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Characteristics of vagal reflex-mediated tracheal response induced by bronchoconstriction in guinea pigs

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Abstract

The reflex tracheal response induced by bronchoconstriction was investigated using a newly devised tracheo-bronchi preparation in anesthetized guinea pigs. Tracheal constriction and subsequent dilatation were observed in response to bronchoconstriction induced by the inhalation of 0.001–0.01% histamine and 0.003–0.03% acetylcholine. These tracheal responses were abolished by cervical vagotomy or treatment of the tracheal site with 1% tetrodotoxin. Tracheal constriction and dilatation were significantly inhibited by 0.1% atropine and 1% propranolol, respectively. When high tracheal tone was induced by 0.01% serotonin, the residual tracheal dilatation observed in the presence of propranolol was enhanced, while dilatation was completely inhibited by 1% hexamethonium. Dilatation was also suppressed by 1% N^{ω}-nitro-L-arginine methyl ester (L-NAME) and 1% methylene blue. The tracheal constriction produced by bronchoconstriction was significantly enhanced by propranolol 2 mg/kg, i.v. and L-NAME 10 mg/kg, i.v. These results demonstrate that a vagally mediated reflex tracheal response (constriction followed by dilatation) is induced by bronchoconstriction in anesthetized guinea pigs. Cholinergic nerves may mediate the constriction, and adrenergic and nonadrenergic noncholinergic (NANC) inhibitory nerves may mediate the dilatation. Furthermore, NO may be involved in the NANC reflex tracheal dilatation.

Keywords: Vagal reflex; Tracheal innervation; Bronchoconstriction; (Guinea-pig)

1. Introduction

The important role of the vagal reflex in the airway is well known. Our previous study demonstrated that a vagus nerve-mediated reflex tracheal constriction and slight dilatation appeared after bronchoconstriction induced by inhalation of histamine or *Ascaris suum* antigen in dogs (Misawa et al., 1992). The results of that study also suggested that cholinergic nerves might mediate the reflex tracheal constriction while both adrenergic and nonadrenergic noncholinergic (NANC) nerves might be involved in the dilatation. This tracheal dilatator reflex may be part of a regulatory mechanism during airway constriction.

It has been demonstrated that transmural electrical stimulation of the trachea and stimulation of the vagus nerve induce a biphasic response, i.e., a contraction followed by a relaxation in guinea pigs (Coburn and Tomita, 1973; Yip et al., 1981; Ellis and Farmer, 1989a; Canning and Undem, 1993a, b). However, Watson et al. (1993) showed that while transmural electrical stimulation of tracheal smooth muscle caused a contraction followed by relaxation, stimulation of the vagus nerve caused only tracheal contraction in an isolated innervated guinea pig tracheal preparation. Thus, the role of the vagus nerves in the trachea is still unclear, especially with regard to tracheal dilatation. Thus far, there have been no reports on the vagally mediated reflex tracheal dilatation in guinea pigs in vivo.

In the present study, we devised a new tracheo-bronchi preparation which enables evaluation of the vagal nerve-mediated tracheal response during bronchoconstriction induced by the inhalation of histamine or acetylcholine in guinea pigs, using a modification of the canine model (Hosokawa et al., 1984). Several investigators have used in vivo tracheal pouch preparations which were perfused or filled with a physiological solution to measure the tracheal response (Chesrown et al., 1980; Yip et al., 1981; Venugopalan et al., 1984, Venugopalan et al., 1991; Canning and Undem, 1993a, b). However, the tracheal response

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was induced by electrical stimulation of the vagus nerve. In contrast, the present model was designed to study the reflex-mediated tracheal response during bronchoconstriction in the guinea pig. In respiratory diseases, e.g., an asthmatic attack, because airway constriction is a major factor in producing severe symptoms, it is important to understand the reflex mechanism(s) during bronchoconstriction.

It is well known that not only adrenergic and cholinergic nerves but also NANC nerves innervate the guinea pig trachea (Coburn and Tomita, 1973; Coleman and Levy, 1974; Richardson and Bouchard, 1975; Chesrown et al., 1980). However, few studies have examined the involvement of adrenergic and NANC pathways in the vagal reflex. Barnes (1992) suggested that NANC nerves, especially inhibitory NANC nerves, could attenuate evoked airway constriction as part of a regulatory mechanism. Furthermore, nitric oxide (NO) has been suggested to be a transmitter for NANC nerves (Tucker et al., 1990; Li and Rand, 1991; Belvisi et al., 1993; Lei et al., 1993). Therefore, we also examined the involvement of NO as a transmitter candidate in the vagally mediated reflex tracheal response.

