ANALYSIS OF EFFERENT DISCHARGES OF THE PHRENIC NERVE DURING THE COUGH REFLEX

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Abstract—The response of the phrenic nerve activities during the cough reflex was investigated in anesthetized dogs. The phrenic nerve activities were recorded from the central cut end of the nerve. Changes in phrenic nerve activities were evaluated by means of computer analysis using the programs for power spectrum, amplitude histogram, pulse density variation, and autocorrelation. The cough reflex was induced by mechanical stimulation of the tracheal mucosa. The cough reflex evoked was accompanied by an increase in the number of spike potentials and shortening of the respiratory burst period of the phrenic nerve activities. There was a significant correlation between the intensity of expiration and the multiplication product of the mean amplitude of spike potentials and spike numbers of the phrenic nerve activities during the cough reflex. Total activity of the discharge, amplitude of spike potentials, and pulse density of the phrenic nerve activities also increased during the cough reflex, while the autocorrelation coefficient decreased. These changes were inhibited by codeine. The responses of the phrenic nerve activity during the cough reflex were also observed under artificial respiration as well as under spontaneous respiration. These findings indicate that the phrenic nerve activity can be an indicator of the cough reflex, and it is useful for the indicator to investigate the central mechanisms of the cough reflex.

For the physiological and pharmacological study of the cough reflex, a pneumotachogram or pneumogram has been frequently used for the indicator of the cough reflex. However, it is impossible to utilize the pneumotachogram and pneumogram for the indicator of the cough reflex under a condition of paralysis and artificial respiration for the study of the central mechanisms of the cough reflex. Therefore, it is necessary to provide some other indicator of the cough reflex.

The efferent discharges of the phrenic nerve can be considered as a pertinent indicator of the output of the respiratory center as a whole. The cough reflex is explosive expiratory movements in nature, while the efferent discharges of the phrenic nerve is the activity of the inspiratory phase. Bucher et al. (1–3) have reported that the inspiration preceding the expiration plays an important role in the cough reflex. It is possible to suggest that the efferent discharges of the phrenic nerve are useful as the indicator of the cough reflex for neuropharmacological study, but detailed investigation on the responses of efferent discharges
of the phrenic nerve during the cough reflex has not been reported.

In the present study, we provided a comparative study of the responses of phrenic nerve activities and those of the pneumotachogram during the cough reflex, and we investigated the usefulness of determining the phrenic nerve activities as an indicator of the cough reflex.

MATERIALS AND METHODS

Male mongrel dogs weighing 10–15 kg were anesthetized with 20 mg/kg pentobarbital-Na given i.v. After an incision in the cervical midline, the cervical trachea was cannulated. Respiratory and cough responses were measured using a pneumotachograph (Nihon Kohden, MFP-1T) via a cannula inserted into the trachea. The systemic arterial blood pressure was monitored from the catheterized left femoral artery.

The right phrenic nerve was dissected free from the surrounding tissue and cut as distally as possible. Fat, connective tissue, and the sheath of the nerve were removed from a 2 cm length of the central cut end of the nerve. This nerve was placed on a bipolar platinum electrode. The exposed nerve and electrode tip were immersed in a pool of paraffin oil at 37°C made in a skin pouch. The efferent discharges of the phrenic nerve were initially amplified by means of a preamplifier (Nihon Kohden, RB-5). The amplified electric signals were led to separate channels of an audiomonitor, an oscilloscope (Nihon Kohden, VC-8), a pen recorder (Nihon Kohden, WI-387G), and a data recorder (TEAC, R-81).

Phrenic nerve activities were processed by means of a digital computer (SAN-El, 7T07A) using programs of the power spectrum, amplitude histogram, pulse density variation, and autocorrelation.

The cough reflex was elicited with mechanical stimuli to the membranous wall of the tracheal mucosa. A stimulating brush (4) of pig bristle was used for the mechanical stimulation. The mechanical stimulation was given to the mucosa for 20 sec at a time.

Some experiments were done under the condition of paralysis and artificial respiration. The animals were immobilized with decamethonium bromide (Tokyo Kasei) (initial dose of 0.4 mg/kg, i.v. and supplemental doses of 0.2 mg/kg, i.v., every hour) and ventilated artificially through a tracheal cannula connected to a positive respiratory pump (Natsume, KN-50) at a constant volume and a frequency of 20 breaths/min.

