Involvement of α_2 -Adrenergic Receptors in the Vagal Reflex-Induced Tracheal Constriction

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The effects of clonidine on the vagal reflex-induced tracheal constriction have been investigated in anesthetized, paralyzed, and artificially ventilated mongrel dogs. The cervical trachea was transected *in situ* into two parts. Responses of the tracheal musculature were measured as changes in the intra-tracheal pressure on an air-filled balloon introduced into the rostral side of the transected trachea. Reflex tracheal constriction was induced by afferent electrical stimulation at the central cut end of the vagus nerve. Drugs were injected or infused close intraarterially (i.a.) into the bilateral cranial thyroid arteries in such a way that each drug was applied just to the rostral trachea. The reflex tracheal constriction was abolished by a close i.a. infusion of 3 μM atropine. The magnitude of the reflex tracheal constriction was slightly reduced by a close i.a. infusion of 10 μM clonidine and was significantly reduced by the infusion of clonidine at a concentration of 100 and 300 μM . The response to 100 μM clonidine was antagonized by a close i.a. infusion of 1 μM yohimbine. The tracheal constriction induced by i.a. injection of 5.5 nmol acetylcholine was unaffected by infusion of 10, 100 and 300 μM clonidine. The vagal reflex-induced tracheal constriction seems to be inhibited by stimulation of prejunctional α_2 -adrenoceptors.

Keywords — reflex tracheal constriction; clonidine; cholinergic neurotransmission; prejunctional α_2 -adrenoceptor

Introduction

Present evidence indicates that the vagal reflex-induced constriction of the airway plays an important role in asthmatic attacks.^{1–3)} It has been reported that endogenously released norepinephrine can partially inhibit bronchoconstrictions induced by stimulation of the cholinergic nerves.⁴⁾ Danser *et al.*⁵⁾ have suggested that norepinephrine, released from sympathetic nerve endings, can activate prejunctional inhibitory β_1 -adrenoceptors to depress cholinergic neurotransmission in isolated canine bronchial segments. It has also been reported that, *in vitro*, relaxation of guinea-pig airways can be induced by stimulation of α_2 -adrenoceptors located prejunctionally on excitatory neurones.^{6,7)}

Recently, Andersson *et al.*⁸⁾ demonstrated an inhibition of the anaphylactic bronchoconstriction in anesthetized and spontaneously breathing guinea pigs pretreated with inhaled or intravenously administered clonidine. In asthmatics, inhaled clonidine caused an inhibition of

the bronchoconstriction induced by allergen bronchoprovocation.⁹⁾ The inhibitory effect of clonidine might be mediated by an inhibitory effect on the release of allergen-induced chemical mediators and/or by an inhibition of the reflex constriction of the airway. The inhibitory effect of clonidine on the release of histamine has been reported in preparations of human basophil and mast cells.¹⁰⁾ The aim of the present study was to determine whether or not clonidine could regulate the vagal reflex-induced constriction of the airway in dogs.

Materials and Methods

Animals — Forty-six male mongrel dogs weighing between 9 and 15 kg were used.

Measurement of Reflex Tracheal Constriction — Experiments were carried out with the preparation used for the evaluation of the vagal reflex-induced responses of the airway, as described previously.^{11,12)} Light anesthesia was induced by the intramuscular in-

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jection of ketamine hydrochloride (20 mg/kg). The cervical trachea was transected at a site about 7 cm caudal to the larynx with the membranous wall left 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 across the wall. A tracheal cannula was inserted into the caudal side of the transected trachea. The left femoral artery was cannulated, and the surgically operated portion was treated with 1% procaine hydrochloride. The systemic arterial blood pressure and heart rate were monitored from the cannulated left femoral artery *via* a pressure transducer (Nihon Kohden, MPU-0.5) and tachometer (Nihon Kohden, AT-600G), respectively. 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) 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 an optimal ventilation level under resting conditions.

An air-filled balloon was introduced into the rostral side of the transected trachea to measure the intratracheal pressure and was 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 mm H₂O.¹³⁾ Reflex tracheal constriction was measured as the change in the intratracheal pressure of the air-filled balloon. Recordings were made on a polygraph (Nihon Kohden, RM-6000). One hour was allowed for stabilization of the preparation after completion of the operation.

Induction of Reflex Tracheal Constriction

— Reflex tracheal constriction was induced by afferent electrical stimulation applied to the central cut end of the vagus nerve, as described previously.¹⁴⁾ The right vagus nerve in the neck was dissected free from 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 the tip of the electrode were immersed in a pool of paraffin oil at 37°C made in a skin pouch. The parameters of the afferent electrical stimuli were as follows: a square-wave pulse with frequency of 20 Hz, a pulse duration of 1 ms, a voltage of 2 V and a duration of application of 60 s. The afferent vagal stimuli were given at intervals of 10, 15, 20, 25 and 30 min after the start of the close i.a. infusion of a drug into the tracheal blood vessel. The right superior laryngeal nerve was cut to avoid any direct effect on the tracheal smooth muscle during the afferent vagal electrical stimulation.

