine interactions of powerful reflex SIs provoked as AspRs with 2 types of reflex apnea induced by mechanical laryngeal stimulation and by inflation of the lungs and with complex motor pattern of cough. We hypothesized that AspR induced during the period of augmenting I activity would enhance and shorten the cough preparatory I phase and vice versa that AspR elicited within the period of active cough expulsion would diminish cough E efforts. We also expected to induce a prolongation of reflex apnea by AspR.
MATERIAL AND METHODS

General procedures and stimulation

All procedures were performed in accordance with the laws, rules, and regulations of Slovakia and EC; the Ethics Committee of JLF UK in Martin approved the protocols. Experiments were performed in 22 cats (3.6±0.3 kg) under pentobarbital anesthesia (Vebral, Polfa; initial i.p. dose 40 mg/kg). Supplementary anesthetic doses were administered intravenously (1-3 mg/kg) as needed. Atropine (0.15 mg/kg, i.v.) was given at the beginning of the experiment to reduce secretions. The animals were breathing spontaneously through a tracheostomy a gas mixture of 25-40% oxygen, balance nitrogen. Arterial blood pressure (BP) was measured via catheter introduced in the femoral artery. Femoral vein was canulated for injections of additional doses of anesthetic and other solutions. End-tidal CO2 concentration (ETCO2), respiratory rate, and body temperature were monitored continuously (temperature was maintained at 37.5±0.5°C). Periodically samples of arterial blood were obtained for blood gas and pH analysis. Electromyograms (EMG) were recorded by bipolar fine wire hook electrodes introduced under visual control in the crural diaphragm (DIA) and in the transversus abdominis or external oblique abdominal muscles (ABD). Intrathoracic pressure changes were detected using a soft balloon inserted in the esophagus (esophageal pressure - EP).

Tracheobronchial cough was induced by mechanical stimulation of the lower airways with a soft polyethylene catheter or multi-loop fiber tool attached to the catheter, inserted into the trachea and moved rostro-caudally and rotated for a periods of 10 s (or longer if necessary). 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 as well as by a corresponding I-E wave of EP. AspR was followed and partially overlapped by a burst of DIA EMG activity immediately

RESULTS

Mechanical tracheobronchial stimulation regularly produced repetitive coughing and tactile or pressure pulses stimulation of the nasopharyngeal mucosa regularly induced AspR in our cats. We found no difference in AspR produced by tactile fiber loop vs. pressure pulses stimulation.

The AspR induced during cough I period significantly altered cough motor pattern in 9 cats tested. In these animals, 6.1±0.5 SIs were induced during 10 s tracheobronchial stimulation, 4.1±0.3 SIs occurred within the I period (71±4% success rate, 20% within quiescent periods between cough, 9% during cough expulsion). After provocation of AspRs during the I phase of cough, the CN (3.98±0.55) was not significantly higher than in the control trials (3.47±0.47; P>0.05; Fig. 1A). The presence of AspRs within the I cough period moderately, but significantly, prolonged CTI, (2.43±0.23 s vs control 1.98±0.18 s; P<0.01) as well as the CTE (3.85±0.25 s vs control 3.29±0.27 s; P>0.05). The amplitudes of DIA EMG moving average (139±28% vs control 81±10%; P<0.01), I deflection of EP during cough (1.17±0.16 kPa vs control 0.72±0.11 kPa; P<0.01), cough ABD EMG moving average (134±28% vs control 93±8%; P<0.05), and E values of EP in cough (1.86±0.57 kPa vs control 1.35±0.44 kPa; P=0.02) were augmented (Fig. 1A). We attempted to induce AspR during the I-E transition period or active cough expulsion in 8 cats (4.0±0.5 SIs during 10 s tracheobronchial stimulation, 3.5±0.5 SIs within the required cough period, success rate 85±3%). No significant changes of CN or any other analyzed spatiotemporal parameter of cough were found (Fig. 1B). An abrupt transient inhibition of cough ABD activity and a split of cough expiratory EP swing were frequently recorded with AspR induced within cough active expiratory period (Fig. 1B). Subthreshold nasopharyngeal stimulation applied during the active cough phases (cough-related DIA and/or ABD activity) had no significant effect on spatiotemporal characteristics of coughing.
Fig. 1. Effects of aspiration reflexes (AspRs) on tracheobronchial cough (TBc) motor pattern. AspR were induced in inspiratory phase of TBc (Panel A) and active expiratory period of repetitive coughs (AspR in E of TBc; Panel B). BP, arterial blood pressure; EP, esophageal pressure; Int DIA, Int ABD, moving averages of diaphragmatic and abdominal muscles EMGs; TB stim, tracheobronchial stimulation; nasoph stim, nasopharyngeal stimulation.

