Involvement of Central Serotonergic Mechanisms in the Cough Reflex

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Abstract—To determine the role of central serotonergic systems in modulating the cough reflex, the effects of serotonergic agonists on the respiration and the cough reflex were comparatively studied. Male and female cats were anesthetized with sodium pentobarbital. Respiration and cough reflex were measured using a pneumotachograph via a cannula inserted into the trachea. The cough reflex was elicited by electrical stimuli to the superior laryngeal nerve. Tranylcypromine, a MAO inhibitor, in a dose of 5 mg/kg, i.v., increased the respiration, but depressed the cough reflex. The serotonin precursor 5-hydroxytryptophan (5 mg/kg, i.v.) depressed the respiration and the cough reflex. Haloperidol (2 mg/kg, i.v.) abolished the tranylcypromine-stimulated respiratory responses, and it intensified the tranylcypromine induced cough depression. It is concluded that the increase in serotonin levels in the brain has a depressant influence on the central generating mechanisms of the cough reflex. Furthermore, central dopaminergic mechanisms seem to play a modulating role on the cough reflex.

There are numerous studies for screening the antitussive agents. However, very few data are available regarding these mechanisms on the control of the cough reflex, despite monumental efforts to define where the antitussive agents act, centrally or peripherally. Furthermore, the importance of neurotransmitter systems in the cough reflex has received limited attention. However, some recent studies have proposed that central serotonergic (1-4) and catecholaminergic (5-9) neuronal pathways may be involved in respiratory control. Both serotonin and dopamine containing cells may function in the peripheral chemoreceptive mechanisms of the carotid body (10-12). On the other hand, both biochemical (13) and histochemical (14, 15) fluorescence studies demonstrated that the nerve terminals containing catecholamine or serotonin were identified in regions of the central respiratory network. In fact, Armijo and Flórez (1) observed that tryptophan and 5-hydroxytryptophan decreased respiratory frequency and minute volume in cats. In the rat anesthetized with halothane, serotonergic agonists like 5-hydroxytryptophan and 5-methoxy-N,N-dimethyltryptamine produced a dose dependent decrease in basal and CO₂-stimulated respiration (3). Thus, it is possible that the central serotonergic systems may modulate the cough reflex.

In response to the afferent electrical stimulation of the vagus nerve, phrenic nerve discharges were facilitated at low frequency and inhibited at high frequency stimulation at constant pulse duration and intensity. This reversal of stimulation effect was designated as "Frequenzeffekt" by Wyss (16) and the stimulation frequency in a series of the afferent vagal stimulation, at which the effect of stimulation was reversed, was called the boundary frequency. It is already verified that a small dose of pentobarbital (17),
morphine (18) and fominoben (19) produced a lowering of the boundary frequency on the reflex responses of the phrenic nerve activity. According to these results, the inhibitory effect on the boundary frequency may be reflected in the inhibitory effect on the central relay organization for the cough reflex.

The aim of the present investigation is to examine the influence of the central serotonergic systems in modulating the cough reflex in cats. In the present study, the effects of tranylcypromine, a nonspecific monoamine oxidase inhibitor, and 5-hydroxytryptophan, a precursor of serotonin, on the respiration and the cough reflex were investigated. In addition, the effect of these drugs on the reflex responses of the phrenic nerve activities to the afferent electrical stimulation of the vagus nerve were also studied.

**Materials and Methods**

Experiments were performed on adult cats of either sex, weighing 2.3–3.6 kg. Anesthesia was produced with sodium pentobarbital (30 mg/kg, i.p.) with supplementary doses given as required (3–4 mg/kg, i.v.). The cervical trachea was exposed, and a trachea cannula was inserted into the caudal site of the transected trachea. The animals breathed with room air through this cannula. Respiration and cough reflex were measured using a pneumotachograph (Nihon Kohden, MFP-1T) via a cannula inserted into the trachea. The left femoral artery and venous were cannulated. The systemic arterial blood pressure and heart rate were measured from the cannulated left femoral artery via a pressure transducer (Nihon Kohden, MPU-0.5) and tachometer (Nihon Kohden, RT-5), respectively. Recordings were made on a polygraph (Nihon Kohden, PM-85).

The cough reflex was elicited by electrical stimuli to the central cut end of the right superior laryngeal nerve (20). A platinum bipolar electrode was used for the electrical stimulation of the superior laryngeal nerve. The exposed nerve and electrode tip were immersed in a pool of paraffin oil at 37°C made in a skin pouch. The parameters of the electrical stimulation used in inducing the coughs were a square-wave pulse with 20 Hz frequency, a pulse duration of 1.0 msec, a voltage of 0.6–1.2 V and an application duration of 10 sec. The cough responses were evaluated by measuring the number of coughs. Respiratory function was assessed by measuring the frequency (breath/min), tidal volume (ml) and minute volume (ml/min).

