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Histamine H_3-receptors inhibit cholinergic neurotransmission in guinea-pig airways

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The histamine H_3-agonist (R)-α-methylhistamine (α-MeHA) caused a dose-dependent inhibition of vagally-mediated contraction of a guinea-pig tracheal tube preparation but did not alter tracheal contraction induced by exogenously-applied acetylcholine. Blockade of H_1- and H_2-histamine receptors, and α- and β-adrenoceptors failed to prevent the inhibitory effect of α-MeHA, whereas the specific H_3-antagonist thioperamide prevented the effect of α-MeHA on tracheal contraction. In the presence of H_1- and H_2-receptor antagonists, histamine also inhibited vagally-mediated tracheal contraction. The inhibitory effect of α-MeHA was greater with preganglionic (vagus nerve) stimulation than with postganglionic stimulation by electrical field stimulation, suggesting that H_3-receptors are localized both to cholinergic ganglia and to post-ganglionic nerve-endings. Our results suggest that H_3-receptors exist on the vagus nerve which modulate cholinergic neurotransmission in the airways.

Introduction Histamine causes bronchoconstriction through stimulation of H_1-receptors, and, in some species, bronchodilatation via H_2-receptors (Chand & DeRoth, 1979; Barnes, 1987). Recently, (R)-α-methylhistamine (α-MeHA), a histamine H_3-receptor agonist, has been developed (Arrang *et al.*, 1983; 1987). H_3-receptors have been localized within the central nervous system, where they appear to be involved in the feedback control of both histamine synthesis and release (Arrang *et al.*, 1987), and have been shown to inhibit sympathetic neurotransmission in perivascular nerves (Ishikawa & Sperelakis, 1987). Their functional significance in autonomic nerves of the airways, however, has not yet been elucidated.

In the airways, vagus nerve stimulation causes bronchoconstriction by releasing acetylcholine. Histamine may enhance vagally mediated bronchoconstriction via H_1-receptors (Kikuchi *et al.*, 1984). In this study, we have investigated whether H_3-receptors are present on airway cholinergic nerves in an innervated guinea-pig tracheal tube preparation (Blackman & McCaig, 1983).

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Methods Male Dunkin-Hartley guinea-pigs (400–600 g) were anaesthetized with an intraperitoneal injection of urethane (2 g kg^{-1}). The trachea was dissected out together with the right vagus nerve supply as previously described (Blackman & McCaig, 1983). The trachea was placed in a tissue bath containing Krebs-Henseleit solution, (mM): NaCl 118, KCl 5.9, $MgSO_4$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.5, and glucose 5.05, maintained at 37°C and bubbled with 95% O_2, 5% CO_2, pH 7.4. Indomethacin (10^{-5} M) was present throughout. The trachea was filled with Krebs-Henseleit solution and mounted vertically at its *in vivo* length. The bottom of the trachea was closed and the top connected to a pressure transducer (Gould) for recording intraluminal pressure. The right vagus nerve was stimulated through a bipolar electrode, and trains of rectangular pulses (40 V, 0.2 ms, 8 Hz) were applied for 5 s every 90 s (Grass SD9). Postganglionic nerves were stimulated by electrical field stimulation with parallel platinum electrodes having the same stimulation parameters.

The following drugs were used: α-MeHA, thioperamide (Bioprojet, Paris); astemizole (Janssen, Wantage); cimetidine (Smith Kline & French); phentolamine (Ciba-Geigy); propranolol (ICI); indomethacin, histamine, acetylcholine chloride (Sigma).

Data were expressed as means ± s.e. and analyzed by Student's *t* test for paired and unpaired data. Probability values of $P < 0.05$ were considered significant.

Results Whilst having no effect on the resting intraluminal pressure, α-MeHA (10^{-12} to 10^{-6} M) gave a concentration-dependent inhibitory effect on vagal nerve-induced tracheal responses, which was maximal within 10 min. The EC_{50} value for this effect was 7.0×10^{-9} M. α-MeHA also reduced the contractile response to electrical field stimulation (10.8 ± 2.8% at 10^{-7} M compared with 27.8 ± 2.0% for vagus nerve stimulation). Matched contractile responses induced by exogenously-applied acetylcholine (10^{-7}–3×10^{-6} M) were unaffected by α-MeHA (Figure 1a).

