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ORIGINAL PAPER

Cough reflex is additively potentiated by inputs from the laryngeal and tracheobonchial receptors and enhanced by stimulation of the central respiratory neurons

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Abstract The cough is an essential airway defense reflex. In this study we investigated the coordination of inputs from the laryngeal and tracheobronchial receptors in the cough reflex. In 15 beagle dogs (7–9 kg) lightly anesthetized with intravenous profobol (20–30 mg/kg/h), the cough response was elicited with mechanical stimulation of either the vocal chord or tracheal bifurcation. Simultaneous stimulation of both sites increased all the parameters of cough strength, that is, mean pleural pressure (P_{pl}), mean expiratory flow, number of cough bouts, and cough duration, in comparison with stimulation of the sites individually. The increases in mean P_{pl} and cough duration reached statistical significance (13.3 vs. 18.4 cmH_2O and 13.3 vs. 18.2 s, respectively). When the anesthetic level became deeper, the prolongation of cough duration almost disappeared, but the augmentation of mean P_{pl} was much less affected. During stimulation of the central respiratory neurons by intravenous dimorphoramine or acute hyperoxic hypercapnia, the cough strength increased significantly. We concluded that inputs from the laryngeal and tracheobonchial cough receptors acted in concert and potentiated the cough reflex. Furthermore, stimulation of the central respiratory neurons may increase the intensity of a cough response.

Keywords Aspiration pneumonia · Hypercapnia · Cough · Anesthesia

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Introduction

The cough has been regarded as an unpleasant airway reflex, and most of the pharmacological and physiological investigations have focused upon the suppression of coughing. However, the primary function of the cough is to clear noxious materials from the airway. Therefore, coughing is an important defense mechanism, and recently this function of the cough has been highlighted [1]. In clinical practice, attenuation of the cough reflex is intimately related to the development of aspiration pneumonia. In elderly persons, especially in those who have experienced cerebrovascular disorders, the cough reflex is attenuated, and the incidence of aspiration pneumonia is considerably high [2]. This high incidence of aspiration pneumonia is attributed to impairment of the upper airway reflexes, such as the coughing and swallowing reflexes [3]. It has been reported that augmentation of the receptor-sensitivity in the upper airway decreased the incidence of aspiration pneumonia [4]. However, concerning the cough reflex, whether only the receptors in the upper airway are responsible for aspiration pneumonia is a matter of question, because there are numerous cough receptors in the lower airway as well. When noxious materials escape from the tall systems in the pharynx, these aspirates then stimulate tracheobronchial cough receptors. One hypothesis of the present study was that inputs from the laryngeal and tracheobronchial cough receptors might additively act to potentiate coughing.

Since the cough plays important roles in the airway defense mechanisms, finding a method to enhance the cough reflex would be beneficial. The neural mechanisms of the cough reflex involve the airway receptors and medullary neurons that control respiration [5]. Therefore, another hypothesis of the present study was that some

respiratory stimulants might potentiate cough reflex to some extent.

Subjects and methods

This study was approved by the Animal Ethics Committee of the Tokai University School of Medicine.

The subjects were 15 beagle dogs (7–9 kg) anesthetized with short-acting intravenous (IV) anesthetics (profobol, 7.5–30 mg/kg/h). A balloon-tipped catheter was placed in the esophagus to measure intrathoracic pressure (P_{pl}). Fine-wire bipolar electrodes were implanted in the external oblique abdominal muscle to record abdominal EMG. Since tracheal intubation hinders vocal cord motions, a tube surrounded by a large balloon was placed in the oral cavity to measure respiratory flow while avoiding tracheal intubation. The tip of the tube stayed between the epiglottis and vocal cord. A pneumotachometer (TV112T, Nihon Kohden, Japan) was connected to the other end of the tube for flow measurement ($\dot{V}$).

The cough response was elicited by mechanical stimulation of the vocal cords or tracheal bifurcation, five times, using a wire inserted through two small holes in the low neck (Fig. 1). Exact position of the tip of each wire was confirmed by a bronchoscopy. To equalize stimulus intensities, one of the authors (N.H.) always acted as a stimulator.

