Effects of Histamine on Neurally Mediated Contraction of Canine Tracheal Smooth Muscle

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Abstract—The neuromodulatory action of histamine was investigated in isolated canine trachea. Histamine potentiated the tracheal contraction induced by electrical field stimulation (EFS). The ACh release induced by EFS was potentiated by histamine. The potentiating effect was blocked by chlorpheniramine. These findings suggest that histamine potentiates the neurally mediated tracheal contraction and the potentiating effect may be related in part to the acceleration of the prejunctional release of ACh, which may be mediated by H1-receptors.

The effects of histamine on airway smooth muscle are due not only to direct effects, but also to the stimulation of sensory receptors in the airways that mediate vagal reflex constriction (1, 2). Evidence for this is based on the fact that atropine and vagotomy reduce histamine-induced or antigen-induced airway constriction (3). Recently, it has been reported that tracheal smooth muscle reactivity to electrical vagal stimulation is potentiated by histamine (4). In the superior cervical ganglion of the rat, activation of H1-receptors facilitates the release of acetylcholine (ACh), whereas H2-receptor activation results in depressed release (5). In the present study, we investigated whether histamine accelerates the release of ACh in the canine trachea.

Male mongrel dogs weighing between 9 and 16 kg were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and killed by bleeding. The cervical trachea was rapidly removed, and rectangular strips of the tracheal smooth muscle, approximately 6 mm long and 3 mm wide, were dissected from the trachea after removing the epithelium. The tracheal strip was suspended in a 20 ml acrylic organ chamber containing modified Krebs-Henseleit solution (117.6 mM NaCl, 5.4 mM KCI, 2.5 mM CaCl2, 0.57 mM MgSO4, 1.03 mM KH2PO4, 25.0 mM NaHCO3, 11.1 mM glucose) maintained at 37°C and gassed with 95% O2-5% CO2 under an initial tension of 2 g. The Krebs-Henseleit solution always contained 20 μM choline chloride as a substrate for acetylcholine biosynthesis, 2 μM indomethacin as an inhibitor of cyclooxygenase for prostaglandin biosynthesis and 10 μM guanethidine as an adrenergic neuron blocker.

Changes in developed tension were isometrically measured with a force-displacement transducer (Nihon Kohden, TB-611T) and recorded on a polygraph (Nihon Kohden, RM-6000) or a desk-top pen recorder (Yokogawa, 3021). Two platinum-ring electrodes (5 mm diameter) were placed parallel to the tracheal strips for electrical field stimulation (EFS). Electrical impulses (1–30 Hz, 0.5 msec, 8 V and 300 pulses) were provided by a stimulator (Nihon Kohden, SEN-3301).

To investigate the release of ACh by histamine, the tracheal strip was mounted in a 1.0 ml acrylic organ chamber of our own making and was perfused at the rate of 1.0 ml/min with modified Krebs-Henseleit solution from the bottom. The perfusate was collected at the top of the organ chamber. All perfusate used in the perfusion study contained 10 mM methanesulfonfonyl fluoride, a cholinesterase inhibitor. The tracheal strip was stimulated at 5 Hz, 0.5 msec and 8 V for 180 sec. The amount
of ACh was determined by radioimmunoassay (RIA). At the end of the experiment, all tissues used were weighed. RIA for ACh was done using the procedure described by Kawashima et al. (6).

Drugs used were histamine dihydrochloride (Wako), chlorpheniramine (Sigma), cimetidine (Sigma) and acetyl[methyl-3H]choline chloride (86 Ci/mmol, Amersham). Anti-ACh antiserum was kindly given to us by Prof. K. Kawashima. All drug concentrations were expressed as final chamber concentrations.

The results shown in the figures are expressed as mean values±S.E. Statistical analyses were performed using Student's t-test, and a P value of <0.05 was considered significant.

When the tracheal strip was stimulated by EFS at varied frequencies from 1 to 30 Hz, a tracheal contraction was obtained in a frequency-dependent manner. The contractile response returned to the resting level immediately after cessation of EFS. Histamine had no effect on the EFS-induced tracheal contraction at a concentration up to 3×10⁻⁸ M. At a concentration range from 10⁻⁸ M to 10⁻⁶ M, histamine significantly potentiated the tracheal contraction induced by EFS (Fig. 1A). The potentiating effect of histamine on the EFS-induced tracheal contraction was obtained at varied frequencies from 1 to 30 Hz (Fig. 1B). Histamine alone induced a tracheal contraction at concentrations >10⁻⁷ M, while tachyphylaxis was obtained by repeated treatment with histamine. Under the tachyphylaxis in response to histamine, the tracheal contractile response to EFS was potentiated (data not shown).