The drug used in this study was codeine phosphate (Sankyo), and the dose of drug used referred to the base. The drug was dissolved in saline solution and injected intravenously.

RESULTS

Increase of the phrenic nerve activities preceded the change of the expiratory phase of the cough reflex elicited by mechanical stimulation on the tracheal mucosa (Fig. 1). The total activity of the phrenic nerve

![Fig. 1. Responses of respiration (Resp.), phrenic nerve activities (PH.), systemic blood pressure (B.P.), and heart rate (H.R.) at the occurrence of the cough reflex. The cough reflex was induced by mechanical stimulation (MS) of the tracheal mucosa. Dots over the recordings of respiration indicate each cough reflex.](image-url)
discharges as measured by means of power spectrum analysis was increased during the cough reflex, especially markedly in the low frequency band (Fig. 2). The period of pulse density variation showed the respiratory phase of phrenic nerve activities. Shortening of the period of pulse density variation was observed during the cough reflex. Furthermore, an increase of pulse density per volley of respiratory discharges of the phrenic nerve was also observed during the cough reflex. A switchover from peak to zero level of pulse density during the cough reflex was steeper than normal respiratory volleys (Fig. 2, P.D.V.).

Both the mean amplitude of spike potentials and spike number of the phrenic nerve activities were increased during the cough reflex as computed using a program for the amplitude histogram (Fig. 3B). The autocorrelation coefficient of the phrenic nerve activity was indicated by a peak value on the autocorrelogram (Fig. 3A). The autocorrelation coefficient of the phrenic nerve activity was decreased during the cough reflex. This observation indicates the disorder of the periodical respiratory activities during the cough reflex.

An i.v. administration of codeine (3 mg/kg) inhibited the cough reflex and the increase of the phrenic nerve activities. The inhibition was recovered in the course of time (Fig. 4).

Fig. 2. Power spectrum (A) and pulse density variation (P.D.V., B) analyses of the phrenic nerve activities during the normal respiration (normal) and the cough reflex (cough). In A: the abscissa, frequency in Hz; the ordinate, power. In B: the abscissa, time course; the ordinate, number of spikes.

Fig. 3. Autocorrelation (A) and amplitude histogram (B) analyses of phrenic nerve activities during the normal respiration (normal) and the cough reflex (cough). In A: the abscissa, delay time (r); the ordinate, autocorrelation function; vertical bar at the left, calibration for correlation coefficient of 1.00. In B: the abscissa, amplitude of spike potentials; the ordinate, number of spikes.

Fig. 4. Effects of codeine on the responses of cough and phrenic nerve activities (PH.) elicited by mechanical stimulation (MS) of the tracheal mucosa. Codeine was injected intravenously. Mechanical stimuli for inducing the cough reflex were given at 5, 15, and 30 min after administration of codeine. Dots over the recordings of respiration (Resp.) indicate each cough reflex.
Codeine in a dose of 3 mg/kg i.v. also inhibited the increase of total activity of discharge observed during the cough reflex. The inhibition disappeared about 30 min after administration (Fig. 5). The response of pulse density variation caused by the cough reflex was inhibited by codeine (3 mg/kg, i.v.) for more than 30 min (Fig. 5).

A significantly positive correlation between the product of the mean amplitude of spike potentials and spike numbers of the phrenic nerve activity and the intensity of the cough reflex measured by pneumotachograph was noticed (Fig. 6).

Fig. 5. Pulse density variation (P.D.V.) and power spectrum analysis of phrenic nerve activities during the cough reflex before and after codeine administration. In P.D.V.: the abscissa, time course; the ordinate, number of spike. In the power spectrum: the abscissa, frequency in Hz; the ordinate, power. Codeine was injected intravenously.