2. Materials and methods

2.1. Experimental procedure

Male Hartley guinea pigs (Japan SLC, Hamamatsu, Japan) weighing 350–820 g were housed under a 12-h light-dark cycle with free access to food and water.

Animals were anesthetized with urethane 500 mg/kg, i.p. and α-chloralose 50 mg/kg, i.p. After cervical incision, with care being taken to preserve the continuity of the vagus nerves and recurrent laryngeal nerves, the trachea was exposed and transected about 4 cm caudal to the larynx with the membranous wall left intact. A Y-shaped respiratory cannula was inserted into the caudal end of the transected trachea. The left femoral artery was cannulated to measure systemic blood pressure with a pressure transducer (Spectramed, P23XL, Statham, USA). Heart rate was monitored by triggering the ECG-II. The left femoral vein was cannulated for drug injection. Animals were immobilized with decamethonium bromide (initial dose of 0.8 mg/kg, i.v. and supplemental dose of 0.4 mg/kg, i.v.). Immediately after immobilization, the respiratory cannula that had been inserted into the trachea connected to the lungs was joined to a respirator (SN-480-7, Shinano, Japan) and artificial ventilation was performed at a frequency of 50 breathes/min with room air.

A schematic diagram of the experimental set up is shown in Fig. 1. To stop blood flow between the upper (tracheal site) and lower (bronchial site) parts of the trachea, the membranous wall was ligated at the transected site by a thread. After another cannula was inserted into the cephalad end of the transected trachea, the tracheal site was filled with warm isotonic saline, a blocker of neural transmission (tetrodotoxin), an acetylcholine receptor antagonist (atropine) and/or adrenoceptor antagonists (phentolamine, pindolol and propranolol), via the cannula, for 30 min to obtain the full effects of these drugs. Thirty minutes after the application of saline or drug to the trachea, the trachea was ligated just above the larynx,

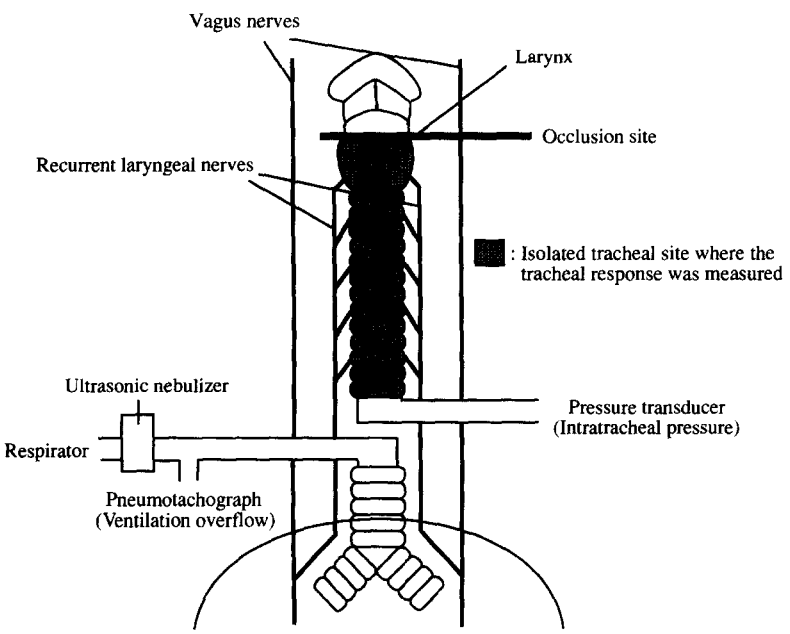

Fig. 1. Diagram of the guinea pig airway preparation.

under the condition that the tracheal site was filled with saline or the drug solution, and the tracheal cannula was connected to a pressure transducer (Spectramed, P23XL, Statham, USA). The initial pressure in the isolated tracheal site containing the pretreatment drug solution was adjusted to 0 cm H_2O immediately after the ligation of the trachea above the larynx.

In the experiment with a high tracheal tone, after the trachea had been treated with a neural ganglion blocker (hexamethonium), an acetylcholine receptor antagonist (atropine), an adrenoceptor antagonist (propranolol), NO modulators (L-NAME and L-arginine) and/or a guanylate cyclase inhibitor (methylene blue) for 30 min with the larynx intact, the drug solution was changed to the new drug solution in which 0.01% (w/v) (ca. 2×10^{-4} mol/l) serotonin (5-hydroxytryptamine, 5-HT) was added to the previous solution. Immediately after that the trachea was ligated just above the larynx and the initial pressure in the tracheal site was adjusted to 0 cm H_2O.