Since the cough response is usually composed of several bouts of coughing, we defined four parameters to assess the cough intensity (Fig. 2). The number of cough bouts was counted on the flow trace. The explosive flow of the cough bout was discriminated from the simple expiration by referring to the activity of the abdominal muscle. The duration of the cough reflex was the period from the onset of the first bout to the termination of the last one. The mean of the peak P_{pl}s was an average of the peak P_{pl}s of the cough bouts. The mean of the peak explosive flows was an average of the peak flows of the cough bouts [5].

The cough parameters under the following conditions were compared in response to (1) stimulation of the vocal cord, tracheal bifurcation, and both sites; (2) either type of stimulation at two different anesthetic levels induced by a two-fold increase of intravenous anesthetics; (3) stimulation of the tracheal bifurcation with the administration of respiratory stimulants, i.e., inhalation of 8% CO_2 + 88% O_2 gas mixture, IV aminophylline (20–30 mg), or IV dimorphoramine (15 mg). Before doubling the dose of anesthetic, eyelash reflex and responsiveness to paw pinch were preserved, and these responses usually disappeared after doubling the dose.

Data were expressed as the mean ± SE. Statistical analysis was performed with a paired *t*-test. A p value < 0.05 was regarded as indicating a significant change.

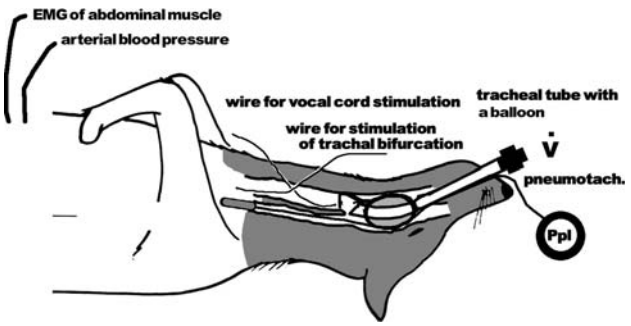

Fig. 1 Experimental setup. Respiratory flow ($\dot{V}$) was measured using an oral tube with a large balloon not to prevent vocal cord motions. Pleural pressure (P_{pl}) was measured with an esophageal balloon-tipped catheter. Electromyogram of the external oblique abdominal muscles was measured with needle electrodes, and arterial blood pressure was also measured

Results

Effects of simultaneous stimulation of the receptors in the upper and lower airways

Stimulation of either the vocal cord or tracheal bifurcation provoked a series of cough bouts (Fig. 3). The response to vocal cord stimulation was almost always preceded by an inspiratory flow, and thus the expiration reflex was rarely provoked. As shown in Fig. 4, when the vocal cord and tracheal bifurcation were simultaneously stimulated, all of the four cough parameters increased compared with measurements during the stimulation of an individual receptor.

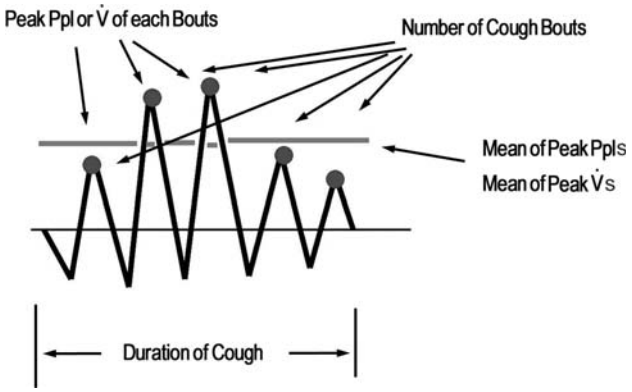

Fig. 2 Cough parameters defined for analysis. The number of cough bouts was counted on the flow trace. The duration of the cough reflex: the period from the onset of the first bout to the termination of the last one. The mean of the peak pleural pressures (P_{pl}s): an average of the peak pleural pressures of the cough bouts. The mean of the peak explosive flows ($\dot{V}$s): an average of the peak flow of the coughing bouts

