INFLUENCE OF TRACHEAL MUSCULAR TONE ON THE INITIATION OF COUGH REFLEX

Saizo YANURA, Tomokazu HOSOKAWA, Harumi KITAGAWA and Yoshikazu YAMATAKE

Department of Pharmacology, Hoshi College of Pharmacy, Ebara, Shinagawa-ku, Tokyo 142, Japan

Accepted January 20, 1978

Abstract—We devised a canine blood-perfusion preparation which made feasible administration of drugs directly at the local tracheal site. The hypothesis of Salem and Aviado on cough mechanism that a local airway constriction induced by stimuli may be a trigger in stimulating cough receptors was investigated using this preparation. Close intraarterial injections of acetylcholine (ACh) and histamine did not elicit a cough although intense tracheal constrictions were evident. The cough reflex elicited by electrical stimulation of the mucosa of isolated upper trachea in situ was accompanied by a slight systemic hypotension, tracheal vasodilatation and tracheal muscular constriction. The latter two changes occurred after a time lag following coughs. Close intraarterial infusions of isoproterenol and papaverine caused a prominent tracheal dilatation, but did not suppress the coughs. Pretreatment with atropine sufficiently inhibited cholinergic tracheoconstriction but had no effect on the electrically induced coughs. Furthermore, an augmentation of the tracheal muscular tone produced by an infusion of ACh did not enhance the cough reflex. In light of our observations, the aforementioned hypothesis should be reconsidered.

The cough reflex is induced by various stimuli to cough receptors in the airways. The existence of two types of cough receptors, mechanical and chemical, has been reported (1). It has hitherto been considered that direct excitation of these receptors by external stimuli leads to activation of the cough center in the medulla and results in production of cough.

However, Salem and Aviado (2) proposed the hypothesis that local broncho constriction induced by stimuli may be a trigger in stimulating cough receptors. Their hypothesis is based on the finding (3-6) that bronchodilators such as ephedrine and isoproterenol can alleviate cough. This hypothesis, however, remains to be substantiated.

We devised a preparation which consists of a direct drug administration at the local site in the airway and of stimulation of the site to induce coughing. The aforementioned hypothesis was investigated using this preparation. Changes in systemic blood pressure and in tracheal muscular and vascular tones during the cough reflex were simultaneously investigated.

MATERIALS AND METHODS

 Forty-five mongrel dogs of either sex, weighing 8-15 kg, were anesthetized with \( \alpha \)-chloralose 100-150 mg/kg given i.p. To administer the drugs directly at the site where cough-induced stimuli were applied we devised the blood-perfusion preparation described...
below (Fig. 1). The upper cervical region was incised in the midline, and the left and right cranial thyroid arteries, the main arteries supplying the upper portion of the trachea, and their branches were exposed and carefully dissected free. The left cranial thyroid artery and muscular, pharyngeal and cricothyroid branches were all ligated with the right cranial thyroid artery left intact. Arterial blood from the right femoral artery was conducted to the right cranial thyroid artery via a peristaltic pump (Tokyo Rikakikai, C-16) and via a water bath at 37°C, and, thus a constant flow perfusion was carried out. The flow was adjusted at the beginning of each experiment so that the perfusion pressure was approximately equal to the systemic arterial blood pressure. The perfusion pressure (PP) was measured between the pump and the perfused artery using a pressure transducer (Nihon Kohden, MPU-0.5), and was used as an index of the change in vascular resistance. Just before start of the perfusion, the animal was given heparin sodium, 500 units/kg i.v., and 100 units/kg were additionally given i.v. at hourly intervals. The systemic arterial blood pressure was monitored from the catheterized left femoral artery.

The cervical trachea was exposed, and transected at about 7 cm caudal to the larynx leaving the membranous wall intact (Fig. 2). Care was taken not to disturb the recurrent

---

**Fig. 1.** Diagram of the preparation for perfusing the isolated canine trachea in situ with the femoral arterial blood in order to investigate the cough reflex.

**Fig. 2.** Schematic illustration of separations of the trachea into the perfused area (trachea A) and non-perfused area (trachea B). Trachea A perfused with femoral arterial blood was electrically stimulated to induce coughs, and a balloon was inserted to measure the intratracheal pressure. Trachea B was cannulated to measure respiratory and cough responses.
laryngeal nerves which arise from the vagus nerve and pass cranially along the dorsolateral aspect of the trachea. The membranous wall at the transected site was ligated with a thread to interrupt blood flow passage across the wall.