The response of the tracheal musculature was measured as a change in the intratracheal pressure of the saline- or drug-filled tracheal site. The response of the bronchial musculature was measured by the modified Konzett-Rössler method (Hosokawa et al., 1984). The lungs were ventilated with a fixed volume of air under a constant pressure (8 cm H_2O), and ventilation overflow was measured using a pneumotachograph (MP-45, Validyne, USA) as an index of the change in the bronchomotor response.

Histamine and acetylcholine were applied (inhaled) to the bronchial site using an ultrasonic nebulizer (TUR-3200, Nihon Kohden, Japan) set in the inspiratory pathway (between the respirator and the tracheal cannula). After a stable tracheal tone was obtained, followed by ligation of the trachea above the layrnx, histamine and acetylcholine were inhaled for 5 min in both the normal and high tracheal tone experiments.

A vagotomy was performed by transection of both the bilateral cervical vagus nerves and the recurrent laryngeal nerves, which are branches of the vagus nerves at the sites close to the bronchi.

In the study with systemic administration of propranolol and L-NAME, these drugs were injected intravenously 15 min before histamine inhalation.

Ventilation overflow and intratracheal pressure were estimated in terms of the respective maximal responses.

2.2. Drugs

Histamine dihydrochloride (Wako Pure Chemicals, Japan), acetylcholine chloride (Ovisot, Daiichi Seiyaku, Japan), 5-hydroxytryptamine (serotonin, 5-HT) (serotonin-creatinine sulphate, Wako Pure Chemicals, Japan), atropine sulphate (Wako Pure Chemicals, Japan), DL-propranolol hydrochloride (Sigma, USA), pindolol (Sigma, USA), phentolamine hydrochloride (Sigma, USA), tetrodotoxin (Sigma, USA), hexamethonium chloride

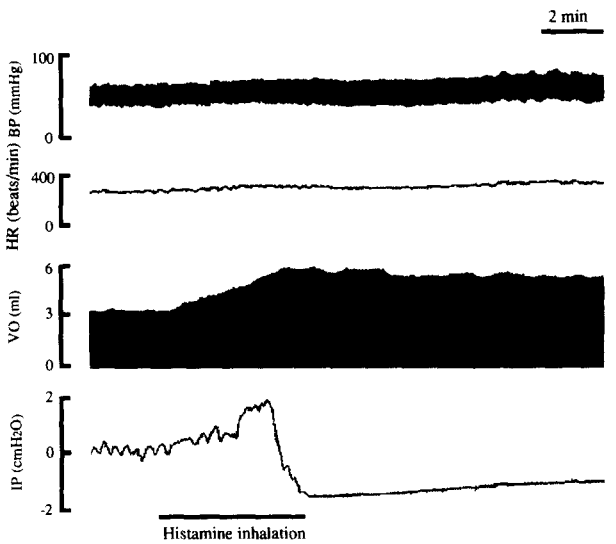

Fig. 2. Typical recordings of bronchial and tracheal responses to the application (inhalation) of 0.01% histamine to the bronchial site for 5 min in anesthetized guinea pigs. Blood pressure (BP), heart rate (HR), ventilation overflow (VO) and intratracheal pressure (IP).

(Sigma, USA), N^{ω}-nitro-L-arginine methyl ester hydrochloride (Sigma, USA), L-arginine hydrochloride (Sigma, USA), methylene blue trihydrate (Sigma, USA), decamethonium bromide (Wako Pure Chemicals, Japan), urethane (Aldrich, USA) and α-chloralose (Wako Pure Chemicals, Japan) were used. DL-Propranolol was dissolved in 0.01 N HCl and diluted with saline. Other drugs were dissolved in saline.

2.3. Statistical analysis

All values are expressed as the means ± S.E.M. Statistical analyses were performed with one-way analysis of variance (ANOVA) or the Kruskal-Wallis test followed by Duncan's test for multiple comparisons.

3. Results

3.1. Reflex tracheal response induced by bronchoconstriction

The application (inhalation) of 0.01% histamine to the bronchial site for 5 min increased ventilation overflow (2.83 ± 0.41 ml, $n = 12$) by causing bronchial constriction (Fig. 2). After bronchoconstriction, intratracheal pressure increased by 1.79 ± 0.31 cm H_2O ($n = 12$) and then decreased by 2.35 ± 0.41 cm H_2O ($n = 12$). Thus, the tracheal response was biphasic (constriction followed by dilatation), while the bronchial response was only constrictive. Tracheal constriction always occurred slightly after bronchoconstriction in all of the animals. Tracheal dilatation occurred about 3 min after the beginning of bronchoconstriction. Similarly, acetylcholine inhalation also in-