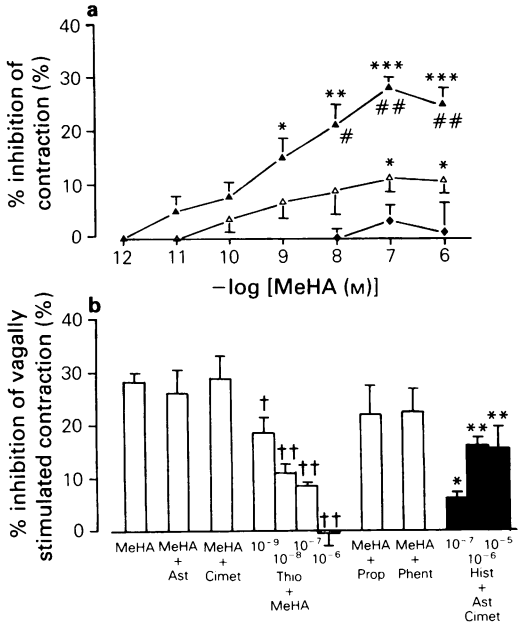

Figure 1 (a) Effect of α-methylhistamine (MeHA) on vagus nerve stimulation (VS; ▲, $n = 5$), electrical field stimulation (EFS; △, $n = 7$), and exogenously-applied acetylcholine (◆, $n = 6$). Values are means with s.e.means shown by vertical bars. (b) Open columns showing the effect of various blockers on the inhibition of VS by MeHA (10^{-7} M). The effects of astemizole (Ast, 10^{-6} M), cimetidine (Cimet, 10^{-4} M), thioperamide (Thio, 10^{-9}–10^{-6} M), propranolol (Prop, 10^{-6} M), and phentolamine (Phent, 10^{-6} M) are shown as mean values of 5 experiments; s.e.mean shown by vertical bars. Solid columns showing the effect of histamine (Hist, 10^{-7}–10^{-5} M, $n = 5$) on VS with Ast and Cimet; $^{*}P < 0.02$; $^{**}P < 0.002$; $^{***}P < 0.001$ as compared with control values; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ as compared with EFS; $\dagger P < 0.05$; $\dagger\dagger P < 0.01$ as compared with MeHA alone.

Neither H_1- nor H_2-receptor antagonism by astemizole (10^{-6} M) and cimetidine (10^{-4} M), respectively, altered the inhibitory effect of α-MeHA but its effect was prevented by the H_3-antagonist, thioperamide ($IC_{50} = 4.2 \times 10^{-9}$ M). Neither the α-blocker phentolamine (10^{-6} M), nor β-blocker propranolol (10^{-6} M) modified the α-MeHA-induced effect. Furthermore, with H_1- and H_2-receptor blockade, histamine (10^{-7}–10^{-5} M) caused significant inhibition of vagally-induced tracheal contraction (Figure 1b). The maximum effect of histamine (16.1 ± 1.0%) was significantly less than that of MeHA (27.8 ± 2.0%).

Discussion We have demonstrated that α-MeHA, a selective H_3-receptor agonist causes dose-dependent inhibition of airway smooth muscle contraction elicited by vagus nerve stimulation in a concentration-range similar to that observed for inhibition of histamine release in the CNS (Arrang *et al.*, 1987). The contractile response of the guinea-pig tracheal preparation is due to release of acetylcholine from cholinergic nerves (Blackman & McCaig, 1983). α-MeHA had no significant effect on matched acetylcholine-induced contraction in this preparation, suggesting that it may be inhibiting acetylcholine release. The inhibitory effect of α-MeHA was greater on vagus nerve stimulation than on electrical field stimulation of postganglionic nerves, indicating that it is acting predominantly on cholinergic ganglionic neurotransmission. The inhibitory effect of α-MeHA was blocked by the H_3-selective antagonist thioperamide with a potency similar to that seen in CNS (Arrang *et al.*, 1987) but not by an H_1-antagonist or an H_2-antagonist, confirming that this effect was mediated via H_3-receptors in the cholinergic pathways.

Cholinergic neurotransmission in airways may be modified by adrenergic mechanisms, such as α_2- (Grundstrom *et al.*, 1981) and β-adrenoceptor stimulation (Rhoden *et al.*, 1988). In this study, however, the inhibitory effect of α-MeHA was not influenced by α_2- or β-adrenoceptor blockade.

Histamine itself also inhibited cholinergic neurotransmission in the presence of H_1- and H_2-antagonists, although the inhibitory effect was less marked than with α-MeHA. The concentration of histamine inhibiting cholinergic neurotransmission is lower than that reported to cause direct contraction of this preparation via H_1-receptors (McCaig, 1986).

Our results suggest that H_3-receptors which inhibit acetylcholine release are present in the vagal pathway and could play a role in modulating neural bronchoconstriction in allergic disease when histamine is released from airway mast cells in the vicinity of airway ganglia and cholinergic nerves.

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