Fig. 6. Correlation diagram between the flow rate (ml/sec) of expiration and the product of the mean amplitude of the spike potentials and spike numbers of the phrenic nerve activities (M x N). Correlation coefficient between the two variables is 0.884 (P<0.001).
In the paralyzed and artificially ventilated animals, an increase of the phrenic nerve activity was observed by the mechanical stimulation of the tracheal mucosa (Fig. 7). When compared by means of the computer using programs for the power spectrum and pulse density variation, the quantitative changes in the phrenic nerve activity under artificial respiration were similar to those observed under spontaneous respiration during the cough reflex. After i.v. administration of codeine (3 mg/kg), the response of

**Fig. 7.** Responses of phrenic nerve activities (PH) and their analyses by means of the pulse density variation (P.D.V.) and power spectrum during the normal artificial respiration (Normal) and the mechanical stimulation (MS) of the tracheal mucosa under artificial respiration. In P.D.V.: the abscissa, time course; the ordinate, number of spike. In the power spectrum: the abscissa, frequency in Hz; the ordinate, power.

**Fig. 8.** Responses of the phrenic nerve activities (PH) and their analyses by means of the power spectrum during the mechanical stimulation (MS) of the tracheal mucosa under artificial respiration before and after codeine administration. In the power spectrum: the abscissa, frequency in Hz; the ordinate, power. Codeine was injected intravenously.
the phrenic nerve activity under artificial respiration induced by mucosal stimulation were inhibited in the same manner as under spontaneous respiration (Fig. 8).

**DISCUSSION**

The quantitative measurement of the ventilation level of animals comes up as an important problem on the research of the central mechanisms of the respiratory reflex when the animal is paralyzed and ventilated artificially because the pneumotachogram or pneumogram is unavailable as an indicator of respiration. Under these conditions, it is considered that efferent discharges of the phrenic nerve are the most suitable indicator for the output of the respiratory center as a whole (5) and consequently for the research on the central mechanisms of the respiratory reflex.

Nishino and Honda (6) reported that the phrenic nerve activities increased during the mechanical stimulation of the tracheal mucosa, equivalent to the stimuli for the cough reflex. In their study, however, a detailed comparison of an increase of the phrenic nerve activity and the cough reflex has not been observed. In the present study, we observed that an increase of the phrenic nerve activity preceded the cough reflex. This result suggests that an increase of the phrenic nerve activity is the initiation of the cough reflex.

It has been reported that the extra burst discharges (7) which do not correspond with the normal respiratory periodical activities of the phrenic nerve were induced by electrical stimulation of the afferent pathway of the cough reflex (8-10). These extra burst discharges disappeared after administration of fominoben (7, 11-13) which has an antitussive effect. However, we could not observe the extra burst discharges when we used the mechanical stimulation of the tracheal mucosa as the cough stimuli. It seems that the above results were ascribed to a difference in the intensity of stimulation.

A switchover from peak to zero level of pulse density of the phrenic nerve activity during the cough reflex was steeper than that of normal respiration. This observation indicates that inspiration to expiration phase-switching during the cough reflex had taken place quickly. Furthermore, it is considered that the rapid switchover from inspiration to expiration was an important factor for the cough reflex. From these results, it is considered that the analysis of the pulse density variation of the phrenic nerve is useful for investigation of the cough reflex.

The dose of codeine (3 mg/kg, i.v.) used in this study was three times as much as the 50% antitussive dose (1 mg/kg, i.v.) (14) in conscious and unrestrained dogs. We observed that an intravenous administration of codeine inhibited an increase of the phrenic nerve activity during the cough reflex which was evaluated by means of a computer using a program for the power spectrum and pulse density variation. Therefore, the inhibitory action of codeine on the response of the phrenic nerve activities might be due to an inhibitory action to the central nervous system, and it was confirmed that the increase of the phrenic nerve activities which was observed in the present study was caused by inducing the coughing. These results suggest that the efferent discharges of the phrenic nerve is available as the indicator of the cough reflex.

In this study, a significant positive correlation between an increase of the phrenic nerve activity and the intensity of expiration during the cough reflex was observed. This finding strongly suggests that the response of the phrenic nerve activity reflects the cough reflex not only qualitatively but also quantitatively. It has been reported that the Hering-Breuer reflex (15, 16) which is elicited by the excitation of the tracheal and pulmonary
stretch receptor (17) plays an important role in the cough reflex (1–3). The efferent discharges of the phrenic nerve reflects the intensity of inspiration and contraction of the striated muscle of the diaphragm (18). Therefore, it is considered that strong contraction of the striated muscle of the diaphragm may play an important role in the cough reflex with a contraction of the expiratory muscle, mainly of the pectoral muscles.