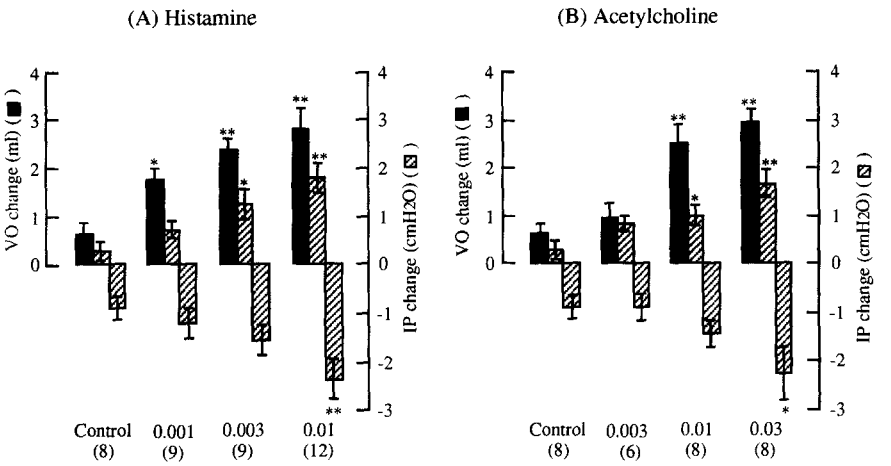

Fig. 3. Effects of the inhalation of 0.001–0.01% histamine (A) and 0.003–0.03% acetylcholine (B) into the bronchial site for 5 min on ventilation overflow (VO, left column) and intratracheal pressure (IP, center and right column) in anesthetized guinea pigs. The IP response appeared biphasically (increase, center column, followed by a decrease, right column). Data are shown as the means ± S.E.M. of the maximal change in each response. The number of animals is shown in parentheses. * $P < 0.05$ and ** $P < 0.01$, significantly different vs. control.

duced a biphasic tracheal response after bronchoconstriction. These bronchial and tracheal responses were concentration-dependent at 0.001–0.01% histamine or 0.003–0.03% acetylcholine (Fig. 3). Both the increase in intratracheal pressure and the decrease in intratracheal pressure correlated with the increase in ventilation overflow, regardless of whether it was induced by histamine inhalation (intratracheal pressure increase vs. ventilation overflow increase; $r = 0.707$, $P < 0.001$ and intratracheal pressure decrease vs. ventilation overflow increase; $r = 0.755$, $P < 0.001$) or acetylcholine inhalation (intratracheal pressure increase vs. ventilation overflow increase; $r = 0.644$, $P < 0.01$ and intratracheal pressure decrease vs. ventilation overflow increase; $r = 0.714$, $P < 0.001$). During histamine or acetylcholine inhalation, systemic blood pressure and heart rate showed only negligible changes, if any (Fig. 2).

3.2. Effects of vagotomy and tetrodotoxin on the tracheal and bronchial responses

After cervical vagotomy, 0.01% histamine-induced bronchoconstriction was slightly but not significantly decreased, whereas both tracheal constriction and dilatation were almost completely abolished (Fig. 4). Treatment of the tracheal site with 1% tetrodotoxin also completely inhibited tracheal constriction and dilatation, but not bronchial constriction.

In the experiment with 0.03% acetylcholine inhalation, bronchoconstriction was significantly inhibited by vago-

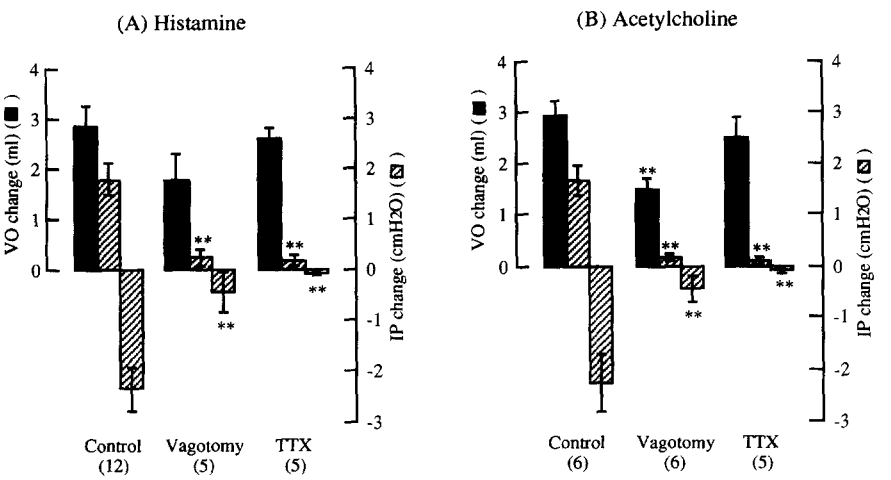

Fig. 4. Effect of cervical vagotomy or 1% tetrodotoxin (TTX) at the tracheal site on the change in ventilation overflow (VO) and intratracheal pressure (IP) induced by the inhalation of 0.01% histamine (A) and 0.03% acetylcholine (B) into the bronchial site for 5 min in anesthetized guinea pigs. Other explanations are the same as in Fig. 3.

