Influence of Carotid Chemoreceptors on the Vagal Reflex-Induced Tracheal Constriction

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Abstract—In this study, the effects of carotid chemoreceptors on reflex tracheal constriction were investigated in anesthetized, paralyzed, and artificially ventilated mongrel dogs. Reflex tracheal constriction was measured as changes in the intra-tracheal pressure of an air-filled balloon introduced into the rostral side of the transected trachea. A hypoxic condition was produced by ventilating the dog with 12% O₂-88% N₂. The reflex tracheal constriction induced by histamine inhalation to the bronchial side was reduced by section of the bilateral sinus nerves. The hypoxic condition significantly potentiated the reflex tracheal constriction induced by histamine inhalation. The potentiated reflex tracheal constriction during hypoxia was abolished by section of the bilateral sinus nerves. The afferent electrical stimulation to the central cut end of the vagus nerve caused a reflex tracheal constriction. The reflex tracheal constriction was significantly potentiated by hypoxia, and the potentiating response was abolished by section of the bilateral sinus nerves. The infusion of NaCN into the bilateral carotid arteries significantly potentiated the reflex tracheal constriction. The NaCN-induced potentiating effect was abolished by section of the bilateral sinus nerves. These results suggest that hypoxia potentiates the vagal reflex-induced tracheal constriction and that the hypoxia-induced potentiating effects may be mediated by carotid chemoreceptors.

Present evidence indicates that the vagal reflex plays an important role in regulating the tension of airway smooth muscle (1–3). Although the existence of the vagal reflex-induced airway constriction in asthmatic attacks has been recognized as an established fact (4–6), there have been few investigations on the analysis of reflex airway responses. In a previous report, we have demonstrated that reflex airway constriction is due to complex effects which may be mediated by sensory receptors in the airways (7).

It is well-known that asthmatic patients are subjected to severe hypoxemia during asthmatic attacks. Sterling (8) has reported that acute hypoxia caused an increase in airway resistance in normal subjects. On the other hand, it has been demonstrated that the airway resistance decreased during hyperoxic conditions in the majority of patients with chronic bronchitis (9), but not in normal subjects (10). Moreover, Vidruk (11) has reported that the magnitude of the reflex airway constriction induced by passive lung deflation was significantly enhanced by hypoxia and attenuated by hyperoxia. Thus, although the airway resistance may be affected by changes in oxygenation, the effects of such changes on the vagal reflex-induced airway constriction are not sufficiently examined.

Recently, it was shown that in dog isolated tracheal segment, a reflex tracheal constriction induced by administration of aerosolized histamine was exaggerated by hypoxia (12). The delivery of aerosolized histamine is known to activate sensory receptors in the airways. However, the influence of hypoxia on the function of sensory receptors in the airways is still unknown. Therefore, it is necessary to investigate the influence of hypoxia on the
reflex airway constriction induced by a method other than the stimulation of sensory receptors in the airways. In this study, the effects of hypoxia on the reflex tracheal constriction induced by afferent electrical stimuli of the vagus nerve were compared with those by histamine inhalation to the bronchial side. In addition, the role of carotid chemoreceptors in modulating reflex airway constriction during hypoxia was also investigated.

Materials and Methods
Forty-five male mongrel dogs weighing between 10 and 13 kg were used. The preparation was used for evaluating the vagal reflex-induced airway responses as described previously (7). Light anesthesia was induced by the intramuscular injection of ketamine hydrochloride (20 mg/kg). The cervical trachea was transected at about 7 cm caudal to the larynx, leaving the membranous wall intact. Care was taken not to obstruct the recurrent laryngeal nerves. The membranous wall at the transected site was ligated with a thread to interrupt blood flow passage across the wall. A tracheal cannula was inserted into the caudal side of the transected trachea. An air-filled balloon was introduced into the rostral side of the transected trachea to measure the intratracheal pressure and connected to a pressure transducer (Nihon Kohden, LPU-0.1) through polyethylene tubing. The volume of air in the balloon was adjusted initially to give a resting intraluminal pressure of 50 mmH2O (13).

The left femoral artery was cannulated, and the portion on which surgery was performed was treated with 1% procaine hydrochloride. The systemic arterial blood pressure was measured from the femoral arterial catheter using a pressure transducer (Nihon Kohden, MPU-0.5). The heart rate was measured with the tachometer (Nihon Kohden, AT-600G) using the systolic blood pressure as the trigger and monitored continuously.

