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COMMUNICATIONS

The presence of inhibitory histamine H_2-receptors in guinea-pig tracheobronchial muscle

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The findings that histamine is released in immediate hypersensitivity reactions and has a potent bronchoconstrictor action on airway smooth muscle of some mammalian species have led to an association of histamine with asthmatic bronchospasms for several decades. Evidence is now accumulating that histamine may produce contraction, or relaxation (or both) of tracheobronchial muscle depending on the species (review: Chand & Eyre, 1976). Contractile responses are in general mediated by histamine H_1-receptors (Ash & Schild, 1966), while inhibitory effects in some instances have been shown to be due to activation of histamine H_2-receptors (Black, Duncan & others, 1972). A relaxant effect of histamine has been demonstrated in tracheobronchial muscles from sheep (Eyre, 1969), cat (Maegwyn-Davies, 1968; Eyre, 1973; Chand & Eyre, 1977a), horse (Chand & Eyre, 1977b, c) and man (Dunlop & Smith, 1977).

Guinea-pig tracheobronchial muscle is sensitive to histamine, and is a popular model for studies of histamine action on airway smooth muscle, and of potential antiasthmatic drugs. We now report that histamine also activates H_2-receptors in the guinea-pig tracheobronchial muscle. We studied the effect of metiamide (H_2-receptor antagonist; Black, Duncan & others, 1973) on histamine contractions of tracheobronchial muscle on the principle that if histamine stimulates two functionally opposite receptors, then a competitive antagonist acting at the 'masked' receptor should cause potentiation of the 'dominant' receptor effect (Szabadi, 1975). Two selective H_1-agonists, 2-methylhistamine (Black & others, 1972) and 2-pyridylethylamine (Durant, Ganellin & Parsons, 1975) were also used.

Helical strips of trachea and bronchi were prepared from 30 guinea-pigs of either sex (300–700g) as described by Eyre (1973) and set up in a 20 ml overflow organ bath containing Krebs-Henseleit solution maintained at 37° and gassed with 5% CO_2 in oxygen. The strips were set up under a tension of 2–3 g and changes in muscle length were measured with an Isotonic Myograph Transducer and recorded on a Physiograph recorder (Desk Model DMP-4B, E & M Instruments Co. Inc. Houston, Texas). Tracheal and bronchial strips were made from the same animal and studied simultaneously. After allowing 1–2 h for equilibration, doses of agonists were added to the bath and allowed a contact time of 5 min. The bath fluid was replaced three times during the ensuing interval of 10–15 min, by which time the muscle had regained its original tone. In one series of experiments, the effect of metiamide on contractile responses to histamine, carbachol and 2-methylhistamine was investigated. Carbachol and 2-methylhistamine (2-MeH) were included as control for time-related changes in muscle sensitivity which might be unrelated to H_2-receptor blockade. Two doses each of carbachol, histamine and 2-methylhistamine producing submaximal contractions were repeatedly applied in a random sequence until each dose had been added 4 times. Then metiamide at a concentration of 4×10^{-5}M was included in the bath. After allowing 30 min for equilibration, the agonists were again administered until four applications of each dose had been completed. The result of one such experiment on the bronchus helical strip is plotted in Fig. 1. After metiamide, contractions to histamine were enhanced

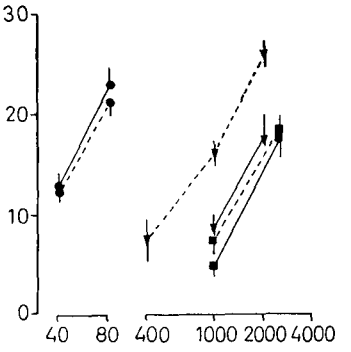

FIG. 1. Guinea-pig bronchus. Effect of metiamide (4×10^{-5} M) on response to carbachol (●) histamine (▼) and 2-methylhistamine (■). Responses to agonists before metiamide are represented by continuous lines. Broken lines are responses after equilibration with metiamide. Each point is a mean of four measurements from one bronchial strip. Vertical bars, s.e.m. Ordinate: Response mm contraction. Abscisssa: Dose (ng ml^{-1}) (log scale).

