Inhibitory Modulation on the Reflex Tracheal Constriction Induced by Afferent Vagal Stimulation

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Abstract—Afferent cervical vagal electrical stimulation caused a reflex tracheal constriction. Atropine changed the tracheal constriction into a tracheal dilatation that was almost inhibited by propranolol. In the hypertonic trachea with 5-hydroxytryptamine, a reflex dilatation following a constriction was observed by afferent vagal stimulation. The reflex dilatation was inhibited about 50% by propranolol and was abolished by hexamethonium. These results suggest that the adrenergic and nonadrenergic inhibitory innervations may mediate the reflex tracheal dilatation, especially in a hypertonic tracheal condition.

Previous investigators have suggested the relaxant role of the sympathetic nervous system in the canine trachea. Castro de la Mata et al. (1) reported that the trachea was dilated by sympathetic neuronal stimulation and by exogenous norepinephrine. Kannan and Daniel (2) and Suzuki et al. (3) demonstrated the tracheal relaxation by field stimulation of the sympathetic nerves in the isolated canine trachea. These results were obtained by stimulating the peripheral cut end of sympathetic nerves or in an in vitro system. However, the involvement of the sympathetic nervous system in the reflex airway responses has not been investigated. In a preliminary study, we confirmed that the adrenergic and nonadrenergic inhibitory innervations are involved in vagal reflex tracheal dilatation induced by transient inflation of the lungs. In the present study, we therefore examined whether or not these inhibitory innervations would mediate the vagal reflex tracheal responses induced by the afferent vagal electrical stimulation, in order to elucidate the reflex mechanism(s) in the airways.

Male mongrel dogs weighing 7–13 kg were used. Light anesthesia was induced by an intramuscular injection of ketamine hydrochloride (20 mg/kg). The cervical trachea was transected at about 7 cm caudal to the larynx, and the tracheal cannula was inserted into the caudal side. The systemic arterial blood pressure was measured from the left femoral artery by a pressure transducer (Nihon Kohden, MPU-0.5). The arterial pressure pulse was used to trigger a cardiotachometer (Nihon Kohden, RT-5), and the heart rate was continuously monitored. The animals were immobilized with dexamethonium bromide (0.4 mg/kg, i.v., and maintained by 0.2 mg/kg/hr, i.v.) and ventilated with room air by an artificial respirator (Natsume, KN-50) at a constant volume and a frequency of 20 breaths/min. End-tidal concentrations of CO₂ and O₂ were continuously monitored with an expired gas monitor (San-ei, 1H21) and were maintained at optimal ventilation levels of 3.5–4.0% and 16.5–17.5%, respectively, under resting conditions.

Responses of the tracheal musculature were measured as changes in the intratracheal pressure of an air-filled balloon introduced into the rostral side of the transected trachea using a pressure transducer (Nihon Kohden, LPU-0.1). The initial intraluminal pressure of the balloon was adjusted to 50 mmH₂O. Responses of the bronchial musculature were measured by a modification of the Konzett-Rössler method (4). The lungs were ventilated at a fixed volume of air under a constant pres-
sure (10 cmH₂O), and the ventilation over-
flow was measured using a pneumotacho-
graph (Nihon Kohden, MFP-1T) as an index
of the change in airway resistance.

The afferent cut end of the left cervical vagus nerve was electrically stimu-
lated to induce a reflex tracheal constriction. The stimulus was a square-wave pulse with a
frequency of 30 Hz, a pulse duration of 0.5
msec and a voltage of 10 V, applied for 10 sec.
From a preliminary experiment, we confirmed
that a frequency of 30 Hz was optimal to get a
strong tracheal response. The left superior
laryngeal nerve was cut beforehand to avoid
any direct effect on the tracheal smooth
muscle during the vagal stimulation.

To administer drugs directly to the upper
tracheal site where the vagal reflex-induced
tracheal responses were measured, we used
the technique for perfusion in situ of the upper
trachea, as described previously (5). The
bilateral cranial thyroid arteries were can-
nulated and perfused with arterial blood
delivered from the right femoral artery, using
a peristaltic pump (Tokyo Rikakikai, MP-
1011) at a constant flow. The perfusion rate
was adjusted at the beginning of each experi-
ment so that the perfusion pressure was ap-
proximately equal to the systemic arterial
blood pressure. Just before the start of the
perfusion, the animal was given heparin
sodium (700 units/kg, i.v., and maintained by
300 units/kg/hr, i.v.).

Drugs used were atropine sulfate (Tokyo
Kasei), propranolol hydrochloride (Inderal,
Sumitomo Chemical Industry), hexametho-
nium bromide (Methobromin, Yamanouchi
Pharmaceutical Co.) and 5-hydroxytrypt-
tamine (5-HT, Sigma). All doses were ex-
pressed in terms of the base. All drugs were
dissolved or diluted in saline solution and in-
jected close-intraarterially into the rubber
tubing just before the Y-shape cannula.

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

The intratracheal pressure was transiently
increased by about 70 mmH₂O by the afferent
electrical stimulation of the vagus nerve (Fig.
1). Simultaneously, the intrabronchial pres-
sure was slightly increased, and systemic
blood pressure and heart rate were reduced by
the afferent vagal stimulation (data not
shown). These responses recovered im-
mediately after cessation of the stimulation.