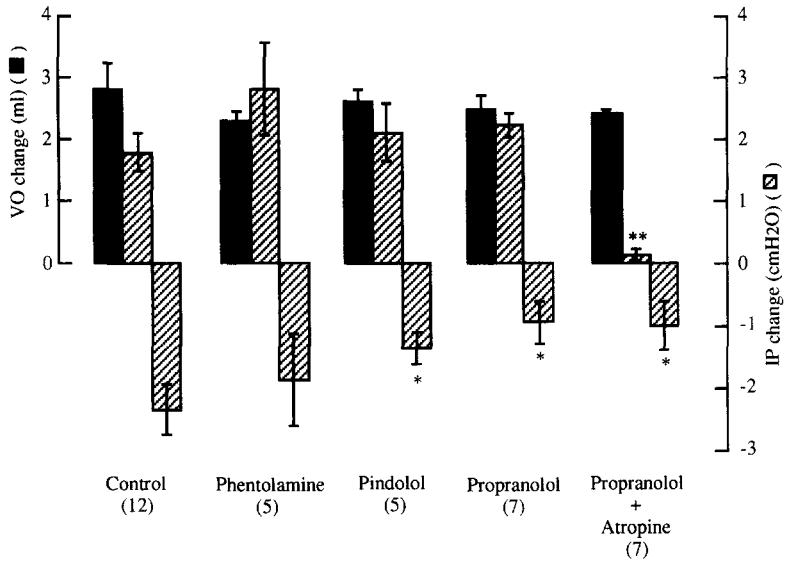

Fig. 5. Effect of the treatment of the tracheal site with 1% phentolamine, 1% pindolol, 1% propranolol or 0.1% atropine combined with 1% propranolol on the change in ventilation overflow (VO) and intratracheal pressure (IP) induced by the inhalation of 0.01% histamine into the bronchial site for 5 min in anesthetized guinea pigs. The tracheal site was treated with phentolamine, pindolol, propranolol and atropine for 30 min before histamine inhalation. Other explanations are the same as in Fig. 3.

tomy. Tracheal constriction and dilatation were abolished by both vagotomy and 1% tetrodotoxin, as they were with histamine inhalation.

3.3. Effects of β-adrenoceptor, muscarinic receptor and α-adrenoceptor antagonists on the reflex tracheal response

Local treatment of the tracheal site with 1% propranolol, a β-adrenoceptor antagonist, inhibited tracheal dilatation by 60% (Fig. 5). Reflex tracheal constriction was slightly but not significantly enhanced by propranolol, while bronchial constriction was not changed. Local treatment with 1% pindolol, another β-adrenoceptor antagonist, also significantly inhibited tracheal dilatation by 41%. The reflex tracheal constriction which was observed in the presence of propranolol was completely inhibited by 0.1% atropine. Treatment of the tracheal site with 1% phentolamine, an α-adrenoceptor antagonist, did not affect the tracheal responses induced by bronchoconstriction.

3.4. Effects of muscarinic receptor and β-adrenoceptor antagonists, and NO synthase inhibitors on the reflex tracheal response in a high tracheal tone preparation

To clarify the role of inhibitory nerves in reflex tracheal dilatation, the basal tone of the isolated tracheal site was increased by treating the site with 0.01% 5-HT. Intratracheal pressure increased immediately and then reached a plateau 5–10 min later at a level of 5–10 cm H_2O. In this high tonus condition, both tracheal dilatation and constriction were greater than without 5-HT (Fig. 6). In this case,

Table 1
Effects of atropine, N^{ω}-nitro-L-arginine methyl ester (L-NAME), L-arginine, propranolol and methylene blue, alone or in combination, on the reflex tracheal constriction and delatation induced by the inhalation of 0.01% histamine in anesthetized guinea pigs

