Influence of Central Respiratory Activity on the Cough Response in Anesthetized Dogs

Hideo SUZUKI, Tetsuri KONDO, Hajime YAMABAYASHI,
Ichiro KOBAYASHI, and Yasuyo OHTA

*Department of Medicine, Tokai University School
of Medicine, Isehara, 259-11 Japan*

Abstract Cough responses evoked by mechanical stimulation of the tracheobronchial mucosa in anesthetized and tracheostomized dogs were studied. The most common response was a group of coughs. Phase relationships between coughing and spontaneous respiration during the cough initiation and resolution periods were categorized as either synchronized or unsynchronized. We defined the synchronization as the coincidence of an expiratory thrust and the early-expiratory phase of respiration. During the cough initiation period, the incidence of synchronization increased as central respiratory activity was enhanced by hypercapnia or as the cough center's activity was suppressed by deep anesthesia. Synchronization decreased as central expiratory activity was enhanced by expiratory threshold loading. During the cough resolution period, synchronization occurred in conjunction with a gradual decrease in the cough center's activity. Coughing could be evoked when the dog was made apneic either by hyperventilation or by the Hering-Breuer reflex. In either case, apnea persisted after coughing subsided. These findings suggest that mechanical stimulation directly activates the cough center rather than the respiratory center; and that synchronization is determined by the relative strengths of the respiratory and cough center's activities.

Key words: respiratory rhythm, synchronization, latent expiratory unit, mechanical stimulation, chemical drive.

Physiological and pharmacological studies with experimental animals have suggested a cough center in the medulla oblongata (KASE, 1980; MORI and SAKAI, 1972), and this center is thought to be functionally independent of the respiratory rhythm generator. However, there are some expiratory neurons at the caudal end of the nucleus ambiguus, which are inactive during quiet breathing and are

recruited during the expiratory phase of cough and sneeze (JAKUS *et al.*, 1985). These neurons are also recruited during expiration while animals were breathing against an expiratory threshold loading (ENGELHORN and WELLER, 1965; JAKUS *et al.*, 1985; PRICE and BATSEL, 1970). These observations suggest that commands for respiratory rhythm and those for the cough reflex share common medullary neurons. In the present study, we analyzed cough coordination with spontaneous respiration under different central respiratory activities.

MATERIALS AND METHODS

The experiments were performed on 14 dogs weighing 10.7–14.5 kg. The animals were initially anesthetized with sodium pentobarbital of 25 mg/kg. During the experiments, light anesthesia, a level at which the ciliary reflex was absent but the light and laryngeal reflexes were preserved, was maintained with intraperitoneal Dial-urethan (Dial 100 mg/ml, urethan 400 mg/ml; 0.6 ml/kg body weight) (ARITA and BISHOP, 1983). Each animal was placed in the supine position. The femoral artery was cannulated to measure arterial blood pressure continuously.

Experimental procedures. Each animal was studied under six conditions: (1) quiet breathing, (2) augmented breathing with CO₂-mixed air (end-tidal CO₂ between 6.5 and 8%), (3) breathing against an expiratory threshold load of 7.5 cmH₂O, (4) apnea induced by artificial hyperventilation, (5) apnea induced by airway obstruction at end-inspiration, and finally (6) deep anesthesia induced with an additional 4 mg/kg of pentobarbital.

In condition (3), the dogs breathed through a one-way breathing valve. A tube connected to the expiratory port of the valve was dipped into a beaker with 7.5 cm under water level. In condition (5), the dogs were manually hyperventilated with a rubber bag (Ambu International Resuscitator, MRK-2).