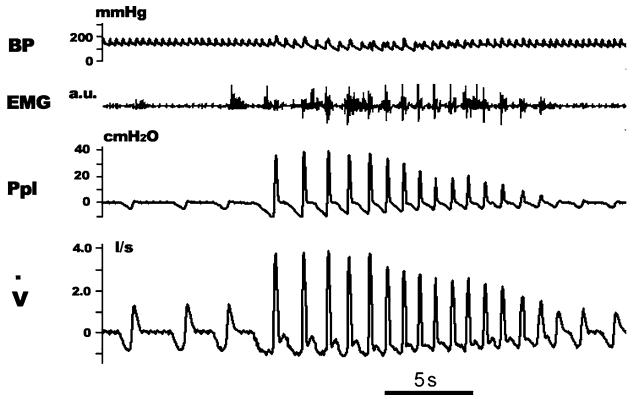

Fig. 3 An example of coughing provoked by simultaneous mechanical stimulation of the tracheal bifurcation and vocal cords. *BP* arterial blood pressure, *EMG* electromyogram of the external oblique abdominal muscle, P_{pl} pleural pressure, $\dot{V}$ respiratory flow

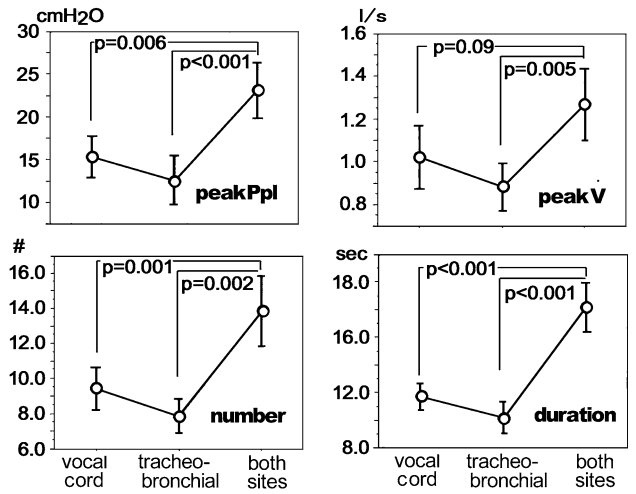

Fig. 4 Response to stimulation of the vocal cord, tracheal bifurcation, and both sites. All of the cough parameters were increased by simultaneous stimulation of both sites compared with those during isolated stimulation. Number, number of cough bouts; duration, duration of coughing

However, the increases in the mean of the peak P_{pl}s (compared with tracheal bifurcation stimulation) and those in the mean of the peak flows (compared with vocal cord stimulation) did not reach statistical significance.

Effect of anesthetic level

The arterial blood gases under light anesthesia (pH 7.280 ± 0.011, PCO_2 44.6 ± 1.0 mmHg, PO_2 98.6 ± 5.1 mmHg) and those at a deeper anesthetic level (pH 7.276 ± 0.012, PCO_2 44.7 ± 1.0 mmHg, PO_2 89.4 ± 2.8 mmHg) were not significantly different. Figure 5 shows the changes in cough parameters with a change in anesthetic level. All the parameters in response to