Respiratory and cough responses were measured using a pneumotachograph (Nihon Kohden, MFP-1T) via a cannula inserted into the caudal site (trachea B in Fig. 2) of the transected trachea. Responses of the tracheal smooth muscle were measured as changes in the intratracheal pressure (IP) of an air-filled balloon (5 cm in length) introduced into the rostral side (trachea A in Fig. 2) of the transected trachea. The air-filled balloon 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. This pressure was found to be suitable for the observation of both dilatation and constriction of the trachea. Recordings were made on a polygraph (Nihon Kohden, RM-150).

The cough reflex was elicited with electrical stimuli to the membranous wall mucosa of trachea A which was perfused with the femoral blood via the pump. An insulated puncture electrode (7) or silver disc electrode was used for the stimulation. The parameters of electrical stimulation used in inducing cough were a square-wave pulse with a 20 Hz frequency, the duration of pulse 1.0 msec, the voltage 8–10 V and the duration of application 10 sec. The sensitivity of α-chloralosed dogs to this electrical stimulation was similar to that of unanesthetized dogs reported in a previous paper (7). The stimuli were given to the animal immediately and at 1, 3, 5, 10 and 20 min after a single close drug injection into the tracheal blood vessel, or at 1, 3 and 5 min after start of and after cessation of a close drug infusion.

Drugs used in this study were acetylcholine chloride (Daichi), 1-isoproterenol hydrochloride (Sigma), papaverine hydrochloride (Wako) and atropine sulfate (Tokyo Kasei). Doses of all drugs used refer to their salts. All drugs were dissolved in saline solution. Drug solutions were closely injected in a volume of 0.05 ml in 20 sec or infused with a pump for 5 min at a rate of 0.17 ml/min into the rubber tubing just proximal to the perfused artery.

RESULTS

Identification of the perfused area

The perfused area in the upper trachea of the cannulated right cranial thyroid artery was confirmed at the end of each experiment by injecting 0.5% pontamine sky blue solution in a volume of 5 ml into the artery. Trachea A was distinctly stained by the dye, whereas trachea B was not. Furthermore, in three animals, another experiment for confirmation of the perfused area was made. Acetylcholine (ACh) was injected into the cannulated artery after insertions of air-filled balloons into both trachea A and B. ACh in a dose range of 0.3 to 3.0 μg produced a dose-dependent increase in intratracheal pressure (IP) in trachea A, with no change occurring in IP in trachea B. Such observations clearly demonstrate that trachea A which was stimulated electrically to induce cough could, in a restricted manner, be the recipient of drugs applied by close i.a. administration.
Muscular and vascular responses of trachea to Ach, histamine and isoproterenol

Close i.a. injections of Ach and histamine produced a decrease in perfusion pressure (PP), viz., vasodilatation in an approximately equal potency (Figs. 3, 4). On the other hand, the potency of Ach to increase IP was about ten times greater than that of histamine. No cough was elicited during the tracheal constrictions induced by Ach and histamine (Fig. 3).

Intraarterial injections of isoproterenol (0.01–1.0 μg) caused decreases in PP (vasodilatation) and IP (tracheal dilatation) (Figs. 3, 4).

The durations of response on PP and IP to Ach, histamine and isoproterenol were relatively short, being about one to four minutes.

FIG. 3. Effects of intraarterial acetylcholine, histamine and isoproterenol on the perfusion pressure (P.P.), intratracheal pressure (I.P.) and respiration (Resp.). Drugs were injected close i.a. into the cranial thyroid artery at each arrow. The systemic blood pressure (B.P.) was unaltered by i.a. administration of the drugs.

FIG. 4. Dose-response curves of the perfusion pressure and the intratracheal pressure to i.a. acetylcholine, histamine and isoproterenol. Each point represents the mean with S.E. for five experiments.
Changes in tracheal muscular and vascular tones and in systemic blood pressure during the cough reflex

An application of 10 sec of electrical stimuli on the tracheal mucosa of trachea A induced 4-8 coughs, which were accompanied by a slight decrease in systemic blood pressure, a decrease in PP and an increase in IP. The systemic hypotension was associated with pressure fluctuations corresponding to each cough and with bradycardia. The changes in PP and IP occurred after a time lag following the cough reflex in most of the 34 animals tested (Fig. 5). Similar response patterns were observed by mechanical stimulation of the bifurcatio tracheae with brushings, and by electrical stimulation (20 Hz, 1.0 msec, 2 V, 10 sec) of the central end of the superior laryngeal nerve through which the stimulation of cough receptors in the mucosa of the upper trachea is transmitted to the cough center in the medulla.