The cough response was evoked by gentle mechanical stimulation of the tracheobronchial mucosa with a polyethylene catheter (o.d. 2 mm) inserted through the tracheostomy. The fine flexible catheter provided light touches on the tracheobronchial mucosa. The catheter length was determined so that it could stimulate the trachea at the level of carina. Mechanical stimulation was always performed by two of the authors, i.e., H. S. and I. K., to provide constant intensity. With higher frequency stimulation, coughing with high airway pressure developed promptly; while with lower frequency, repeated mechanical stimulation was necessary and the evoked cough did not have high airway pressure. The stimulus strength was classified into three grades according to stimulus frequency: light stimulus represented a frequency less than 1 Hz; moderate stimulus represented a frequency between 1 and 4 Hz; strong stimulus represented a frequency higher than 4 Hz.

As will be described later in the RESULTS (see Fig. 2), we defined a cough as a rapid expiration preceded by a preparatory inspiration. However, a preparatory inspiration is occasionally absent in our study. When the rapid inspiration is not clear, high-amplitude spikes in expiratory muscle EMGs are useful to discriminate

Fig. 1. Schematic representation of the technique to implant electrodes into the diaphragm. Dogs were placed in supine position. The electrodes with their tip hooked were implanted into the costal diaphragm under laparoscopic observation.

a cough. Thus, the cough response was characterized by high-amplitude EMG spikes with an abrupt increase in esophageal pressure and gastric pressure.

Esophageal pressure (P_{pl}) and gastric pressure (P_g) (Toyo Baldwin, LPU-0.1-350-0-II), as well as air flow (Minato Medical, RF-L) and CO_2 concentration at the orifice of the endotracheal tube (F_{CO_2}) (Beckman, LB-2), were continuously monitored. The electrical activity of five respiratory muscles, i.e., the diaphragm, the internal intercostal muscle (IIC), the rectus abdominis (RA), the transverse abdominis (TA) and the external oblique (EO) abdominal muscle, were recorded using pairs of fine-wire ($30\ \mu m$ in diameter), hook-shaped electrodes (BASMAJIAN and STECKO, 1962). The technique used to implant the recording electrodes into the diaphragm without a laparotomy, was previously described in detail (KONDO, 1989) (Fig. 1). The electrodes were delivered through a spinal-tap needle (21 G). After injecting 300 ml of air intraperitoneally, the needle was introduced percutaneously through abdominal wall. The exact location of the needle tip was directly observed via laparoscopy (Machida, FLX). Once the electrodes were properly implanted into the costal diaphragm, the delivering needle and the laparoscope were removed and abdominal air was evacuated. The P_{pl} , P_g , air flow, F_{CO_2} , and EMGs of the respiratory muscles were displayed on a chart recorder (Nihon Kohden, WS682G) with a response frequency of 2.8 kHz.

In each experimental condition, the characteristics of the evoked cough were analyzed with respect to the following: (1) the phase relationship between the cough and spontaneous respiration, (2) the initial phase of the evoked cough, i.e., whether the cough started with preparatory inspiration or rapid expiration, and (3) the changes in respiratory rhythm during cough resolution.

RESULTS

Responses during quiet breathing

Figure 2 illustrates a typical record during quiet breathing. The most common response to mechanical stimulation was a series of coughs. An individual cough consisted of the preparatory inspiration followed by rapid expiration, i.e., an expiratory thrust. However, the initial cough in series occasionally lacked a preparatory inspiration. High-amplitude spikes were consistently observed in expiratory muscle EMGs during the early-expiratory phase of a cough. Thus, the cough response was characterized by high-amplitude EMG spikes with an abrupt increase in P_{pl} and P_g .

We divided the time course of a series of cough responses into three stages as shown in Fig. 2. In the initiation stage (stage 1), the increases in pleural and abdominal pressures were small (less than 50% of peak P_{pl} in stage 2); but high-amplitude spikes of the expiratory muscle EMGs indicated that the responses were coughs. In stage 2, P_{pl} and P_g were greater ($P_{pl} > 5.0 \text{ cmH}_2\text{O}$); and high-amplitude spikes were consistently observed in the expiratory muscle EMGs. In stage 3, stimulation was discontinued; but the responses of high P_{pl} and P_g persisted. In this stage, the responses of P_{pl} and P_g gradually became less and the

Fig. 2. Coughing during normocapnia. The traces indicate pleural pressure (P_{pl}), gastric pressure (P_g), respiratory flow, EMG of the external oblique (EO-EMG), and EMG of the transverse abdominis (TA-EMG). A group of coughs is divided into three stages. Recruitment of the expiratory muscles in early-expiratory phase of coughs is indicated by high-amplitude EMG spikes.