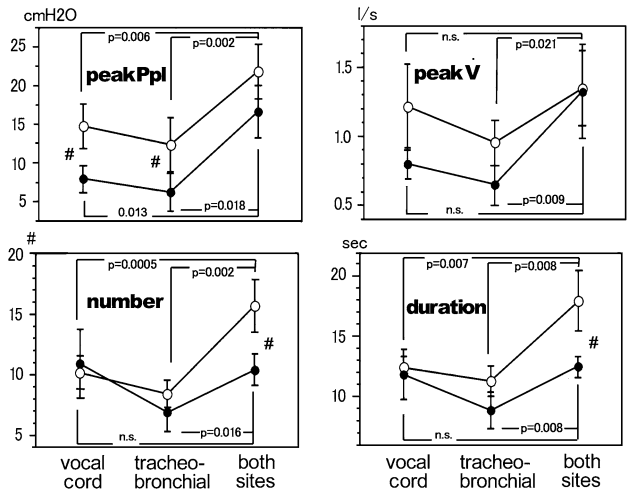

Fig. 5 Changes in cough parameters with a change in anesthetic level. All of the parameters in response to any of the stimulus sites tended to decrease with deeper anesthesia (*filled circles*) compared with those at the light anesthetic level (*open circles*). *Hash symbol* indicates the difference between light and deep anesthesia was significant

stimulation at either site tended to decrease with deeper anesthesia (filled circles) compared with those at the light anesthetic level (open circles). However, there were some differences in the effect of anesthetic level on individual parameters. Concerning the cough frequency and cough duration, the additive effects of simultaneous stimulation to both sites were significantly attenuated by deeper anesthesia (Fig. 5, lower two panels). In contrast, the anesthetic effects were not apparent in the responses of peak P_{pl}s and peak flow (upper two panels in Fig. 5).

Influence of respiratory stimulants

Figure 6 shows the effects of acute hyperoxic hypercapnia on the cough parameters. The arterial blood gases during the breathing of room air were pH 7.293 ± 0.017, PCO_2 43.1 ± 1.7 mmHg, and PO_2 94.4 ± 4.4 mmHg, and those 10 min after the onset of the mixed gas inhalation were pH 7.189 ± 0.013, PCO_2 54.5 ± 1.9 mmHg, and PO_2 527.5 ± 14.3 mmHg. The minute volume (MV) of respiration was increased almost two-fold (3.92 ± 0.52 vs. 8.05 ± 1.60 l/min), and the increase was statistically significant. The increase in the mean of the peak P_{pl}s was two-fold (10.49 ± 2.13 vs. 18.82 ± 2.11 cmH_2O) and that in the mean of the peak flows was 1.5-fold (0.62 ± 0.11 vs. 0.85 ± 0.10 l/s). These changes were statistically significant.

Figure 7 shows the effects of IV dimorphoramine on the cough parameters. The MV of respiration was significantly increased (4.05 ± 0.37 vs. 6.75 ± 1.28 l/min). Both the mean of the peak P_{pl}s (11.31 ± 2.50 vs. 17.67 ± 3.85

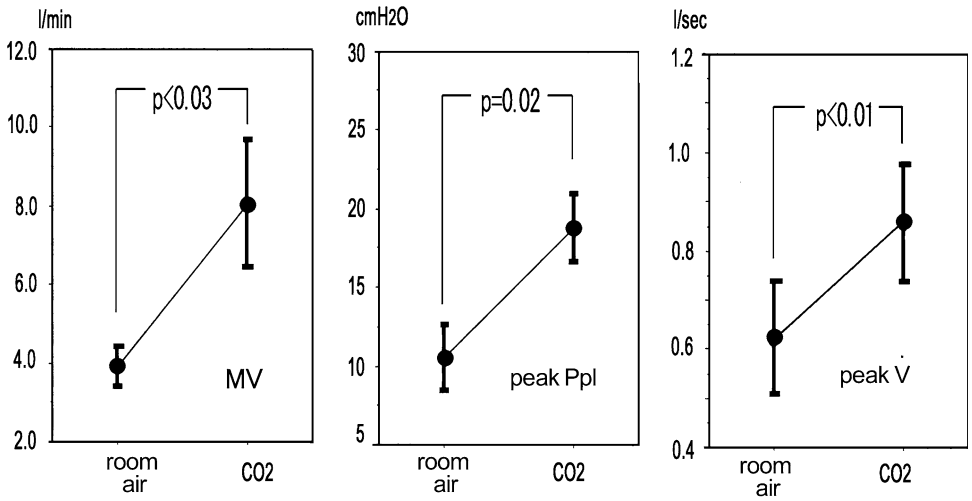

Fig. 6 Effects of acute hyperoxic hypercapnia. The minute volume (MV) was significantly increased during acute hyperoxic hypercapnia (CO_2). The increase in the mean of the peak P_{pl} was almost twofold and that in the mean of the peak explosive flows (peak $\dot{V}$) was 1.5-fold. These changes were statistically significant