Technique for Perfusion of the Bilateral Cranial Thyroid Arteries — To administer the drugs directly at the site where the vagal reflex-induced tracheal constriction was measured, we used the preparation for perfusion *in situ* of the upper trachea, as described by Yanaura *et al.*¹¹⁾ After an incision was made in the cervical midline, the muscular, pharyngeal and cricothyroid branches of the bilateral cranial thyroid arteries were all ligated. The bilateral cranial thyroid arteries were cannulated and perfused with arterial blood delivered from the right femoral artery, using a micro-tube pump (Tokyo Rikakikai, MP-1011). Just before the start of the perfusion, the animal was given heparin sodium, 500 units/kg i.v., and 100 units/kg of heparin sodium were additionally given i.v. at hourly intervals.

Identification of the Blood-Perfused Area

— The perfused area in the upper trachea of the cannulated bilateral cranial thyroid arteries was confirmed at the end of each experiment by injection of a 0.5% solution of pontamine sky blue in a volume of 5 ml into the arteries.

Chemicals — Drugs used were atropine sulfate (Sigma), clonidine hydrochloride (Sigma), yohimbine hydrochloride (Sigma) and acetylcholine hydrochloride (Ovisot, Daiichi Seiyaku). All drugs were dissolved and diluted in saline. Solutions of drugs, except acetylcholine, were close-infused with a pump for 30 min at a rate of 2.5 ml/min into the rubber tubing just proximal to the perfused arteries. Infusion of yohimbine was commenced 10 min before infusion of clonidine. Acetylcholine was injected

Fig. 1. Effects of Clonidine on the Reflex Tracheal Constriction Induced by the Afferent Vagal Electrical Stimulation

Clonidine was infused close i.a. at concentrations of 10 μM (▨), 100 μM (▧) and 300 μM (■) for 30 min into the bilateral cranial thyroid arteries. Each column is the mean value with S.E. of results from five experiments. The changes are significant at a) $p < 0.05$ and b) $p < 0.01$ when compared to values for saline controls (□).

in aliquots of 50 μl into the rubber tubing. All doses are expressed as final concentrations.

Statistical Analysis — The results shown in the figures are expressed as mean values $\pm$ S.E. Statistical analyses were performed using the unpaired Student's t -test.

Results

The application for 60 s of the afferent vagal electrical stimulation induced an increase in intratracheal pressure, *viz.*, reflex tracheal constriction, which was accompanied by a slight increase in systemic blood pressure and a slight decrease in heart rate. The reflex tracheal constriction occurred after a brief lag period following the application of afferent vagal electrical stimulation in most of the animals used. A close i.a. infusion of saline affected neither the reflex tracheal constriction nor the change in systemic blood pressure and heart rate induced by afferent vagal electrical stimulation ($n=5$). A close i.a. infusion of 3 μM atropine slightly reduced the basal intratracheal pressure. The reflex tracheal constriction was abolished by infusion of 3 μM atropine (data not shown). This experiment was carried out in a total of five animals.

Effects of clonidine on the reflex tracheal

constriction were examined using five animals for each dose. A close i.a. infusion of 10 μM clonidine slightly inhibited the reflex tracheal constriction (Fig. 1). As shown in Fig. 1, an infusion of 100 and 300 μM clonidine significantly inhibited the reflex tracheal constriction in a dose-

Fig. 2. Effects of Clonidine Alone and of Clonidine in the Presence of Yohimbine on the Reflex Tracheal Constriction

Clonidine and yohimbine were infused i.a. into the bilateral cranial thyroid arteries. Each point is the mean value with S.E. of results from four to five experiments. The changes are significant at a) $p < 0.05$ and b) $p < 0.01$ when compared to values for saline controls. ▲, saline; ○, clonidine 100 μM ; ●, clonidine 100 μM + yohimbine 1 μM .

dependent manner. The inhibitory effects of 100 and 300 μM clonidine were observed for 30 min during the infusion, and the inhibitory effects almost disappeared about 15 min after cessation of the infusion. Clonidine alone, at the concentrations of 10 and 100 μM , had no effect on the basal intratracheal pressure, systemic blood pressure and heart rate, but 300 μM clonidine slightly reduced the systemic blood pressure and heart rate.

A close i.a. infusion of 1 μM yohimbine reversed the inhibitory effect of 100 μM clonidine on the reflex tracheal constriction ($n=4$) (Fig. 2). Yohimbine alone, at the concentration used in this study, showed a tendency to enhancement of the reflex tracheal constriction. This experiment was repeated in four animals in all.

A close i.a. injection of 5.5 nmol acetylcholine into the bilateral cranial thyroid arteries caused a marked increase in intratracheal pressure, *viz.*, tracheal constriction. The tracheal constriction induced by acetylcholine was unaffected by a close i.a. infusion of 10, 100 and 300 μM clonidine, but was abolished by infusion of 3 μM atropine (data not shown). These experiments were repeated in three to five animals in all.