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 with room air by an artificial respirator (Shinano, SN-480-4). End-tidal CO2 and O2 concentrations were continuously monitored by an expired gas monitor (San-Ei, 1H21), and they were maintained at optimal ventilation levels of 3.5–4.0% and 16.5–17.5%, respectively, under resting conditions. Reflex tracheal constriction was measured as changes in the intratracheal pressure of an air-filled balloon introduced into the rostral side of the transected trachea. Recordings were made on a polygraph (Nihon Kohden, RM-6000). One hour was allowed for stabilization of the preparation after completion of the operation.

Reflex tracheal constriction was induced by administration of aerosolized histamine to the bronchial side or by afferent electrical stimulation to the central cut end of the right vagus nerve. Inhalation of histamine was carried out for 10 min with about 1 ml of a 0.003% inhalant liquid in the bronchial side using an ultrasonic nebulizer (Nihon Kohden, TUR-3200), which was set into the circuit of artificial ventilation (7). The right vagus nerve in the neck was dissected free from the surrounding tissue and cut. 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 parameters of afferent electrical stimulation were a square-wave pulse with a frequency of 5Hz, pulse duration of 1 msec, voltage of 2 V and duration of application of 60 sec. The right superior laryngeal nerve was cut to avoid any direct effect to the tracheal smooth muscle during the afferent vagal electrical stimulation.

The hypoxic condition was produced by ventilating the dog with a hypoxic gas mixture (12% O2, balance N2) for 10 min. A catheter was inserted into the abdominal aorta through the right femoral artery for blood sampling. The blood samples were analyzed at 37°C for PaO2, PaCO2 and pH with a blood gas analyzer (Corning, 158 pH/Blood Gas Analyzer).

In five experiments, the peripheral cut end of the right recurrent laryngeal nerve was electrically stimulated with a square-wave pulse with a frequency of 20 Hz, pulse duration of 1 msec, voltage of 2 V and duration of application of 30 sec.
Drugs used were histamine dihydrochloride (Wako Pure Chemicals) and sodium cyanide (NaCN, Wako Pure Chemicals). All doses were expressed in terms of the base. All drugs were dissolved in saline solution. NaCN solution was infused with a pump for 65 sec at a rate of 0.052 ml/min into the bilateral carotid arteries to apply drug just to the carotid chemoreceptors.

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

Results

Effects on the reflex tracheal constriction induced by histamine inhalation to the bronchial side: Inhalation of saline solution to the bronchial side had no effect on the parameters measured. Typical recordings of the changes in intratracheal pressure, systemic blood pressure and heart rate by inhalation of a 0.003% solution of histamine for 10 min to the bronchial side are shown in Fig. 1. Histamine inhalation caused an increase in intratracheal pressure, viz., reflex tracheal constriction (Fig. 1, left panel).

Arterial blood gas and pH values under basal conditions were as follows: $P_{O_2}$ 98.0±3.4 mmHg; $P_{CO_2}$ 34.3±2.2 mmHg; pH, 7.39±0.02. After inhalation of histamine to the bronchial side, these values changed to 83.7±7.8 mmHg, 35.6±2.6 mmHg and 7.35±0.01 for $P_{O_2}$, $P_{CO_2}$ and pH, respectively. Blood gas measurements were performed at the end of a 10 min period of histamine inhalation. This suggested that carotid chemoreceptors could be stimulated during histamine inhalation. In fact, the histamine-induced reflex tracheal constriction was reduced but not completely abolished by section of the bilateral sinus nerves (Fig. 2). The section of the bilateral sinus nerves had no effect on the intratracheal basal pressure. Arterial blood gas and pH values during hypoxic conditions were 51.5±1.9 mmHg, 34.2±2.3 mmHg and 7.40±0.02 for $P_{O_2}$, $P_{CO_2}$ and pH, respectively. The hypoxic condition by itself used in this study usually had no detectable effect on the intratracheal basal pressure. The magnitude of the reflex tracheal constriction induced by histamine inhalation was significantly potentiated by hypoxia (Fig. 1, right panel and Fig. 3). The potentiating effects during hypoxia disappeared after restoring normoxia. The onset time of the reflex tracheal constric-
tion induced by 0.003% histamine inhalation was significantly shortened during hypoxia. The potentiated reflex tracheal constriction during hypoxia was abolished by section of the bilateral sinus nerves. This experiment was repeated in four other animals.