Fig. 3. Schematic representation of synchronized and unsynchronized coughs. We compared the phases of abdominal and pleural pressures to the expiratory muscles EMGs to determine whether the expiratory thrusts are coincident with the early-expiratory phase of spontaneous breathing or not.

amplitudes of the expiratory EMG spikes in individual coughs gradually decreased as well.

Since the most essential part of a cough is an expiratory thrust, we characterized the phase relationship between the coughs and spontaneous respiration by the following definitions. "Synchronized" indicates the coincidence of an expiratory thrust and the early-expiratory phase of spontaneous respiration. "Unsynchronized" indicates an arbitrary relationship between the expiratory thrust and the respiratory cycle, not including early-expiration (see Fig. 3). Even if a single unsynchronized cough occurred, the group was classified as unsynchronized. The cough in stage 3 was defined as synchronized or unsynchronized by the same criteria: If the difference between the expiratory thrusts and the expiratory phase of the spontaneous respiration was unclear the relationship was regarded as synchronized and if not it was regarded as unsynchronized. With these definitions for phase relationships, we classified the groups of coughs in stage 1 as synchronized in 41 of 119 (34%) provocations and unsynchronized in 64 of 119 (54%). In stage 3, synchronization was observed in all provocations.

Fig. 4. Coughing during hypercapnia. The traces show *Ppl*, *Pg*, respiratory flow, EMG of diaphragm (Diaph.-EMG), and EO-EMG. A high incidence of synchronization occurred during stage 1.

Responses during hypercapnia

Amplitude spikes in expiratory muscle EMGs was higher during moderate hypercapnia (end-tidal CO_2 between 6.5 and 8%) than those during normocapnia. However, when mechanical stimulation was applied during moderate hypercapnia, *Ppl* and *Pg* increased abruptly and they were always associated with two- or threefold higher-amplitude spikes in expiratory muscle EMGs (Fig. 4). In this condition, light or moderate stimulation, the same strength as those applied for normocapnia, could evoke coughing. The intensity of the evoked cough, as expressed by an increase in *Ppl* and *Pg*, was similar to the cough during quiet breathing (11.4 ± 4.6 (S.D.) cmH_2O during quiet breathing vs 12.3 ± 4.5 cmH_2O during hypercapnia, $p=0.65$ by *t*-test). The coughs in stage 1 were classified as synchronized in 29 of 49 provocations (59%), and the incidence of synchronization during hypercapnia was greater than during quiet breathing in 11 of 14 dogs. When stimulation was discontinued, the coughs resolved gradually and synchronized with spontaneous respiration.

Responses during expiratory threshold loading

Ten animals were studied for expiratory threshold loading. While the animals breathed against a load of 7.5 cmH_2O , their respiratory frequency reduced and their expiratory muscles were recruited for expiration (Fig. 5). In this condition, strong stimulation was required to evoke coughing. A cough could be evoked during either expiratory or inspiratory phase of spontaneous respiration of expiratory

Fig. 5. Coughing while breathing against expiratory threshold loading of 7.5 cmH₂O. There is no synchronization of the coughs and spontaneous respiration in stage 1, but synchronization occurred in stage 3.

threshold loading. In stage 1, the coughs were classified as synchronized in only 4 of 32 provocations (12%), and the incidence of synchronization did not increase in all of the 10 dogs. Synchronization frequently occurred in stage 3.