In order to examine the effect of drugs on the reflex responses of the phrenic nerve activities, the animals were immobilized with decamethonium bromide (initial dose of 0.4 mg/kg, i.v., and supplemental doses of 0.2 mg/kg, i.v., every hour) and ventilated artificially with room air through the tracheal cannula connected to an artificial respirator (Natsume, KN-56) at a constant volume and a frequency of 30 breaths/min. End-tidal O₂ and CO₂ levels were continuously monitored by an expired gas monitor (San-ei, 1H21) and were maintained at an optimal level of ventilation under resting conditions. The right phrenic nerve was dissected free from the surrounding tissue and cut as distally as possible. The afferent activities of the phrenic nerve were induced using a bipolar platinum electrode. Bilateral vagus nerves were also cut. The afferent fiber groups of the vagus nerve were stimulated electrically using a bipolar electrode with frequency ranging from 5 to 80 Hz at constant pulse duration (0.1 msec) and intensity (0.4–0.6 V). The nerve stimulation was performed under controlling the evoked potentials of the central part of the vagus nerve to the stimulation site in order to avoid undesirable excitation of the afferent fiber groups in the vagus nerve except for the Aα and Aβ groups. The exposed nerve and electrode tip were immersed in a pool of paraffin oil at 37°C made in a skin pouch. The response of the phrenic nerve activities was assessed by the changes in period of the phrenic nerve activities before and during the electrical stimulation of the vagus nerve.

Drugs used in this study were 5-hydroxytryptophan (Sigma), tranylcypromine (Sigma) and haloperidol hydrochloride (Serenace, G.D. Searle & Co.). Drugs were dissolved in a saline and injected into the
femoral vein. The doses of 5-hydroxytryptophan and tranylcypromine used were in the range previously used to elicit respiratory effects (1) and to increase the level of brain serotonin (21).

The results shown in the figures are expressed as the mean value±S.E. Statistical analyses were made using the Student's t-test.

**Results**

An i.v. injection of saline solution had no effects on the cough reflex and the respiration. An i.v. administration of drugs used in this study had no severe effect on the systemic blood pressure and heart rate. These responses were recovered to the control level within 5 min even when the systemic blood pressure and heart rate were affected by these drugs. As shown in Fig. 1, 5 mg/kg of tranylcypromine (TCP) produced excitation of respiration. The significant increase in the respiratory frequency was observed 5–15 min after TCP injection. The maximum effect on tidal volume and minute volume was observed 30 min after TCP injection. An application of 10 sec of electrical stimuli to the superior laryngeal nerve induced 4–6 coughs. Administration of TCP in a dose of 5 mg/kg produced inhibition of the cough number, and the maximum effect was observed 15 min after TCP injection (Fig. 1). The cats did not recover from cough depression within 60 min when TCP was injected i.v.

5-Hydroxytryptophan (5-HTP) in a dose of 5 mg/kg caused a slight reduction of respiration (Fig. 2). As shown in Fig. 2, the cough reflex was also depressed by 5-HTP. These changes, however, were not significant. In MAO inhibition studies, TCP was given to achieve magnification of the 5-HTP action. 5-HTP was injected 75 min after pretreatment with TCP. 5-HTP in a dose of 10 mg/kg with combination of TCP disrupted the respiratory movements. The respiratory movements were not disrupted but depressed significantly by 5 mg/kg of 5-HTP (Fig. 3). The significant decrease in minute volume and tidal volume were observed for at least 60 min after 5-HTP (5 mg/kg) injection. In contrast to the difficulty in obtaining a

![Fig. 1. Effects of tranylcypromine on the respiratory frequency (△), minute volume (■), tidal volume (●), and cough number (○). Tranylcypromine was injected i.v. in a dose of 5 mg/kg. Cough reflex was elicited by electrical stimulation of the superior laryngeal nerve. The stimuli were given at 5, 10, 15, 30, 45, and 60 min after drug administration. Each point represents the mean with S.E. for five experiments. Significant difference from control value is depicted as *(P<0.05).](image-url)
recovery from minute volume and tidal volume depression, it was easier to obtain a recovery from respiratory frequency depression. As shown in Fig. 4, the cough depressant effect of 5-HTP was enhanced by pretreatment with TCP. Recovery from the

![Graph](image1)

**Fig. 2.** Effects of 5-hydroxytryptophan on the respiratory frequency (□), minute volume (○), tidal volume (●) and cough number (○). 5-Hydroxytryptophan was injected i.v. in a dose of 5 mg/kg. For other explanations, see Fig. 1.

![Graph](image2)

**Fig. 3.** Effects of 5-hydroxytryptophan in combination with tranylcypromine on the respiratory frequency (△), minute volume (□), tidal volume (●) and cough number (○). 5-Hydroxytryptophan (5 mg/kg) was injected 75 min after i.v. administration of tranylcypromine (5 mg/kg). Significant differences from the control value are depicted as *(P<0.05) and **(P<0.01). For other explanations, see Fig. 1.
cough depression was also prolonged by TCP.