Fig. 2. Effects of aspiration reflexes (AspRs) on reflex apnea produced by mechanical laryngeal stimulation (LAR; Panel A) and by lung inflation (HBIR; Panel B). BP, arterial blood pressure; EP, esophageal pressure; DIA, ABD, EMG activities of the diaphragm and abdominal muscles; nasoph stim, nasopharyngeal stimulation.
We induced 3.7±0.6 AspRs (1-8 SIs) during the period of apnea caused by continual laryngeal stimulation in 5 cats. The duration of DIA respiratory quiescence indicating the duration of apnea induced by laryngeal stimulation was not significantly affected by the presence of SIs (4.94±0.84 s vs. control 5.25±0.38 s; P>0.05; Fig. 2A). On the other hand, when 5.4±0.5 (3-9) SIs were elicited within the first 1.3-s of HBIR in 7 cats, we saw a moderate, but significant, increase of the duration of reflex apnea (13.30±1.51 s vs. control 11.06±1.34 s; P<0.05).

DISCUSSION

The primary finding of our study is that SIs are regularly inducible by mechanical stimuli to the nasopharyngeal mucosa (AspR) in anesthetized cats during cough (including cough expulsion) and during reflex apnea. AspR induced during I cough periods enhanced I, but also E component of cough with little effect on temporary characteristics of coughing. AspR is steadily inducible by touching or by pressure pulses to the nasopharyngeal mucosa in cats under very variable conditions (2, 10, 14). Our results confirm high excitability of SIs of the AspR during cough characterized by massive I DIA as well as E activation of the ABD muscles. Based on an occurrence and a superposition of reflex SIs during regular breathing, we expected high excitability of AspR during cough I. The I efforts of the laryngopharyngeal cough are thought to be enhanced by possible activation of pharyngeal afferents when the stimuli are not restricted to the laryngeal area, possibly resulting in more pronounced I effort of laryngopharyngeal compared to tracheobronchial cough (2). We detected increased I cough-related EP and DIA EMG moving average amplitudes when AspR occurred within the I phase of the tracheobronchial coughs. It has been reported that the powerful I activation during the AspR may enhance other I pattern, e.g., apneusis or eupneic I (2, 6). Central neuronal mechanisms for generation of cough motor pattern significantly overlap with that for breathing (3, 4, 12, 15, 16). The frequency composition of I motor output signals suggest similarities in mechanisms generating I in eupnea and cough (17). A massive neuronal excitation is widely spread on virtually all I neurons within the brainstem during AspR including powerful excitation of I premotor and motor output (2-4). It is not clear which particular cellular and/or neuronal mechanism at the brainstem level can possibly "transmit" and "extend" the short-lasting AspR-related I neuronal excitation to enhance longer-lasting respiratory or cough I. Rapid and abrupt alterations of intrathoracic pressure provided by SIs vigorously activate PSRs located within the lower airways, rapidly adapting receptors, and possibly other mechanoreceptors. Afferent input resulting from activation of these sensory afferents may contribute to enhancement of I cough component by AspR, too.

The AspR induced within the I period of cough did not "prematurely" terminated the cough I phase either with an earlier occurrence of cough expiration or with the prolongation of I-E cough transition. Stable temporary, but also spatial, I characteristics of cough were reported under several conditions, such as suppression of cough by central antitussive drugs (13, 18), activation of central cough suppressive mechanism (19), or E neuronal populations (20) supposedly involved in the generation of cough E phase (12, 15). It seems that the crucial component determining cough I phase is a neuronal population of I-driver neurons which are a part of CPG neuronal network (15). We propose that "secondary" afferent inputs may as those stimulated by the occurrence of AspRs, which project into the CPG or cough motor output, can only modulate the basic cough motor pattern. SIs of the AspR induced during the cough I period enhanced cough E amplitudes of the EP and ABD EMG moving average. Cough E phase depends on the initial preparatory I phase (2, 11). The generation and shaping of powerful ballistic-like cough expulsive E is not fully understood. According to the model of cough pattern generating network (12, 15) interactions of several populations of E neurons determine the temporary feature of cough E activity. However, additional E drives are proposed to magnify motor output. Stimulation of primary cough-related afferents may generally increase the excitability of E motor output. Thus, the expiration reflex (21) and tonic-like ABD activity may occur during tracheobronchial stimulation that extends beside the cough expulsion (22). The central excitatory drive to E motor output during cough was also proposed (18). A significant role in I-E transition and E phase generation may have PSR inflation mechanism (11, 12). The E excitatory component of inflation response can provide an additional excitatory drive of E motor output during coughing. PSRs including their subset of rapidly adapting PSR are vigorously stimulated during the AspR (23). Some of these receptors could represent also primary cough-related afferents participating in initiation of coughing.