	Change in intratracheal pressure (cm H_2O)	
	Increase	Decrease
Control (6)	4.83 ± 1.08	−4.75 ± 0.59
Atropine (7)	0.43 ± 0.17 [b]	−4.07 ± 0.43
Atropine + L-NAME (8)	0.81 ± 0.39 [b]	−2.31 ± 0.63 [a]
Atropine + L-NAME + L-arginine (7)	1.00 ± 0.44 [b]	−3.64 ± 0.60 [c]
Atropine + propranolol (7)	0.29 ± 0.29 [b]	−2.79 ± 0.47 [a]
Atropine + propranolol + L-NAME (5)	0.10 ± 0.10 [b]	−0.30 ± 0.30 [b,c]
Atropine + propranolol + methylene blue (8)	0.75 ± 0.75 [b]	−1.31 ± 0.61 [b,d]

The tracheal site was treated with 0.1% atropine, 1% L-NAME, 5% L-arginine, 1% propranolol or 1% methylene blue for 30 min. The site was then treated with 0.01% 5-HT. When the tone of the trachea reached a plateau, 0.01% histamine was inhaled into the bronchial site for 5 min. Other explanations are the same as in Fig. 3. [a] $P < 0.05$ and [b] $P < 0.01$, significantly different vs. control. [c] $P < 0.05$ significantly different vs. atropine plus L-NAME. [d] $P < 0.05$ and [e] $P < 0.01$, significantly different vs. atropine plus propranolol.

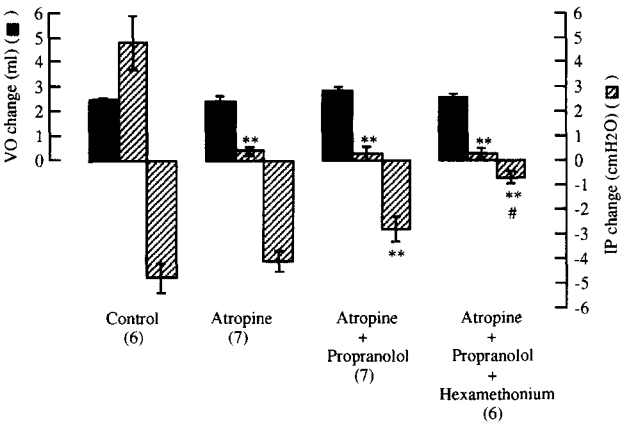

Fig. 6. Effects of treating the tracheal site, which had a high basal tone, with 0.1% atropine, 1% propranolol and 1% hexamethonium, alone or in combination for 30 min on the change in ventilation overflow (VO) and intratracheal pressure (IP) induced by the inahalation of 0.01% histamine into the bronchial site for 5 min in anesthetized guinea pigs. Histamine was inhaled after the tracheal tone had stabilized after treatment with 0.01% 5-HT. # $P < 0.05$, significantly different vs. atropine plus propranolol. Other explanations are the same as in Fig. 3.

tracheal constriction and dilatation were abolished by vagotomy (data not shown).

The tracheal constriction produced by histamine-induced bronchoconstriction was almost completely abolished by treatment of the tracheal site with 0.1% atropine. Tracheal dilatation was significantly inhibited by 36% with the further addition of 1% propranolol (Fig. 6). The residual tracheal dilatation in the presence of atropine and propranolol was completely inhibited by 1% hexamethonium (Fig. 6).

This reflex tracheal dilatation was also significantly inhibited by treatment with 1% L-NAME, a NO synthase inhibitor (Table 1). The inhibitory effect of L-NAME was reversed by simultaneous treatment with 5% L-arginine. Methylene blue (1%), a guanylate cyclase inhibitor, also

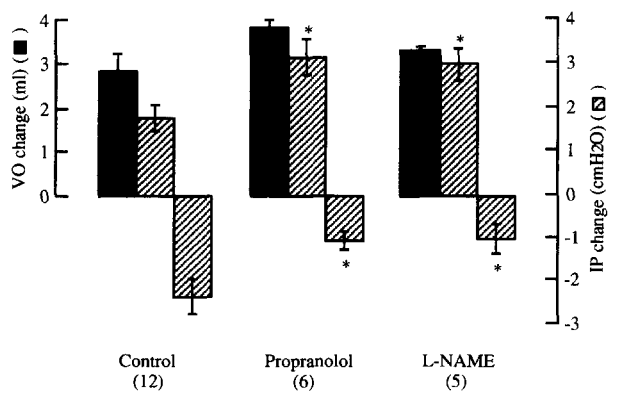

Fig. 7. Effects of propranolol 2 mg/kg, i.v. and N^{ω}-nitro-L-arginine methyl ester (L-NAME) 10 mg/kg, i.v. on the change in ventilation overflow (VO) and intratracheal pressure (IP) induced by the inhalation of 0.01% histamine into the bronchial site for 5 min in anesthetized guinea pigs. Propranolol and L-NAME were administered 15 min before histamine inhalation. Other explanations are the same as in Fig. 3.

inhibited tracheal dilatation in the presence of atropine and propranolol.

