Involvement of Inhibitory Innervation in Reflex Tracheal Dilatation Induced by Lung Inflation

Miwa MISAWA, Yoshinori TAKAHASHI, Tomokazu HOSOKAWA and Saizo YANAURA
Departments of Applied Pharmacology and Pharmacology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan
Accepted January 12, 1990

Abstract—We investigated the involvement of inhibitory innervation in reflex tracheal dilatation (RTD) induced by inflating the lungs in dogs. RTD was inhibited about 50% by 100 μg propranolol injected into the cranial thyroid artery, but was unaffected by adrenalectomy. Residual RTD under β-blockade was abolished by sections of both the bilateral superior laryngeal nerves and spinal cord at the C1 level. These findings suggest that RTD may be mediated by adrenergic innervation and partly by nonadrenergic inhibitory innervation.

It is well-known that several airway reflexes such as cough, bronchoconstriction and bronchodilatation occur by the stimulation of airway sensory receptors (i.e., irritant receptors, pulmonary stretch receptors and C-fiber endings) (1). In a previous paper (2), we have developed a preparation for evaluating the vagal reflex-induced airway responses and demonstrated that the reflex tracheal constriction and dilatation are induced by transient lung deflation and inflation, respectively. Widdicombe and Nadel (3) have reported that an increase in activities of pulmonary stretch receptors caused by lung inflation decreased the efferent vagal excitatory activity to the airway smooth muscle. On the other hand, it has been found that activations of β-adrenoceptors cause the tracheal dilatation in dogs (4). However, it has not yet been investigated whether or not β-adrenoceptor activation is involved in the RTD induced by lung inflation.

Involvement in RTD of nonadrenergic inhibitory innervation, which have been demonstrated to be present in the airways of cats (5), guinea pigs (6, 7) and humans (8), is also interesting. Nonadrenergic inhibitory innervation is known to be recognized in the tracheobronchial tree through the cervical vagus nerves. Recently, Yip et al. (9) suggested that preganglionic nerves of these nerves run not only in vagus nerves, but also in sympathetic preganglionic ones. In the present study, we therefore investigated the involvements of adrenergic innervation and/or nonadrenergic inhibitory innervation in RTD evoked by transient lung inflation in dogs.

Twenty-five male mongrel dogs weighing 8–16 kg were used. The preparations were used to evaluate the vagal reflex-induced airway responses as described previously (2). 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 animals were immobilized with decamethonium 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. The systemic arterial blood pressure was measured through the femoral arterial catheter by a pressure transducer (Nihon Kohden, MPU-0.5). The heart rate was
monitored with the tachometer (Nihon Kohden, RT-5) using the systolic blood pressure as the trigger.

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 intratracheal pressure of the balloon was adjusted to 100 mmH₂O. On the other hand, intraluminal pressure of the caudal side of the transected trachea was measured as the intrabronchial pressure through the tracheal cannula inserted into the caudal side using a pressure transducer (Nihon Kohden, MPU-0.5). When the manipulation of a transient inflation of the lungs was necessary, 100 ml of air was insufflated with a calibrated syringe. We demonstrated previously that the extravagal pathway consisting of the recurrent and superior laryngeal nerves plays a role in part of the afferent pathway of the vagal reflex airway responses (2). Therefore, the bilateral recurrent laryngeal nerves were cut beforehand in all animals to avoid any direct effect on the upper trachea smooth muscle during the lung inflation.

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 (10). Two arms of a Y-shape cannula were connected to the bilateral cranial thyroid arteries, and the arterial blood from the right femoral artery was perfused into the cranial thyroid arteries via a peristaltic pump (Tokyo Rikakikai, MP-1011) at a constant flow. The perfusion rate was adjusted at the beginning of each experiment so that the perfusion pressure was approximately equal to the systemic arterial blood pressure. Just before starting the perfusion, the animal was given heparin sodium (700 units/kg, i.v., and maintained by 300 units/kg/hr, i.v.). Adrenalectomy was performed bilaterally through an abdominal midline incision.

Drugs used were propranolol hydrochloride (Inderal, Sumitomo Chemical Industry), phentolamine mesylate (Regitin, Ciba-Geigy) and hexamethonium bromide (Methobromin, Yamanouchi Pharmaceutical Co.). All doses were expressed in terms of the base. All drugs were diluted in saline solution and injected close-intraarterially into the rubber tubing just before the Y-shape cannula.

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.