Effects of i.a. drug administrations on the cough reflex

Effects of isoproterenol, papaverine, atropine and ACh on the initiation of cough reflex were examined using 5-6 animals for each agent.

A close i.a. injection or infusion of saline affected neither the cough response nor changes in PP, IP and systemic blood pressure elicited by electrical stimulation of the mucosa of trachea A.

The systemic hypotension observed following the cough reflex was hardly influenced by any drug given into the cranial thyroid artery.

Typical recordings of effects of a close i.a. infusion of isoproterenol (12 μg/min) for 5 min are shown in Fig. 6. This dose of isoproterenol caused marked decreases in PP and IP during infusion, and inhibited the tracheal constriction elicited by the mucosal stimulation. The cough response was, however, not altered by isoproterenol.

When papaverine (120 μg/min) was infused i.a., the cough response elicited by the
mucosal stimulation was not inhibited, although the tracheal constriction observed following the coughs was considerably reduced.

A close i.a. injection of atropine (30 µg) hardly altered PP and IP. The vasodilatation and cough response elicited by the mucosal stimulation were also unaltered, while the tracheal constriction induced by electrical stimulation was markedly inhibited for at least 10 min (Fig. 7).

**Fig. 6.** Effects of isoproterenol on the responses of cough, systemic blood pressure (B.P.), perfusion pressure (P.P.) and intratracheal pressure (I.P.) elicited by electrical stimulation (ES) of the tracheal mucosa. Isoproterenol was infused close i.a. in a dose of 12 µg/min for 5 min. Dots over the recordings of respiration (Resp.) indicate each cough response.

**Fig. 7.** Effects of atropine on the responses of cough, systemic blood pressure (B.P.), perfusion pressure (P.P.) and intratracheal pressure (I.P.) elicited by electrical stimulation (ES) of the tracheal mucosa. Atropine was injected close i.a. in a dose of 30 µg at the arrow. Dots over the recordings of respiration (Resp.) indicate each cough response.
FIG. 8. Effects of acetylcholine on the responses of cough, systemic blood pressure (B.P.), perfusion pressure (P.P.) and intratracheal pressure (I.P.) elicited by electrical stimulation (ES) of the tracheal mucosa. Acetylcholine was infused close i.a. in a dose of 4 μg/min for 5 min. Dots over the recordings of respiration (Resp.) indicate each cough response.

Effects of a close i.a. infusion of ACh, a tracheal constrictor, in a dose of 4 μg/min are illustrated in Fig. 8. The dose of ACh produced a sustained tracheal constriction and a vasodilatation, but did not enhance the cough response elicited by the mucosal stimulation (Fig. 8).

DISCUSSION

It was recently reported that blood perfusion of the dog trachea enabled measurement of responses of tracheal musculature and vasculature in situ (8). Our newly devised preparation involves a more restricted perfused area (trachea A in Fig. 2) in the upper trachea and is suitable for analysis of direct drug action on the local site around the tracheal mucosa, this site being electrically stimulated to induce coughing. Since trachea A is isolated from trachea B through which respiratory and cough responses are measured, vascular and muscular responses of trachea A can be investigated without being disturbed by the airflow from the lungs during cough reflex and respiration.

In the present experiment, ACh produced a greater tracheal constriction than did histamine. ACh and histamine are reportedly equipotent in increasing pulmonary resistance in dogs (9). It has been reported, however, that vagotomy or atropine pretreatment reduces or nearly abolishes the increase in pulmonary resistance evoked by histamine (10–12), and it was suggested that the airway constriction induced by histamine may be explained by a cholinergic bronchoconstrictor reflex in addition to the direct effect of histamine on smooth muscle. Mills and Widdicombe (12) suggested that the vagal reflex arc was activated by stimulation of so-called ‘lung irritant receptors’ in the bronchial epithelium. On the other hand, in the isolated dog trachea, bronchus or bronchiole, ACh always evokes a much greater
constriction than histamine (13). The present finding that ACh had a more potent tracheal muscular effect than histamine may be explained by differences in the direct musculotropic activity of these drugs.

Systemic hypotension was observed during coughs evoked by mechanical stimulation of the tracheal mucosa or by electrical stimulation of the medulla (14). We confirmed in our experiments that coughing produces a systemic hypotension, since various stimuli for cough induction produced a similar hypotension.