Discussion

Canine tracheal smooth muscles are innervated by cholinergic excitatory and adrenergic inhibitory system,^{15,16)} but not by non-adrenergic and non-cholinergic systems.¹⁷⁻¹⁹⁾ It has been reported that electrical stimulation of thoracic sympathetic nerves after vagotomy has no effect on the dimensions of the airway in dogs. However, when vagal bronchoconstriction was present, supramaximal sympathetic stimulation inhibited the constriction of airways from the bronchi to the bronchioles.¹⁷⁾ In the present study, the afferent vagal electrical stimulation produced a constriction phase only in the reflex tracheal response. Atropine completely abolished the reflex tracheal constriction. A relaxant response was not observed during the afferent vagal electrical stimulation when atropine was administered. The above findings suggest that the conditions of electrical stimulation used in

this study may be unaffected upon induction of a reflex adrenergic inhibitory response.

There is evidence that both α_1 - and α_2 -adrenoceptors may be present in the airways. α_1 -Receptors are the classic α -receptors which are postsynaptic, whereas α_2 -receptors are presynaptic and inhibit the release of norepinephrine from synaptic nerve terminals. In human airways, it appears that no postjunctional, bronchoconstrictive α_2 -adrenoceptors are present on the bronchial smooth muscle.²⁰⁾ However, it has been demonstrated, using the canine trachea treated with a β -adrenergic blockade, that tracheal constriction induced by sympathomimetic amines is mediated by α_1 - and α_2 -adrenoceptors on tracheal smooth muscle.²¹⁾ Langer *et al.*²²⁾ have suggested that postsynaptic α_2 -receptors may be localized extrasynaptically and may be preferentially regulated by circulating catecholamines. In the present study, clonidine at the dose used had no effect on basal tracheal tone. Further study seems necessary to examine the pathophysiological significance of the coexistence of postsynaptic α_1 - and α_2 -adrenoceptors in the smooth muscle of the airway.

Grundström *et al.*⁶⁾ showed, in the isolated guinea-pig trachea, that noradrenaline inhibits cholinergic neurotransmission by acting on prejunctional α_2 -adrenoceptors. In the guinea-pig bronchi, prejunctional α_2 -adrenoceptors mediate the inhibitory control of non-adrenergic and non-cholinergic neurotransmission.⁷⁾ Grundström and Andersson²³⁾ demonstrated that exogenous noradrenaline inhibits the electrically evoked cholinergic contractions in ring preparations of human bronchi. They concluded that the cholinergic neurotransmission can be inhibited by stimulation of presynaptic α_2 -adrenoceptors. The reflex tracheal constriction observed in the present study was inhibited by clonidine, but clonidine had no effect on the tracheal constriction induced by exogenous acetylcholine. Moreover, yohimbine, a selective α_2 -antagonist, antagonized the inhibitory effect of clonidine on the reflex tracheal constriction. It is, therefore, considered that the inhibitory effect of clonidine on the reflex tracheal constriction may be mediated by an effect on pre-

junctional α_2 -adrenoceptors.

In the present study, 1 μ M yohimbine was used to inhibit the α_2 -adrenoceptors. Azuma *et al.*²⁴⁾ reported that yohimbine has local anesthetic properties on nerve at dose of 10^{-5} – 10^{-4} M. It has been demonstrated that 3×10^{-7} M yohimbine abolished contraction of norepinephrine (10^{-8} – 10^{-4} M) in dog trachealis strips.²⁵⁾ Therefore, the effect of 1 μ M yohimbine observed in this study seems to inhibit α_2 -adrenoceptors. When yohimbine and clonidine were used, the reflex tracheal constriction was not significantly enhanced. Yohimbine alone, at the dose used in this study, enhanced the reflex tracheal constriction (data not shown), indicating that circulating catecholamines may regulate the tracheal tone *via* α_2 -adrenoceptors. Therefore, it appears that yohimbine inhibits the inhibitory effect of both clonidine and circulating catecholamines on tracheal tone.

The site of action of the inhibitory α_2 -effect in the airways has not been conclusively determined, although a presynaptic site seems likely. Baker *et al.*²⁶⁾ found that norepinephrine blocked the action potentials evoked by stimulation of fiber tracts that enter the ganglia in the ferret trachea. Phentolamine reversed this blockage. These findings are interpreted as indicating the presence of α -adrenoceptors in airway ganglia. It is, therefore, possible that the inhibitory α_2 -modulation of the reflex tracheal constriction takes place at the ganglionic level. Further work is necessary to determine whether the ganglionic site does, in fact, mediate the inhibitory α_2 -effect on the airways, because in the present preparation the reflex tracheal constriction is blocked by ganglion blockers such as hexamethonium.

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