Typical recordings of the changes in intrabronchial pressure and intratracheal pres-

![Fig. 1. Effects of propranolol, section of the bilateral superior laryngeal nerves (S.L.N.) and transection of the spinal cord at the C1 level on the responses of intratracheal pressure and intrabronchial pressure to lung inflation. Lung inflations were applied at 5, 10 and 30 min after propranolol injection, section of S.L.N. and transection of the spinal cord, respectively.](image-url)
sure induced by lung inflation (100 ml) are shown in the left portion of Fig. 1. Transient inflation of the lungs in a volume of 100 ml caused a decrease in intratracheal pressure, viz., RTD following a transient increase in intrabronchial pressure. The increase in intrabronchial pressure by lung inflation was about 7.3±2.1 mmHg, and the reflex decrease in intratracheal pressure was 61.2±5.8 mmH2O.

Propranolol in a dose of 100 µg decreased the RTD by about 50% (Fig. 1). Upon treatment with 300 µg of propranolol, the RTD was inhibited to the similar degree as that by 100 µg (data not shown). The inhibitory effect of propranolol lasted for more than 60 min. Basal intratracheal and intrabronchial pressures were unchanged by the administration of propranolol. The remaining RTD after an application of 100 µg propranolol was only partly removed by section of the bilateral superior laryngeal nerves. The residual RTD was abolished following a consecutive transection of the spinal cord at the C1 level (Fig. 1). The RTD was unaffected by 200 µg phentolamine or the bilateral adrenalectomy (Fig. 2). On the other hand, the RTD in the presence of 100 µg propranolol was abolished by 100 µg hexamethonium (Fig. 2). All these experiments were repeated in five animals.

It is well-known that pulmonary stretch receptors are responsible for the Hering-Breuer inflation reflex. Widdicombe and Nadel (3) reported that stimulation of these receptors reflexly relaxed airway smooth muscle by the reduction of the efferent vagal excitatory activity to the musculature. In the present study, the RTD induced by lung inflation was inhibited by 100 µg propranolol, but was not completely blocked. Russel (4) demonstrated the dilatatory role of β-adrenoceptors in the canine trachea. A dose of 100 µg of propranolol was used to inhibit the β-adrenoceptors in his study. Himori and Taira (11) reported that a dose of 60 µg of propranolol injected into the cranial thyroid artery exerted complete β-blocking action in the canine trachea. Furthermore, an increase in the propranolol to 300 µg produced no greater effect compared with that of 100 µg, indicating that the dose of propranolol used is sufficient to completely block the β-adrenoceptors in this preparation. It is, therefore, suggested that a part of the RTD occurs through the activation of β-adrenoceptors in the trachea. Propranolol is known to have membrane stabilizing effects. However, in the present study, the tonus of the tracheal musculature was unaffected by propranolol in the dose used.

Phentolamine and adrenalectomy had no effect on the RTD. Because it has been reported that phentolamine in a dose of 200 µg was sufficient to block α-adrenoceptors in the canine trachea (11), α-adrenoceptors may not be related to the RTD. The circulating catecholamines liberated from the adrenal medulla also may not be involved in the RTD. Therefore, the adrenergic nerves (β-action) by themselves seem to be involved in the RTD, but circulating catecholamines released from the adrenal medulla do not.

Residual RTD under β-blockade was completely abolished by a combination of section of the bilateral superior laryngeal nerves and transection of the spinal cord at the C1 level. These results indicate that the observed RTD may be mediated through both the pre-ganglionic sympathetic and parasympathetic

---

Figure 2. Effects of propranolol (prop.), hexamethonium (C6), phentolamine (phen.) and the adrenalectomy on the reflex tracheal dilatation induced by lung inflation. Each column is the mean value with S.E. on the basis of the control value. The symbol (***) denotes the change is significant at P < 0.01.
innervations to airways. Yip et al. (9) suggested that preganglionic nonadrenergic inhibitory innervation to the tracheal musculature runs in the vagus nerves and the sympathetic trunks in guinea pigs. Therefore, the inhibitory innervation in question that runs in the superior laryngeal nerves and the spinal cord may resemble a nonadrenergic pathway. Diamond and O’Donnell (5) reported that nonadrenergic bronchodilatation is abolished by hexamethonium in anesthetized cats. In the present study, the RTD under β-blockade was completely inhibited by hexamethonium furthermore indicating that the nonadrenergic inhibitory innervation may be involved in the RTD. In dogs, nonadrenergic inhibitory innervation to airway smooth muscle is, however, still unclear. Ressel (4) observed a propranolol-resistant inhibition for which a nonadrenergic inhibitory system may be responsible. On the other hand, Fleetham et al. (12) denied the possibility of these innervations in dogs. Further study will be necessary to verify the presence of nonadrenergic inhibitory innervation in the canine trachea.

In conclusion, it is suggested that the RTD induced by a transient inflation of the lungs may be mediated by both the adrenergic innervation and, probably, nonadrenergic inhibitory innervation.

References