3.5. Effects of the systemic administration of a β-adrenoceptor antagonist and a NO synthase inhibitor on tracheal and bronchial constriction

The reflex tracheal constriction induced by the inhalation of 0.01% histamine was significantly potentiated by the systemic administration of propranolol 2 mg/kg, i.v., while the bronchoconstriction was slightly, but not significantly, enhanced (Fig. 7). The tracheal dilatation was inhibited by propranolol. Injection of L-NAME 10 mg/kg, i.v., also potentiated the reflex tracheal constriction and inhibited the dilatation, but did not affect the bronchoconstriction.

4. Discussion

A biphasic reflex tracheal response (constriction followed by dilatation) was observed during bronchoconstriction induced by histamine inhalation. These two successive tracheal responses were abolished by local treatment of the tracheal site with tetrodotoxin or by vagotomy. These findings indicate that the constriction and dilatation are mediated by the vagus nerves and recurrent laryngeal nerves innervating the cervical trachea. These responses are thought to be one of the vagal reflexes evoked by bronchial constriction. Therefore, this preparation allows one to evaluate vagally mediated tracheal responses induced by bronchoconstriction in anesthetized and immobilized guinea pigs. Furthermore, this is the first demonstration of a vagally mediated reflex tracheal response induced by bronchoconstriction in guinea pigs.

The tracheal and bronchial responses both depended on the concentration of histamine. The tracheal constriction and dilatation both correlated quite well with the magnitude of the bronchial constriction. In addition, the reflex tracheal constriction and subsequent dilatation seen after acetylcholine inhalation were quite similar to those seen with histamine inhalation. Therefore, these vagally mediated reflex tracheal responses do not depend on the bronchoconstrictor agent used. The reflex tracheal responses may be part of positive and negative feedback mechanisms which are induced by constriction of the bronchus and lungs. Tracheal constriction may be mediated by activation of irritant receptors and C-fiber endings in the bronchi (Karczewski and Widdicombe, 1969; Coleridge et al., 1982) and tracheal dilatation may be mediated by activation of lung stretch receptors (Widdicombe and Nadel, 1963; Bartlett et al., 1976). Widdicombe and Nadel (1963) reported that not only irritant receptors but also lung stretch receptors were stimulated by airway constriction induced by histamine in dogs. The initiation of tracheal dilatation through activation of stretch receptors may re-

quire contraction of the bronchial muscle above some threshold and an increase in intrabronchial pressure, so that the tracheal dilatation observed in this study appeared after bronchoconstriction. The reflex tracheal dilatation during bronchoconstriction may be part of a mechanism for limiting the development of airway constriction.

In the experiment with the high tracheal tone preparation, both of the reflex tracheal responses were much stronger than those in the normal tone preparation. Reflex dilatation may have been greater because tracheal smooth muscle is believed to relax easily in the hypertonus condition. 5-HT is known to increase tracheal tone by stimulation of 5-HT_2 receptors (Selig et al., 1988; Buckner et al., 1991). Macquin-Mavier et al. (1991) reported that 5-HT increased cholinergic transmission in guinea pig trachea partly through activation of 5-HT_2 receptors, and Rizzo et al. (1993) demonstrated that the activation of 5-HT_3 receptors potentiated endogenous cholinergic constriction in isolated guinea pig trachea. In our experiments with the high tracheal tone preparation, reflex tracheal constriction may have been increased due to an interaction between 5-HT and cholinergic nerve activation.

The reflex tracheal constriction was almost completely abolished by treatment of the tracheal site with atropine, either with or without 5-HT. This suggests that the reflex tracheal constriction induced by bronchoconstriction may be mediated only by a cholinergic pathway. NANC nerve-mediated tracheal constriction induced by electrical stimulation is reportedly mediated by both NK_1 and NK_2 receptors in guinea pigs (Irreland et al., 1991; Charette et al., 1994). However, Grundström et al. (1981) and Lundberg and Saria (1982) reported that an excitatory NANC neuronal pathway, in which substance P is the transmitter, is involved in bronchial muscle contraction, but not in tracheal muscle contraction, induced by electrical field stimulation in guinea pigs. However, our results suggest that the reflex tracheal constriction induced by bronchial constriction in anesthetized guinea pigs is not mediated by an excitatory NANC pathway.