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approximately two-fold. 2-Methylhistamine was not potentiated. Carbachol responses were slightly reduced. Potentiation of histamine by metiamide (10^{-6}–10^{-5} M), but not of 2-methylhistamine or 2-pyridylethylamine was consistently observed in all experiments (n = 10). Higher concentrations of metiamide (>10^{-4} M) usually inhibited contractions to all agonists (see also Chand & Eyre, 1977a).

In another series of experiments, dose-response curves were constructed to histamine and carbachol in paired helical strips from trachea and bronchus before and in the presence of metiamide 4×10^{-5} M. From each of five separate dose-response curves, dose-ratios were evaluated from doses producing a constant response of 25 mm before and after metiamide. Mean dose-ratios on trachea were $1{\cdot}25 \pm 0{\cdot}4$ and $0{\cdot}59 \pm 0{\cdot}059$ for carbachol and histamine respectively and on bronchus, $1{\cdot}24 \pm 0{\cdot}06$ and $0{\cdot}38 \pm 0{\cdot}03$ for carbachol and histamine respectively. Compared with carbachol in both bronchus and trachea, histamine was significantly potentiated by metiamide (paired Student's *t*-test, $P < 0{\cdot}001$). This potentiation was attributed to block of H_2-receptors sub-serving relaxation.

In five paired tracheal and bronchial strips, three doses each of histamine and 2-methylhistamine ($2{\cdot}5 \times 10^{-7}$, 5×10^{-7} and 10^{-6} M) producing submaximal contractions were randomly given. From dose-response curves obtained in each experiment, 2-methylhistamine was found to have 61% (range, 41–81) and 70% (50–82) of the contractile activity by histamine in the trachea and bronchus respectively. In the guinea-pig ileum, 2-methylhistamine is less than 20% as active as histamine (Black & others, 1972). The difference may be explained on the basis of the presence of inhibitory H_2-receptors which are stimulated by histamine, but not by 2-methylhistamine in tracheobronchial muscle. The presence of inhibitory H_2-receptors has either not been demonstrated in the guinea-pig ileum (Black & others, 1972) or when they have been found (Bareicha & Rocha ē Silva, 1976) these receptors appeared to exert an inhibitory effect only on responses to low concentrations of histamine.

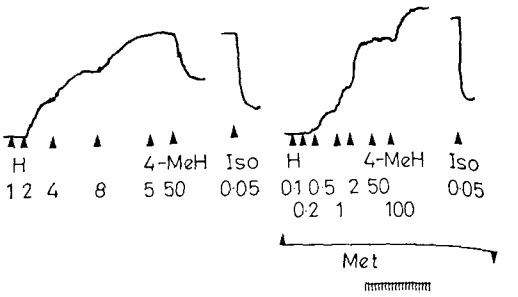

FIG. 2. Guinea-pig tracheal spiral strip in Krebs Henseleit solution mixed with 5% CO_2 in oxygen at 37° under a resting tension of 3 g. 4-Methylhistamine (4-MeH: a specific H_2 agonist) and isoprenaline (Iso) relax trachea partially contracted to histamine (H). Metiamide (5×10^{-5} M) potentiated the histamine-induced contraction and caused reversal of 4-methylhistamine-induced relaxation to a contraction without altering the isoprenaline response. This shows clear evidence for the presence of H_2-inhibitory receptors in this tissue. Agonist concentrations are $\mu g\ ml^{-1}$ of bath fluid. Time marker indicates min.

Finally it has been observed (Chand & Eyre, unpublished data) that large doses of histamine and 4-methylhistamine (a selective H_2 agonist:— Black & others, 1972) can relax guinea-pig tracheal and bronchial helical strips which are partially contracted by carbachol in the presence of mepyramine. We also show in Fig. 2, that tracheal and bronchial helical strips contracted by 2-methylhistamine relax under the influence of 4-methylhistamine. This effect is specifically prevented by pretreatment with metiamide (7 out of 9 expts). We conclude that the guinea-pig tracheobronchial muscle contains H_2-receptors which modulate the effects of H_1-receptor activation.

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