The potentiating effect of histamine on the EFS-induced tracheal contraction was obtained at varied frequencies from 1 to 30 Hz (Fig. 1B). Histamine alone induced a tracheal contraction at concentrations >10⁻⁷ M, while tachyphylaxis was obtained by repeated treatment with histamine. Under the tachyphylaxis in response to histamine, the tracheal contractile response to EFS was potentiated (data not shown).

The potentiating effect of histamine on the EFS-induced tracheal contraction was blocked by 10⁻⁶ M chlorpheniramine, but was unaffected by 10⁻⁵ M cimetidine (Fig. 2A). Chlorpheniramine and cimetidine at the doses used had no effect on the EFS-induced tracheal contraction.

Under the present experimental conditions, the amounts of spontaneous ACh release from the tracheal strips were approximately 1.0 pg/mg tissue/min (n=5). EFS caused an increase in the release of ACh and a contractile response. At the concentration of 3×10⁻⁷ M, histamine potentiated the ACh release induced by EFS (Fig. 2B). The potentiating effect of histamine on the EFS-induced ACh release was inhibited by 10⁻⁶ M chlorpheniramine (Fig. 2B).

In the present investigation, it was shown that histamine potentiated the tracheal contraction induced by EFS. The EFS-induced tracheal contraction was abolished by tetrodotoxin (10⁻⁷ M) and atropine (10⁻⁶) (data not shown), indicating the mediation by cholinergic nerve stimulation. Canine tracheal smooth muscles are innervated by cholinergic excitatory and adrenergic inhibitory systems (7), but not by non-adrenergic and non-cholinergic systems (8). In the present study, to block adrenergic neurons, guanethidine was always treated in the Krebs-Henseleit solution.

At concentration >10⁻⁷ M, histamine caused a tracheal contraction. The tracheal reactivity may be influenced by change in the basal tone of the smooth muscle. In contrast, histamine showed a tachyphylaxis by repeated treatment. Under this condition, the potentiating effect of histamine on the EFS-induced tracheal contraction was still observed, indicating that the potentiating effect of histamine may be related to a change in the basal tone of the smooth muscle.

The potentiating effect of histamine on the EFS-induced tracheal contraction was abolished by chlorpheniramine, a H₁-blocker, but cimetidine, a H₂-blocker, had no effect. Histamine causes airway contraction through H₁-receptors; and in some species, it causes airway dilatation via H₂-receptors (9). In the canine airway, however, histamine receptors are exclusively of the H₁-type which mediate constrictions (10, 11). Recently, it has been reported that H₃-receptors which inhibit ACh release are present on the vagus nerve in guinea pig and human airways (12, 13). It is possible that H₃-receptors may act on cholinergic neurotransmission in dog airways.

Benson and Graf (14) demonstrated the interaction between electrical stimulation of the vagus nerve and histamine which was greater than the additive responses in dogs. However, it has been reported that there is no interaction between histamine and vagal stimulation in dogs (15). Kikuchi et al. (4) showed that histamine potentiates tracheal
smooth muscle reactivity to electrical vagal stimulation in dogs. The mechanisms of interaction between vagal and histamine contraction have not yet been elucidated. The interaction might occur at receptor sites on the smooth muscle. Further work will be necessary to examine the interaction with receptor sites on the smooth muscle.

Fig. 1. Effects of histamine on the electrically induced tracheal contraction. A: Concentration-dependent effect of histamine on the contractile responses of tracheal strips stimulated at 5 and 10 Hz. For each electrical field stimulation, isometric tension is expressed as a percentage of the pre-control response obtained prior to saline (Control) and histamine treatment. B: Effects of $3 \times 10^{-7}$ M histamine (Hist) on noncumulative electrical field stimulus frequency-response relationships. Isometric tension is expressed as percentage of the contractile response obtained at 30 Hz prior to saline (Control) and histamine treatment. In A and B, each point shows the mean value with S.E. of results from five preparations. The changes are significant at $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ when compared to the value for the control.
The ACh release induced by EFS from the tracheal strip was potentiated by histamine. The potentiating effect of histamine was inhibited by the H₁-blocker chlorpheniramine. These findings indicate that H₁-receptors exist in the presynapses of vagus nerves and may mediate the acceleration of ACh release. We conclude that histamine potentiates the neurally mediated tracheal contraction, and the potentiating effect may be related in part to acceleration of the prejunctional endogenous neural release of ACh which may

Fig. 2. Effects of histamine (Hist) on the tracheal contraction induced by electrical field stimulation (A) and the electrically induced acetylcholine (ACh) release from the canine tracheal strip (B). The tracheal strip was stimulated at 5 Hz, 0.5 msec and 8 V for 60 sec (A) and 180 sec (B). Isometric tension is expressed as a percentage of the pre-control response obtained prior to saline (Control) and histamine treatment. Each column shows the mean value with S.E. of results from five preparations. The changes are significant at *P<0.05 and ***P<0.001 when compared to the value for the control.
be mediated by $H_1$-receptors.

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