An infusion of 5 μg/min of atropine into
the cranial thyroid arteries changed the reflex
tracheal constriction by afferent vagal stimu-
lation into a tracheal dilatation (Fig. 1). The
maximum magnitude of the tracheal dilatation
was 3.2±1.6 mmH₂O (n=5). The basal tonus
of bronchial musculature and the bronchial
constriction induced by vagal stimulation
were unaffected by atropine infusion. This
reflex tracheal dilatation was almost inhibited
by injection of 100 μg propranolol into the

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Fig. 1. Effects of atropine, propranolol and hexamethonium (C₆) on the responses of intratracheal
pressure to the afferent vagal stimulation. The afferent vagal electrical stimulations (U) were carried
out by a square-wave pulse (30 Hz, 0.5 msec, 10 V, 10 sec). Atropine was infused close i.a. in a dose of
5 μg/min into the cranial thyroid arteries. Propranolol and C₆ were injected close i.a. at the arrows.
cranial thyroid arteries, and it was completely abolished by the additional injection of 100 µg hexamethonium.

On the other hand, by an infusion of 10 µg/min of 5-HT into the cranial thyroid arteries, the basal intratracheal pressure increased and became stable at about 250 mmH₂O. In this hypertonic tracheal musculature, afferent vagal stimulation caused a tracheal dilatation following an initial constriction (Fig. 2). An infusion of 5 µg/min of atropine abolished only the constriction, but rather enhanced the dilative responses from 34.6±10.6 mmH₂O (n=5) to 44.3±14.6 mmH₂O (n=5), although it was not significantly different. This reflex tracheal dilatation appeared as a much larger response than that in the 5-HT-untreated, normotonic condition. The dilative responses of 44.3±14.6 mmH₂O (n=5) were inhibited to 20.7±5.4 mmH₂O (n=5) by 100 µg propranolol, but not significantly. Residual dilatation was completely abolished by 100 µg hexamethonium.

Systemic arterial blood pressure and heart rate were unaffected by the administrations of 5-HT, atropine, propranolol and hexamethonium.

We reported that the histamine inhalation-induced reflex airway constriction was due to complex effects which may be mediated by plural sensory receptors in the airways (6). By electrical stimulation of the central cut end of the cervical vagus nerve, a distinct reflex tracheal constriction was induced in the present study. This reflex tracheal constriction was changed into a tracheal dilatation by atropine, indicating that the constrictive response may be mediated fully by a cholinergic pathway. In the canine trachea, a constrictive role of α-adrenoceptors was suggested (7, 8). However, α-adrenoceptors seem not to be involved in the vagal reflex tracheal constriction. The reflex dilatation actualized after treatment with atropine was almost inhibited by 100 µg propranolol. This dosage of propranolol was demonstrated to completely block β-adrenoceptors in the tracheal site (7). Therefore, the dilatation induced by afferent vagal stimulation may be mostly mediated by β-adrenoceptors in the trachea.

In the presence of tracheal hypertonus made with an infusion of 5-HT, half of the reflex tracheal dilatation was inhibited by β-blockade. In a preliminary study, we observed the involvement of the sympathetic nervous system (i.e., activation of β-adrenoceptors) in the reflex tracheal dilatation induced by transient lung inflation. Taking this into consideration, too, it is suggested that sympathetic nerves may mediate the vagal reflex-induced tracheal dilatation as one of the efferent pathways.

The residual component of the tracheal dilatation induced by afferent vagal stimulation under β-blockade was completely abolished by hexamethonium. Chesrown et al. (9) and Diamond and O'Donnell (10) suggested that a nonadrenergic inhibitory pathway runs in vagus nerves. They furthermore demonstrated that the bronchodilatation mediated by

![Fig. 2](image-url) Fig. 2. Effects of atropine, propranolol and hexamethonium (C₆) on the responses of intratracheal pressure to the afferent vagal stimulation, in the presence of tracheal hypertonia. The hypertonia of trachea was induced by 5-hydroxytryptamine (5-HT) infusion in a dose of 10 µg/min into the cranial thyroid arteries. Other explanations are as in Fig. 1.
the nonadrenergic pathway is abolished by hexamethonium. In a preliminary study, we confirmed that nonadrenergic inhibitory innervation may, in part, mediate the reflex tracheal dilatation induced by lung inflation in dogs. Therefore, nonadrenergic inhibitory innervation may be responsible for the present response which was abolished by hexamethonium. Aizawa et al. (11) suggested that the nonadrenergic inhibitory nervous system does not play a role until the airway smooth muscle tone becomes higher. The present finding that much more prominent propranolol-resistant tracheal dilatation was observed in the hypertonic tracheal smooth muscle would support this concept.

In the present study, tracheal hypertonus was induced by 5-HT. Altiere et al. (12) reported that 5-HT has no effect on nonadrenergic inhibitory system function in the cat trachea. Therefore, it is indicated that the tracheal dilatation mediated by the nonadrenergic inhibitory system is not a specific response for the 5-HT-induced hypertonus.

In conclusion, afferent vagal stimulation causes reflex tracheal dilatation, as well as constriction, which may be mediated by sympathetic and partly by nonadrenergic innervations. These innervations seem to serve as an efferent pathway in the vagal reflex to act as a protective mechanism(s) against over-constriction of the respiratory tract.

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