Responses under deep anesthesia

Six animals were studied under deep anesthesia. Strong stimulation was required to evoke coughing while the dogs were under deep anesthesia (Fig. 6). Frequently an expiratory thrust was not clear in each cough. Although recruitment of large motor units and increased firing frequency of on-going motor units in the expiratory muscles were observed, high-amplitude spikes in the EMGs of expiratory muscles were not limited to the early-expiratory phase. The most prominent change observed during stimulation was the shortening of both the inspiratory and expiratory time. In stage 1, the coughs were classified as synchronized in 10 of 15 provocations (67%); and the incidence of synchronization increased in 5 of 6 dogs.

Figure 7 summarizes the incidence of synchronization in the four conditions. The differences in synchronization were considered significant at the $p < 0.05$ level with a χ^2 test.

Responses during post-hyperventilation apnea

During passive hyperventilation, tonic activity was observed in the EMGs of expiratory muscles for 100 to 200 s. This was then followed by an apneic period with no respiratory muscle activities. If hyperventilation was discontinued at this

Fig. 6. Coughing under deep anesthesia. The invoked coughs are weak and are difficult to discriminate from spontaneous respiration.

Fig. 7. Incidence of synchronization between coughing and spontaneous respiration during phase 1.

moment, the complete apnea persisted for 20 to 40 s. During this apnea, mechanical stimulation evoked coughs but strong stimulation was required. The cough developed in 5–10 s after stimulation. The initial cough in a group of coughs invariably lacked preparatory inspiration. Spontaneous respiration did not resume immediately after the coughing subsided (Fig. 8). Tonic activity of the expiratory

Fig. 8. Coughing during post-hyperventilation apnea. The data were obtained after 4 min of passive hyperventilation. The invoked cough always starts with an expiratory thrust. Apnea remains after coughing subsides and the expiratory muscles remains tonically active.

muscles developed during the apneic interval and continued until spontaneous respiration resumed. However, with a few exceptions, complete apnea recurred after several coughs.

Responses during Hering-Breuer reflex

When the airway was obstructed at end-inspiration, spontaneous respiration ceased for 20 to 50 s (Fig. 9). After approximately 0.5 s of silence, during which the inspiratory and expiratory muscles were inactive, the expiratory muscles began firing in coincidence with increases in *Ppl* and *Pg*. During this apneic interval, stimulation could evoke coughing, but strong stimulation was required. The initial cough invariably lacked preparatory inspiration. A single cough without preparatory inspiration could be evoked.

DISCUSSION

A cough is typically composed of three phases: a preparatory inspiration, compression of intrathoracic gas, and an expiratory thrust. In the present study the evoked cough lacked the compressive phase because of the existing tracheostomy. However, in most of the previous studies, intubated or tracheostomized animals have been used for cough analysis (LUNTEREN *et al.*, 1988 and see its references). Furthermore, tracheostomy has an advantage of preventing upper airway obstruction in the supine position when air flow traverses the upper airway (LUNTEREN *et al.*, 1988).

The most essential part of a cough is an expiratory thrust (SANT'AMBROGIO and REMMERS, 1985), i.e., an expulsive expiration. In the present study, the

Fig. 9. Coughing during the Hering-Breuer reflex. A cough can be invoked during the apneic interval of the Hering-Breuer reflex. Apnea persists after coughing subsides.

expiratory thrust was invariably associated with high-amplitude spikes on the expiratory muscle EMGs which represent recruitment of large motoneurons (HANNEMAN *et al.*, 1965). Although amplitude spikes in the expiratory muscle EMGs during moderate hypercapnia were higher than those during normocapnia, those with the expiratory thrust was always two- or threefold higher. Thus, the high-amplitude EMG spikes were a reliable indicator of the cough response. We propose that an analysis of respiratory muscle EMGs, as well as changes in *Ppl* and *Pg*, provides a complete set of information about the cough response.