Effects on the reflex tracheal constriction induced by afferent vagal electrical stimulations: The afferent electrical stimulation to the central cut end of the right vagus nerve caused a reflex tracheal constriction (Fig. 4). The afferent vagal electrical stimulation used in

Fig. 2. Effects of section of the bilateral carotid sinus nerves (S.N.) on the reflex tracheal constriction induced by histamine inhalation. A 0.003% histamine solution was inhaled for 10 min to the bronchial side. Each column is the mean value with S.E. for five experiments.

Fig. 3. Effects of hypoxia on the reflex tracheal constriction induced by histamine inhalation. A 0.003% histamine solution was inhaled for 10 min to the bronchial side. Each column is the mean value with S.E. for five experiments. The change is significant at \( *P<0.05 \) against control values.

Fig. 4. Effects of hypoxia on the responses of systemic blood pressure (B.P.), heart rate (H.R.) and intratracheal pressure (I.P.) to the afferent vagal electrical stimuli (5 Hz, 1 msec, 2 V, 60 sec). Hypoxic condition potentiated the reflex tracheal constriction. Hypoxia alone had no effect on B.P., H.R. and I.P.
this study had no effect on the systemic blood pressure and heart rate. Moreover, the arterial blood gas and pH values did not change during the reflex tracheal constriction induced by the afferent vagal electrical stimulation.

As shown in Figs. 4 and 5, the magnitude of the reflex tracheal constriction induced by afferent vagal electrical stimulation was significantly greater during hypoxia than normoxia. The potentiated reflex tracheal constriction during hypoxia was abolished by section of the bilateral sinus nerves (Fig. 5).

An i.a. infusion of NaCN into the bilateral carotid arteries at the concentration used had no effect on the measured parameters. NaCN infusion at rate of 0.1 μg/sec had no effect on

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**Fig. 5.** Effects of hypoxia (---) and hypoxia with section of the bilateral carotid sinus nerves (----) on the reflex tracheal constriction induced by the afferent vagal electrical stimuli. Each point is the mean with S.E. for five experiments. The change is significant at *P<0.05 against pre-control values.

**Fig. 6.** Effects of NaCN infusion on the reflex tracheal constriction induced by afferent vagal electrical stimuli. NaCN solution was infused into the bilateral carotid arteries at the rate of 0.1, 0.3 and 1.0 μg/sec. Each point is the mean with S.E. for five experiments. The change is significant at *P<0.05 against pre-control values.
the reflex tracheal constriction induced by afferent vagal electrical stimulation (Fig. 6). On the other hand, the reflex tracheal constriction was slightly increased by NaCN infusion at the rate of 0.3 μg/sec and was significantly potentiated by 1.0 μg/sec (Fig. 6). The potentiating effect of NaCN infusion was abolished by section of the bilateral sinus nerves. This experiment was repeated in four other animals.

The efferent electrical stimulation of the peripheral cut end of the right recurrent laryngeal nerve caused a tracheal constriction. The hypoxic condition used in this study had no effect on the tracheal constriction.

Discussion

The results of the present investigation suggest that an acute exposure to alveolar hypoxia potentiates the reflex tracheal constriction induced by both histamine inhalation to the bronchial side and afferent vagal electrical stimulation of the central cut end of the vagus nerve.

In a previous report (7), we confirmed that the tracheal constriction observed in the preparation used in this study is mediated by an arc of the vagal reflexes. It is known that histamine inhalations stimulate irritant receptors and c-fibers and evoke a reflex constriction of airway smooth muscle (14-16). In this study, histamine inhalation to the bronchial side caused a change in arterial blood gas and pH values. The reflex tracheal constriction induced by histamine inhalation was reduced by section of the bilateral carotid sinus nerves. These findings therefore indicate that the magnitude of the reflex tracheal constriction induced by histamine inhalation is mediated not only by sensory receptors in the airways but also by the carotid chemoreceptors. The reflex tracheal constriction induced by histamine inhalation was potentiated by hypoxia, and the potentiating response was abolished by section of the bilateral carotid sinus nerves. It was reported that the effect of oxygenation on the reflex response to histamine is mediated by an interaction between sensory receptors in the airways and carotid chemoreceptors (12). There is no evidence that the activities of sensory receptors in the airways are affected by changes in oxygenation. Therefore, to confirm an involvement of the carotid chemoreceptors during hypoxia, it seems to be necessary to investigate whether vagal reflexes induced by some procedures other than histamine inhalation would be similarly affected. Thus, we attempted to induce the reflex tracheal constriction by afferent electrical stimulation of the vagus nerve.