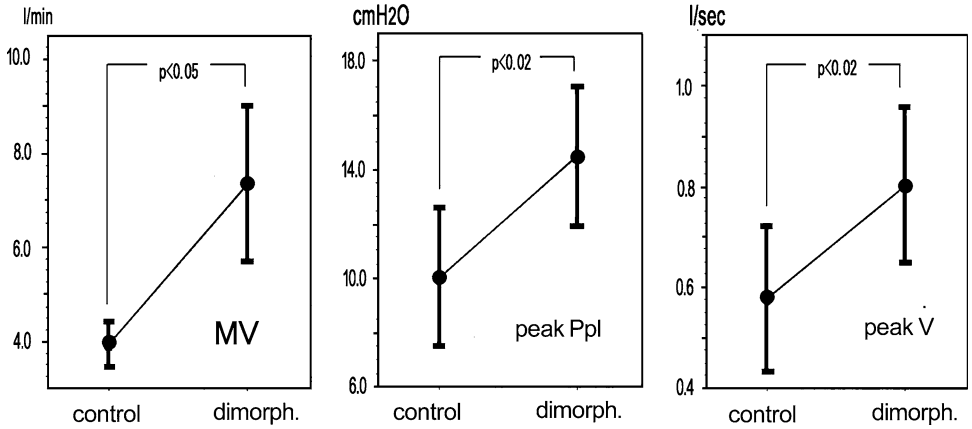

Fig. 7 Effects of IV dimorphoramine. The minute volume (MV) was significantly increased by intravenous administration of dimorphoramine (dimorph). Both the mean P_{pl} and the mean of the peak explosive flows (peak $\dot{V}$) were significantly increased

cmH_2O) and the mean of the peak flows (0.59 ± 0.12 vs. 0.82 ± 0.13 l/s) were also significantly increased.

Serum concentration of theophylline after IV aminophylline was 14.4 ± 1.7 μg/ml. Figure 8 shows the effects of IV aminophylline on the cough parameters. The MV was significantly increased (4.00 ± 0.80 vs. 5.87 ± 1.12 l/min), but the increase was less than those produced by acute hypercapnia or IV dimorphoramine. The increases in the mean of the peak P_{pl}s (17.26 ± 3.26 vs. 17.55 ± 3.22 cmH_2O) and the mean of the peak flows (0.86 ± 0.13 vs. 0.90 ± 0.12 l/s) were so small that the increases did not reach statistical significance.

Discussion

This study revealed that (1) simultaneous stimulation of cough receptors in the upper and lower airways elicited stronger coughing in terms of intensity and duration; (2) the prolongation of the cough almost disappeared when the anesthetic level was deeper, but the augmentation of cough intensity was much less affected by deeper anesthesia; (3) stimulation of the central respiratory neurons increased the intensity of the cough response.

Simultaneous stimulation of the upper and lower airways

Receptors initiating the cough reflex are primarily located in the larynx, trachea, and large bronchi. It is generally agreed that rapidly adapting receptors (RARs), which are sensitive to mechanical stimulation, are responsible for initiating the cough reflex. C-fiber endings are also involved in the cough reflex as either sensory terminals for chemical stimulants or modulators of the cough reflex [6]. In the present study, mechanical stimulation was adopted for cough provocation, and thus RARs might have acted as the primary receptors. Bolser et al. proposed a model of a central cough generation system [7] in which inputs from the laryngeal RARs and tracheobronchial RARs are mediated through different relay neurons. In this sense it is not surprising that inputs from the laryngeal and tracheobronchial receptors had additive effects on the intensity, number, and duration of coughing bouts. However, there were