Synchronization of coughing and respiration. In the cough initiation stage, the cough and respiratory phases were synchronized more often during hypercapnia than during quiet breathing. Hypercapnia, which enhances both inspiratory and expiratory activity in the automatic respiratory system, does not excite cough center activity since the intensity and probability of coughing did not increase during hypercapnia. On the other hand, deep anesthesia, which suppresses the cough center more than the respiratory center (YANAURA *et al.*, 1982), produced a higher incidence of synchronization. These findings suggest that phase synchronization occurs when stimuli from the cough center are weaker than stimuli from the respiratory rhythm generator.

In the cough resolution stage, synchronization invariably occurred. In this stage, the parameters of evoked coughs became less apparent suggesting that the cough commands gradually decreased. The spontaneous respiration replaced coughing without boundary, suggesting that the respiratory commands gradually increased. Therefore, synchronization of coughing and spontaneous respiration in

stage 3 confirms our hypothesis that phase synchronization occurs when stimuli from the cough center are weaker than stimuli from the respiratory rhythm generator.

Cough during post-hyperventilation apnea. Extreme hypocapnia suppresses activities of both the central inspiratory and expiratory neurons (COHEN, 1968). Thus, the absence of respiratory motoneuronal activities during post-hyperventilation apnea probably reflects inactivity of the medullary respiratory neurons. However, mechanical stimulation still evoked coughs during post-hyperventilation apnea. This finding suggests that the cough center is functionally independent of respiratory rhythm generator. In addition, the initial response to mechanical stimulation invariably lacked preparatory inspiration. This finding also suggests that the neurons, which are stimulated by the cough center, are expiratory neurons.

Afferents from slowly adapting receptors (SARs). It was difficult to evoke coughing during Hering-Breuer reflex. However, in the report by HANACEK *et al.* (1984), when the Hering-Breuer reflex was inhibited by chemical blocking of SARs in anesthetized rabbits, the incidence and intensity of the cough response were reduced. The discrepancy between our findings and those of HANACEK *et al.* (1984) may be explained by two possible mechanisms. One is the inputs from lung C-fibers. TATAR *et al.* (1988) reported that selective stimulation of lung C-fibers suppressed mechanically-provoked cough in anesthetized cats. Large static lung inflation stimulates bronchial C-fiber as well (COLERIDGE *et al.*, 1978). The other is dilatation of tracheal smooth muscle during lung inflation (MITCHEL *et al.*, 1985). When tracheal caliber becomes larger during lung inflation, the possibility to stimulate the "cough receptor" may be reduced.

During expiratory threshold loading, the SARs are continuously stimulated. Thus, decreased probability of evoking coughs may also be due to mechanisms similar to the Hering-Breuer reflex.

Expiratory activities. Since expiratory neurons fire tonically, rather than being silent during the Hering-Breuer reflex, the activity of expiratory neurons during the Hering-Breuer reflex was essentially different from their activity during post-hyperventilation apnea. The expiratory neurons were silent during post-hyperventilation apnea. Despite the differences in expiratory activity prior to the cough response, the initial cough response always lacked preparatory inspiration for both the Hering-Breuer reflex and post-hyperventilation apnea. This finding suggests that the expiratory neurons which are excited by the Hering-Breuer reflex do not play a significant role in evoking the cough response.

Deep anesthesia. Under deep anesthesia, mechanical stimulation evoked high-amplitude spikes on the EMG of the expiratory muscles but an abrupt increase in Ppl and in Pg were unremarkable. The most prominent finding was an increase in the respiratory frequency, which may be due to the response of rapidly adapting receptors (RARs) to stimulation. The RARs in larger airways do not differ histologically from RARs in smaller airways which cause hyperpnea (WIDDI-

COMBE, 1981). Therefore, when cough center is not active, the respiratory center may control the response to the stimulation of RARs.

In conclusion, this study confirms that the cough center is functionally independent of respiratory rhythm generator. The cough center may elicit cough response through the particular expiratory neurons which are not excited during the Hering-Breuer reflex. Phase synchronization occurs when stimuli from the cough center are weaker than those from the respiratory rhythm generator.

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