AspRs induced during the I cough period slightly (23%) prolonged CTE (23). We observed prolonged inter-cough periods (the time of relative motor quiescence between cough efforts within the cough attack) that are part of CTE (23), when repetitive reflex SIs were induced during these periods (24), but the coughs with AspR in "inter-cough" intervals were eliminated from the present analysis. The relation between the intensity of cough I and the duration of cough E phase is unknown. However, apparently no I neuronal mechanism of CPG (4, 12, 15) determine the duration of cough E phase. We speculate that prolonged CTE is related to increased E drive and to the enhanced cough E activity. The AspRs induced during the cough expulsion transiently inhibited the cough E activity providing evidence that reflex SI of AspR is inducible during massive cough E activation. However, single or double AspR was not capable of significantly reducing the peak E cough-related activation or alter its temporary characteristics (e.g., earlier termination of cough E) probably due to a very short duration of reflex SI. Cough E activity (ABD EMG activity as well as a positive swing of EP) typically continued after AspR (Fig. 1B). Whether multiple SIs placed over the whole active E period of cough would diminish cough expulsion is unclear. Nevertheless, our data suggest a stable (probably "pre-programmed") temporary feature of cough motor component.

The effects of SIs on cough motor pattern are associated with an expression of AspR motor pattern. We found no difference in coughing affected by sub-threshold stimulation of nasopharyngeal mechanosensitive afferents with pressure pulses that failed to elicit signs of AspR (sometimes very weak form of SIs only during the I phase of respiration was seen). The "subthreshold" pressure within the airways was confirmed by its measurement in the nostril and by an abrupt activation of laryngeal motor output (EMG activity of laryngeal adductors and abductors) related to laryngeal and/or nasopharyngeal stimulation. It is worth noting, however, that we applied only mechanical stimuli and they were delivered for a very short time. Different results might have been obtained with continual stimulation and/or other than mechanical stimuli to the nasopharyngeal surface. Nevertheless, our data provide evidence that reflex responses (the cough in our experiment) and/or their modulation by stimulation of additional afferent input (the nasopharyngeal mechanoreceptors in our experiment) can differ substantially depending on the occurrence of motor pattern of additional behavior (AspRs in our experiment).

The larynx represents a significant part of the airways with high density of multiple types of receptors (2, 4). A number of respiratory related reflexes including protective and defensive behaviors could be induced by stimulation of laryngeal mucosa. Protective apnea with concomitant laryngospasm and some cardiovascular changes occur when mechanical, and/or irritant
stimuli are applied to the larynx (2). We found no difference in excitability of AspR during LAR compared to other conditions tested in our experiments with cough and apnea. Vigorous excitation of I related neurons and I motor output during SIs provide arousal reaction on CNS resulting in a recovery and consolidation of cardiorespiratory parameters under a hypoxic apnea and other severe conditions (7-10). Possible neuronal interactions during the simultaneous occurrence of AspR and the protective reflex apnea are unknown. We saw very variable durations of LAR with AspRs induced during this apnea (among the animals as well as the trials). The actual duration of LAR may result from the balance of very unstable components involved: uneven stimulation and its time-course, sensitivity changes of mechanoreceptors, central interaction of afferent inputs and motor outputs, various levels of arousal reaction provided by AspR, stimulation of PSR by AspR, development of hypoxia and hypercapnia during the apnea, additional ventilation by SIs made during the apneic period, and others.

HBIR represents an important mechanism preventing the damage of lungs by their hyperinflation. It is also an important reflex mechanism of respiratory control providing I-inhibitory and E-excitatory action on respiratory output. We repeatedly induced AspR during HBIR with a prolongation of the apneic period. Long-lasting apneic periods are regularly terminated by large sigh and gasp caused by an increase of chemoreceptoratory respiratory drive during developing hypoxia and hypercapnia. AspRs in our experimental design did not provide any ventilation because the tracheal tube was blocked by the syringe delivering the inflating volume of air. It seems that the additional stimulation of PSR during SI efforts producing vigorous intrathoracic pressure changes and possibly also stimulation of chest wall muscles afferents, both known to be capable to delay the "breaking point" for restarting the respiration (25), account for the effect.

CONCLUSIONS

AspRs manifesting as reflex SIs can be provoked by mechanical stimulation of the nasopharynx in various phases of cough and during 2 different types of reflex apnea LAR and HBIR. Both I and E components of cough were enhanced by AspRs within the cough I period suggesting that the expression of cough expulsion depends on the intensity of preceding preparatory inspiration and that vigorous stimulation of PSRs may participate in the development of cough E phase. SIs induced either during the cough I period or during cough expulsion had very limited effect on temporary characteristics and the rhythmicity of cough, which supports the concept that separate neuronal circuits control the expression (the excitability) of cough and the generation of temporarily relatively stable (possibly "pre-programmed") cough central motor pattern. The efficacy of subthreshold stimulation of nasopharyngeal afferents in modulation of cough reflex is significantly lower when motor pattern of AspR is not expressed. Reflex effect of pulmonary hyperinflation causing inflation apnea was not inhibited by SIs, vice versa additional massive activation of PSRs by AspRs resulted in prolongation of HBIR. Reflex SIs during the LAR postponing the development of hypoxia and hypercapnia tended, however, to shorten LAR probably by an excitatory effect of AspRs on brainstem respiratory neuronal circuits.

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