Mechanical stimulation of the tracheal mucosa with brushings evokes a bronchoconstriction via a vagal reflex (15). A tracheal constriction was also observed during coughs induced by electrical stimulation of trachea A mucosa and of the superior laryngeal nerve and by mechanical stimulation of trachea B mucosa. The tracheal response arose after a time lag following coughs and not during coughing demonstrating that at least the upper tracheal constriction did not correspond to each inspiration and expiration that was comprised in the coughs. Since an i.a. injection of atropine almost blocked the tracheal constriction following coughing, the constriction may be attributed to a vagal activity.

The decrease in PP, viz., vasodilatation evoked by the mucosal stimulation was not inhibited by atropine. The mechanism of the vasodilatation is now being studied.

Salem and Aviado presented a hypothesis that bronchoconstriction may be a trigger in initiating the cough reflex (2). Their hypothesis was born out of the finding (3–6) that some bronchodilators such as ephedrine and isoproterenol possess an alleviating effect on the cough, and, in addition, out of the finding by Tiffeneau (16) that inhalation of ACh, a bronchoconstrictor, evokes coughing in humans. This author stated that inhalation of ACh might provoke excitation of the cough reflex via the pulmonary sensory nerve endings in the lungs. A close i.a. injection or infusion of ACh as well as histamine into the cranial thyroid artery never produced coughing in the present experiments, despite a strong tracheal constriction. Furthermore, large doses of isoproterenol and papaverine applied locally at the site of trachea A relaxed considerably the tracheal smooth muscle and suppressed the tracheal constriction following coughs, whereas neither drug had any effect on the cough response. The mild antitussive effect by systemic administrations of bronchodilators may be accounted for by an alleviation of excessive airflow, which occurs during coughing.

In light of our observations herein, the hypothesis on cough mechanism proposed by Salem and Aviado should be given further consideration.

REFERENCES

1) Widdicombe, J.G.: Receptors in the trachea and bronchi of the cat. J. Physiol. 123, 71-104 (1954)
2) Salem, H. and Aviado, D.M.: Antitussive drugs. With special reference to a new theory for the initiation of the cough reflex and the influence of bronchodilators. Am. J. med. Sci. 247, 585-600 (1964)
3) Kasi, Y.: Pharmacological studies on cough reflex. Part 2, Cough depressing actions of various drugs. Japan. J. Pharmacol. 4, 118-129 (1955)
4) Hara, S. and Yanaura, S.: A method of inducing and recording cough and examination of the action of some drugs with this method. Japan. J. Pharmacol. 9, 46-54 (1959)
5) Sokoloff, M.J.: Mechanisms and management of cough. Med. clin. N. Amer.ica 45, 1437–1442 (1961)
6) Kalish, J.: Modern cough mixtures. Drug Cosmet. Indust. 92, 696–812 (1963)
7) Yanaura, S., Iwase, H., Sato, S. and Nishimura, T.: A new method for induction of the cough reflex. Japan. J. Pharmacol. 24, 453–460 (1974)
8) Himmori, N. and Taira, N.: A method for recording smooth muscle and vascular responses of the blood-perfused dog trachea in situ. Brit. J. Pharmacol. 56, 293–299 (1976)
9) Wasserman, M.A.: Bronchopulmonary responses to prostaglandin F2α, histamine and acetylcholine in the dog. Europ. J. Pharmacol. 32, 146–155 (1975)
10) Dekock, M.A., Nadel, J.A., Zwi, S., Colebatch, H.J.H. and Olsen, C.R.: New method for perfusing bronchial arteries: histamine bronchoconstriction and apnea. J. appl. Physiol. 21, 185–194 (1966)
11) Simonsson, B.G., Jacobs, F.M. and Nadel, J.A.: Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease. J. clin. Invest. 46, 1812–1818 (1967)
12) Mills, J.E. and Widdicombe, J.G.: Role of the vagus nerves in anaphylaxis and histamine-induced bronchoconstrictions in guinea-pigs. Brit. J. Pharmacol. 39, 724–731 (1970)
13) Yamatake, Y., Sasagawa, S. and Yanaura, S.: Drug responses of canine trachea, bronchus and bronchiole. Chem. Pharm. Bull. (in press)
14) Chou, D.T. and Wang, S.C.: Studies on the localization of central cough mechanism; site of action of antitussive drugs. J. Pharmacol. exp. Ther. 194, 499–505 (1975)
15) Widdicombe, J.G.: Regulation of tracheobronchial smooth muscle. Physiol. Rev. 43, 1–37 (1963)
16) Tiffenau, R.: The acetylcholine cough test. Dis. Chest. 31, 404–422 (1957)