Characteristic responses of the phrenic nerve activity during the cough reflex, i.e., an increase in the total activity of the phrenic nerve activities and rapid switchover from inspiration to expiration evaluated by means of pulse density variation, were observed under artificial respiration as well as under spontaneous respiration. Furthermore, an intravenous administration of codeine had effects on these responses as well as on spontaneous respiration.

These results substantiate the usefulness of phrenic nerve activities as the indicator to investigate the central mechanisms of the cough reflex.

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REFERENCES

1) Bucher, K. and Jacot, C.: Zum Mechanism des Hustens. Helv. physiol. pharmacol. Acta 9, 454–462 (1951)
2) Bucher, K.: Tessalon, ein hustenstillendes Mittel von neuerzeitigem Wirkungsmechanismus. Schweiz. med. Wschr. 86, 94–96 (1956)
3) Bucher, K.: Pathophysiology and pharmacology of cough. Pharmacoel. Rev. 10, 43–58 (1966)
4) Kasé, Y.: New methods of estimating cough depressing action. Japan. J. Pharmacol. 2, 7–13 (1952)
5) Hukuhara, T., Jr.: Activity of respiratory center during asphyxiation. Brain and Nerve 22, 13–21 (1970) (Abs. in English)
6) Nishino, T. and Honda, Y.: Effects of $\text{Paco}_2$ on inspiratory activity during cough reflex. Kokyu to Junkan 29, 173–176 (1981) (Abs. in English)
7) Hukuhara, T., Jr., Takano, K., Kiguchi, Y. and Kamei, J.: Effects of fominoban-HCl (PB 89 CI), PB 88 BS and PB 1058 BS on the central respiratory mechanisms in the brain stem of the rabbits as measured by efferent activity of the phrenic, hypoglossal and facial nerves. Folia pharmacol. japon. 76, 125P (1980) (in Japanese)
8) Widdicombe, J.G.: Respiration reflexes. In Handbook of Physiol. Sect. 3. Respiration, Edited by Fenn, W.O. and Rahn, H., Vol. 1, p. 585–830, Am. Physiol. Soc. Washington, D.C. (1964)
9) Kuntz, A.: The autonomic nervous system. p. 687, Lea and Febiger, Philadelphia (1947)
10) Tomori, Z., Korpas, J. and Ivanco, I.: Über die Bedeutung der afferenten Innervation bei dem aus verschiedenen Gebieten der Luftwege ausgelosten Husten. Physiologia bohemoslov. 6, 175–178 (1957)
11) Püschman, S. and Engelhorn, R.: Pharmakologische Untersuchungen über eine Substanz mit antitussiven und atemungsanregenden Eingenschaften. Arzneim.-Forsch. 23, 295–305 (1973)
12) Engelhorn, R., Trieb, G. and Weller, E.: Einfluss einer neuen antitussiv and atmungsanregend wirkenden Verbindung auf die Aktivität respiratorischer Ej-neurone in der medulla oblongata der Katze. Arzneim.-Forsch. 23, 305–311 (1973)
13) Hukuhara, T., Jr.: Effects of fominocen on the functional organization of the central respiratory neuronal mechanisms in the brain stem. Tokyo Jikeikai Medical Journal 91, 236–251 (1976) (Abs. in English)
14) Yanaura, S., Iwase, H., Sato, S. and Nishimura, T.: A new method for induction of the cough reflex. Japan. J. Pharmacol. 24, 453–460 (1974)
15) Horing, E.: Die Selbststeuerung der Atmung durch den Nervus vagus. Sitzber. Akad. Wiss. Wein. Math-Naturwiss. kl. (Abt II) 57, 672–677 (1868)
16) Breuer, J.: Die Selbststeuerung der Atmung durch den Nervus vagus. Sitzber. Akad. Wiss. Wein. Math-Naturwiss. kl. (Abt II) 58, 909–937 (1868)
17) Widdicombe, J.G.: Receptors in the trachea and bronchi of the cat. J. Physiol. 123, 71–104 (1954)
18) Lourenco, R.V., Cherniack, N.S., Malm, J.R. and Fishman, A.P.: Nervous output from the respiratory center during obstructed breathing. J. appl. Physiol. 21, 527–533 (1966)