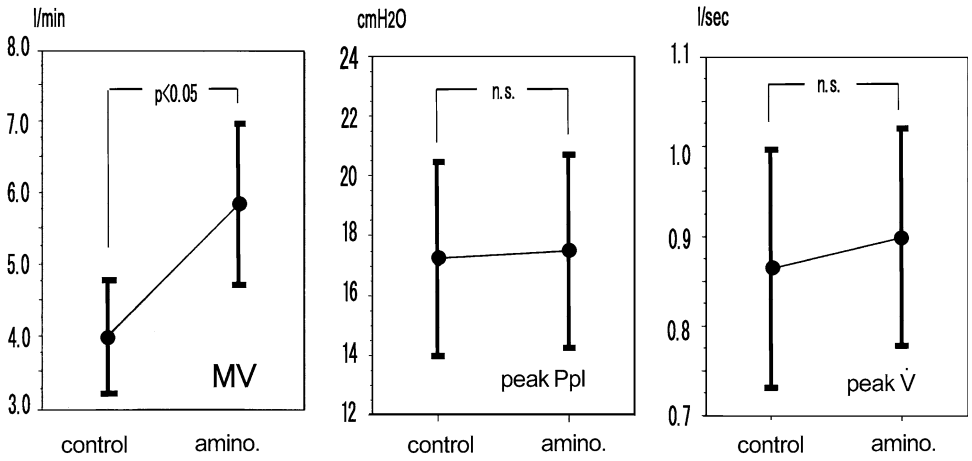

Fig. 8 Effects of IV aminophylline (amino). The minute volume (MV) was significantly increased, but the increase was less than those produced by acute hypercapnia or IV dimorphoramine

some differences in changes of the four parameters. Simultaneous stimulation of the laryngeal and tracheobronchial receptors did not always significantly increase the intensity parameters (the mean of peak P_{pl} and that of the peak flows). In contrast, the time parameters (the number of cough bouts and the duration of the cough reflex) were always significantly increased with simultaneous stimulation. This difference is pertinent because when noxious materials have invaded both the upper and lower airways, an increase in cough duration rather than intensity may be effective for sequential ejection of such materials.

Effects of anesthetic level on coughing

The results of our study suggested that the inputs from the laryngeal and tracheobronchial receptors additively acted to develop stronger and longer coughing. We analyzed the mechanism of this coordination by further administration of short-acting anesthetics. Arterial blood gases did not significantly change with deeper anesthesia, suggesting that suppression of the respiratory neurons was minimal. In deeper anesthesia, the additive effects of inputs from the laryngeal and tracheobronchial receptors on the intensity parameters were maintained, while those on the duration parameters almost disappeared. The effect of anesthesia on the laryngeal and tracheobronchial receptors has not been studied. Intravenous anesthesia may have acted on various sites of the cough reflex loop. For example, the threshold of the cough receptors may be increased with deeper anesthesia. The effect of anesthesia may reflect suppression of either the forebrain or the airway receptors.

Effects of respiratory stimulants on the cough reflex

From the results described above, we speculated that the inputs from the laryngeal and tracheobronchial receptors work in concert to augment the cough reflex. There was a worldwide study examining the effect of an oral ACE inhibitor, which has been reported to increase the sensitivity of the laryngeal receptors [8] on the prevention of pneumonia in people with a history of stroke or ischemic attack [9]. However, the results failed to provide a conclusive benefit. Therefore, we hope to find another method to potentiate the cough reflex. The neural mechanisms of the cough reflex involve the medullary respiratory neurons [10] as well as receptors in the airways. We stimulated the medullary respiratory neurons using three different methods: acute hyperoxic hypercapnia, IV dimorphoramine, and IV aminophylline. These stimulants significantly increased the dogs' MV, and these increases were associated with increases in the cough-intensity parameters (the mean of the peak P_{pl}s and that of the peak flows). Thus, stimulation of the respiratory neurons is a promising method for augmenting the cough reflex.