Haloperidol (2 mg/kg) administered 15 min prior to TCP (5 mg/kg) abolished the TCP induced increase in respiratory frequency, tidal volume and minute volume. Further-

Fig. 4. Effects of tranylcypromine and 5-hydroxytryptophan on the cough reflex. Tranylcypromine (TCP, 5 mg/kg) was injected 75 min prior to 5-hydroxytryptophan (5-HTP, 5 mg/kg). Drugs were injected i.v. at the arrow. The stimuli for inducing cough were given at 5, 10, 15, 30, 45 and 60 min after TCP and at 5, 10, 15, 30, 45 and 60 min after 5-HTP injection. Upper shows typical recordings. Lower shows time course of the responses of coughs. For other explanations, see Fig. 1.

Fig. 5. Effects of tranylcypromine in combination with haloperidol on the respiratory frequency (△), minute volume (□), tidal volume (●) and cough number (○). Haloperidol (2 mg/kg) was injected 15 min prior to tranylcypromine (5 mg/kg). Significant differences from the control value are depicted as *(P<0.05) and **(P<0.01). For other explanations, see Fig. 1.
Fig. 6. Effects of tranylcypromine and 5-hydroxytryptophan on the reflex responses of the phrenic nerve activities to the afferent vagal stimulation. Tranylcypromine (5 mg/kg) and 5-hydroxytryptophan (5 mg/kg) were injected i.v. Each column represents a percentage of the boundary frequency in each stimulation frequency. For other explanations, see the text.

Discussion

The central mechanisms regulating the cough reflex involve a large number of neurons interconnected with the respiratory center of the brain. It may be assumed that the central integrating mechanisms of the respiratory reflexes consist of 3 mechanisms: 1) afferent input receptive mechanisms, 2) respiratory rhythm generating mechanisms, and 3) output propagative mechanisms. The site of action of antitussive drugs may include all portions of these mechanisms. Among the many central-acting antitussive drugs, fominoben (22) and 2-aminoindane (23) are typical examples as they have a potent antitussive activity in spite of a potent respiratory stimulating effect with antitussive doses. The reason for this differential effect of the antitussive drugs on the respiration and the cough reflex is not clear. However, it is possible that different neuronal mechanisms and/or the neurotransmitters may act on the central integrating mechanisms of the respiration and cough reflex. This hypothesis was confirmed by the present study that TCP stimulated the respiration and depressed the cough reflex. In the present study, TCP induced increases in respiratory frequency, minute volume and tidal volume were abolished by haloperidol, a recognized dopamine receptor blocker. It is well-known that an administration of TCP, a MAO inhibitor, results in elevation of cerebral serotonin, dopamine and noradrenaline concentration (24). In rats, furthermore, apomorphine produced a dose dependent increase in the respiration (8). Therefore, the excitatory effect of TCP upon the respiration may be partially dependent on the dopaminergic mechanisms. The cough depressant effect of TCP was enhanced by haloperidol. Although the data were not shown in this paper, L-dopa (300 mg/kg, i.p.) stimulated the respiration, but had no effect on the cough number. Thus, the haloperidol induced increase in the cough depressant effect of TCP might be caused by its inhibitory effect on the TCP-stimulated respiratory responses.

5-HTP in combination with TCP rapidly caused a profound depression of the respiration and cough reflex. 5-HTP in a dose of 10 mg/kg in combination with TCP caused the disruption of the respiratory movements resulting in apneustic breathing or gasping. Since this respiratory pattern was not available for quantitative evaluation of respiration and cough reflex, the effect of 5 mg/kg of 5-HTP was examined. In regards, to 5-HTP, this study did not verify the possibility that 5-HTP per se directly depressed the respiration and the cough reflex within the central nervous system. However, inhibition of central I-aromatic...
amino acid decarboxylase with Ro 4-4602 prevented the depression of the respiration induced by 5-HTP in combination with TCP (1). It seems likely, therefore, that an inhibition of the respiration and cough reflex by 5-HTP in the present study may be due to its conversion to serotonin.

Afferent impulses from the receptors in airways, which in regard to the cough reflex, projected to the nucleus of the solitary tract in the dorsal medulla passing through the vagus and the superior laryngeal nerve (17, 25). The nucleus of the solitary tract projects to cell groups in the brain stem, including the A1 and A5 catecholamine cell groups (26). Calza et al. (27) evidenced the presence of serotonin both in the terminal axonal endings and cell bodies in the nucleus of the solitary tract. In this study, the boundary frequency in reflex responses of the phrenic nerve activities shifted to the lower side of stimulation frequency after TCP and 5-HTP in combination with TCP. These observations indicate that the increase in brain serotonin level depresses the reflexogenous responses of the phrenic nerve activities induced by the afferent vagal stimulation. This finding suggests that the serotonergic mechanisms have an inhibitory effect on the central relay organization for the respiratory reflexes mediated by the vagus nerve. This effect might play an important role in the anti-tussive effect.

In conclusion, our pharmacological study suggest that the central monoaminergic system may be involved in the process of the integrating mechanisms for the cough reflex and may act in a different manner on each portion of these mechanisms.

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