Since reflex tracheal dilatation was inhibited by propranolol by almost 50%, an adrenergic pathway may mediate this dilatation. High doses of propranolol sometimes have a local anesthetizing effect. However, since pindolol, a β-adrenoceptor antagonist which has no substantial local anesthetizing effect, also inhibited the reflex tracheal dilatation, this reflex tracheal dilatation appears to be mediated by β-adrenoceptors. It is widely accepted that the guinea pig trachea is innervated by sympathetic nerves. Therefore, sympathetic nerves may be involved in the reflex tracheal dilatation. However, since the cervical sympathetic nerve trunks run separately from the vagus nerves in guinea pigs (Chesrown et al., 1980; Canning and Undem, 1993a), the reflex tracheal response may be mediated by the afferent vagal pathway and efferent vagal and sympathetic nervous pathways. However, since the reflex dilatation persisted for a long time, the involvement of circulating adrenaline released from the adrenal glands can't be ruled out.

The residual reflex tracheal dilatation in the presence of atropine and propranolol was completely prevented by hexamethonium. The NANC nerve is known to run through the ganglion of the vagus nerve innervating the trachea. Yip et al. (1981) reported that NANC-mediated tracheal relaxation was inhibited by hexamethonium. Therefore, in the present study, the reflex tracheal dilatation may have been mediated by a NANC neuronal pathway through the vagal nerve and recurrent laryngeal nerve ganglia.

Recently, NO has been reported to be a neurotransmitter in NANC neurons in the guinea pig airway (Tucker et al., 1990; Li and Rand, 1991; Belvisi et al., 1993; Lei et al., 1993). In our study, the reflex tracheal dilatation observed with atropine and propranolol was strongly inhibited by L-NAME. Even without propranolol, L-NAME significantly inhibited the tracheal dilatation by 50%, and this effect was reversed by L-arginine. Furthermore, methylene blue also inhibited the NANC-mediated reflex tracheal dilatation. These results suggest that NO may be involved in reflex tracheal dilatation through the activation of guanylate cyclase. Since the NANC-mediated tracheal dilatation was completely inhibited by L-NAME, NO would be expected to play a predominant role among the putative transmitters of inhibitory NANC nerves. Meulemans et al. (1993) and Desai et al. (1994) reported that NO is the main transmitter of NANC dilatation; vasoactive intestinal peptide (VIP) plays only a minor role in the guinea pig stomach. Therefore, the results of our study suggest that VIP may play only a limited role, if any, in the reflex tracheal dilatation induced by bronchoconstriction. However, VIP has been shown to play a role in NANC dilatation in the guinea pig airway by Matsuzaki et al. (1980) and Ellis and Farmer (1989a, b). Furthermore, Lei et al. (1993) reported that both NO and VIP mediated the NANC response in guinea pig trachea. Tucker et al. (1990) reported that NO and VIP were equally involved in the NANC response. In isolated guinea pig trachea, Li and Rand (1991) demonstrated that the VIP-mediated NANC tracheal dilatation induced by electrical stimulation at a frequency of 5 Hz was abolished by L-NAME. Lilly et al. (1993) found that VIP-induced bronchodilation was inhibited by a NO synthase inhibitor in guinea pigs. Therefore, further studies of the involvement of VIP in reflex tracheal dilatation and of the interaction between NO and VIP may be necessary.

The reflex tracheal constriction induced by bronchoconstriction was enhanced by the systemic administration of propranolol and L-NAME. Potentiation of histamine-induced bronchoconstriction by propranolol has been described previously (McCulloch et al., 1967; Diamond, 1972). Belvisi et al. (1993) reported that NO modulated cholinergic neurotransmission in guinea pig trachea. Furthermore, Yan et al. (1994) demonstrated that L-NAME enhanced histamine-induced constriction in isolated guinea

pig trachea. Therefore, both adrenergic and NANC neural pathways may be involved in a regulatory system in the guinea pig trachea. However, neither propranolol nor L-NAME significantly potentiated histamine-induced bronchoconstriction in our study. These results may suggest that these inhibitory nerves may be less responsible for bronchoconstriction than for reflex tracheal constriction, or that the inhibitory reflex may have been masked by bronchoconstriction, and that histamine caused a very potent bronchoconstriction (Misawa et al., 1992).

In conclusion, a biphasic vagally mediated reflex tracheal response (constriction followed by dilatation) was provoked by bronchoconstriction in anesthetized guinea pigs, which suggests that a cholinergic neural pathway might mediate the reflex tracheal constriction, while an adrenergic and a NANC pathway might mediate the reflex tracheal dilatation. Furthermore, NO may be involved in NANC-mediated reflex tracheal dilatation.

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