The increases in cough-intensity parameters were significant during acute hypercapnia or IV dimorphoramine, while the changes after IV aminophylline were not. It is known that hypercapnia stimulates central respiratory neurons via changes in cerebrospinal fluid pH. Although many of neurons for respiration and coughing are overlapped in the brainstem, their integration is not the same. For example, some expiratory neurons are excited by carotid chemoreceptor stimulation, while they are suppressed during fictive cough [11]. Dimorphoramine has been used for more than 50 years as a respiratory stimulant. This drug acts on the central neurons, but the precise mechanism of its action is not clear because modern analyses of the pharmacologic mechanism of this drug have not been performed. Intravenous aminophylline enhances respiration at the level of the brainstem [12], but the induced hyperventilation is not associated with changes in the ventral medullary extracellular fluid PCO_2 or hydrogen ion concentration [13]. Among the other respiratory effects of aminophylline, muscular enforcement [14] could potentiate

cough intensity, but in our study the increase in cough strength did not reach statistical significance after IV aminophylline. Its bronchodilating effects may not be concerned in our results because aminophyilline is a cough depressant rather than enhancer in clinical use for bronchial asthma. Therefore, the major sites of three stimulants examined in this study may be the central architecture. Either acute hypercapnia or IV dimorphoramine augmented the MV two-fold, while IV aminophylline changed it only 1.5-fold. Thus, the respiratory-stimulating effect of IV aminophylline was less than that of the other two stimulants. Further injection of aminophylline was not possible because the serum concentration had already reached a higher level in the therapeutic range. We speculate that the differences in cough response to these drugs are related to the strength of the respiratory stimulation.

There remains a question about whether an increase in MV is always accompanied by augmentation of the cough reflex. For example, Tatar et al. [15] reported that in acute hypoxia (F_IO_2, 0.11–0.04) MV increased, but intensity of the cough reflex diminished in the anesthetized and endotracheally intubated cat. However, in their study, increases in MV produced hypocapnia, which might have caused suppression of the cough reflex. Eckert et al. [16] reported similar results in human subjects. In that report, acute hypocapnic hypoxia (SpO_2 80%, i.e., $PaO_2 \sim 44$ mmHg, and end-tidal $CO_2 \sim 35$ mmHg) suppressed both the cough threshold to inhaled capsaicin and increases in cough number to a higher concentration of capsaicin. Although capsaicin stimulated chemical receptors rather than mechanical ones, afferent signals from either receptor were transmitted to the same central neurons [17]. Similar to Tatar's study [15], results from hypocapnic hypoxia may not be adopted to our study.

Several studies suggested that hypercapnia suppressed cough reflex [18, 19], and these are contradictory to our results. However, in the study by Nishino and Honda, the authors studied not cough reflex, but expiration reflex. The function of the former reflex is to dump noxious materials from the lower airway by explosive air flow, but function of the latter one is to prevent external material to pass through the larynx. Furthermore, we should not forget that the function of dog's vocal cord was preserved in our study. In cough reflex, vocal cord closure has significant function by compression of thoracic gas, which strongly enforces explosive flow. Increases in P_{pl} and chest wall compression during cough, stimulating the intra- and extra-thoracic mechanical receptors such as pulmonary stretch receptors, significantly modify the strength of cough [20, 21]. We also experienced that the MV was increased by acute hypercapnia (end-tidal CO_2 6.5–8.0%), but P_{pl} of the cough bouts did not significantly increase in the anesthetized and tracheostomized dog [22]. We found synchronization of the respiratory phase to cough attenuated cough intensity at acute hypercapnia.

In conclusion, inputs from the laryngeal and tracheobronchial cough receptors acted in concert and thereby potentiated the cough reflex in terms of intensity and duration. Intravenous anesthesia selectively suppressed the prolongation of the cough during simultaneous stimulation of the upper and lower airways, presumably by the suppression of forebrain or cough-receptor sensitivity. Although some method used to stimulate respiratory neurons in this study is not clinically feasible in human patients, stimulation of the central respiratory neurons may be a promising method for increasing the intensity of the cough response.

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