The afferent electrical stimulation of the central cut end of the right vagus nerve induced the tracheal constriction. In a preliminary study, it was found that the constractive response induced by afferent vagal electrical stimulation was abolished by the section of both the left superior laryngeal and cervical vagus nerves or was reduced by pentobarbital at a low dose (5 mg/kg, i.v.) (data not shown). Hukuhara (17) has reported that the low dose of pentobarbital showed an inhibitory action on neuronal discharges of the respiratory center without cardiovascular effects. In a previous report, we demonstrated that the reflex tracheal constriction induced by histamine inhalation was reduced by a low dose of pentobarbital (7). It is therefore suggested that the tracheal constriction induced by afferent vagal electrical stimulation is mediated by a vagal reflex.

No changes in the arterial blood gas and pH values were observed during the reflex tracheal constriction induced by afferent vagal electrical stimulation. As described above, histamine inhalation caused a change in arterial blood gas and pH values. This discrepancy seems to come from the differences in the constractive effect on the bronchial smooth muscle. In a preliminary study, we have confirmed that histamine inhalation to the bronchial side caused a strong bronchoconstriction, while afferent vagal electrical stimulation had only a little constractive effect. The reflex tracheal constriction induced by afferent vagal electrical stimulation was potentiated by hypoxia. The potentiating response was abolished by section of the bilateral carotid sinus nerves, suggesting that the hypoxia-induced potentiation in the reflex tracheal constriction induced by afferent vagal electrical stimulation is mediated by the carotid chemoreceptors.

Comroe (18) has reported that the aortic
chemoreceptors play a major role in the initiation of powerful reflexes in the vasomotor center during anoxemia. However, Nadel and Widdicombe (19) have reported that the constrictive responses of the upper and lower airways induced by inhalation of 10% O$_2$ in N$_2$ were prevented by tying the glossopharyngeal nerves that innervate the carotid chemoreceptors, suggesting that those responses are mediated by the carotid chemoreceptors. To clarify an involvement of carotid chemoreceptors during hypoxia, the effect of NaCN infused into the carotid arteries on reflex tracheal constriction was investigated. The results revealed that reflex tracheal constriction is potentiated by NaCN infusion into the carotid arteries. It is known that the afferent discharges of the carotid sinus nerve are increased by NaCN. Lahiri and DeLaney (20) have demonstrated that single chemoreceptor afferent fibers responded to changes in arterial P$_{O_2}$. Although the afferent discharge of carotid sinus nerve was not measured in this study, the potentiating effect of NaCN in the reflex tracheal constriction was abolished by section of the bilateral carotid sinus nerves, suggesting that the potentiating response is mediated by the carotid chemoreceptors. Further work will be necessary to examine the relationship between the activity of carotid chemoreceptors and the magnitude of hypoxic potentiation on the vagal reflex-induced airway constriction.

It has been reported that the efferent vagal activities were augmented by various chemical mediators such as serotonin (21), histamine (22), PGF$_{2\alpha}$ (23), thromboxane A$_2$ (24), PGD$_2$ (25) and PAF (26). Recently, Ahmed and Marchette (27) demonstrated that alveolar hypoxia induces a release of mediators from mast cells. The hypoxic condition (12% O$_2$, balance N$_2$) used in this study had no effect on the intratracheal basal pressure and the tracheal constriction induced by the efferent recurrent laryngeal nerve stimulation. However, the hypoxic gas mixture of 10% O$_2$ in N$_2$ caused a tracheal constriction (data not shown). These results suggest that the vagal reflex-induced tracheal constriction may be potentiated by the hypoxic condition which does not induce a direct airway constriction. We conclude that the carotid chemoreceptors may play an important role in the hypoxia-induced potentiating airway responses.

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