Is There Loss of a Protective Muscarinic Receptor Mechanism in Asthma?*

L. Eduardo Ayala, M.D.;† and Tahir Ahmed, M.D., F.C.C.P.

We investigated the hypothesis that prior airway muscarinic receptor stimulation (with aerosolized methacholine) would modify the bronchoconstrictor response to histamine, which is, in part, vagally mediated. On four different experiment days, the following combinations of methacholine and histamine inhalation challenges were performed in 15 subjects (nine normal and six asthmatic) in a random fashion: methacholine-histamine, histamine-methacholine; methacholine-methacholine and histamine-histamine. Cumulative provocative dose of each agonist which caused a 50 percent decrease in SGaw was estimated (PD₅₀). The second challenge was performed approximately 1 hour after the first challenge, when SGaw had returned to baseline. In normal subjects, prior muscarinic stimulation with methacholine suppressed the subsequent bronchoconstrictor response to histamine (mean ± SE PD₅₀ histamine-increased from 13.7 ± 3.1 to 28.4 ± 7.2 breath units), without modifying the bronchoconstrictor response to methacholine. In asthmatic subjects, prior methacholine exposure failed to modify the bronchoconstrictor responses to histamine and methacholine. In contrast, prior challenge with histamine did not modify the subsequent bronchoconstrictor responses to histamine and methacholine in both normal and asthmatic subjects. Pretreatment with ipratropium bromide attenuated the histamine-induced bronchoconstriction, suggesting that airway effects of histamine, in part, are vagally mediated. These data suggest that prior muscarinic stimulation has a protective effect on histamine-induced bronchoconstriction in normal subjects and the absence of this inhibitory effect in asthmatic patients may represent loss of a protective muscarinic receptor mechanism. (Chest 1989; 96:1285-91)

Various types of agonists are capable of contracting the airway smooth muscle through different mechanisms of action. Among these agents, histamine and methacholine are the most widely used in the investigation of airway hyperreactivity. The pharmacodynamic properties of these agents have shown that histamine acts through H₁ and H₂ receptors, whereas methacholine stimulates muscarinic receptors on smooth muscle and possibly on autonomic ganglia.

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Repetitive stimulation of histamine receptors elicits tachyphylaxis in vitro and in vivo, whereas repetitive administration of methacholine generally has not shown any tachyphylaxis. Although the mechanism of action of these two agonists in terms of specific receptor stimulation and blockade by specific receptor antagonist is well established, any interaction between these two agonists if not well known.

The classic mode of mediator action has been described in terms of agonist-antagonist interaction at the receptor level. Recently, Ishii and Kato have proposed a new type of drug interaction at the cellular level, ie, "functional antagonism" between agonists for two distinct receptor system, exhibiting physiologic responses in the same direction (smooth muscle contraction). These authors observed a suppression of H₁ histamine-receptor function by prior stimulation of muscarinic receptors in the longitudinal muscle of guinea pig ileum. Although such an interaction, so far, has not been described in airway smooth muscle, Minette and Barnes have observed that the muscarinic agonist, pilocarpine, attenuated the contractile responses to electrical field stimulation in the guinea pig airway smooth muscle possibly via stimulation of inhibitory M₄-muscarinic receptors. Since airway effects of histamine, in part, are mediated indirectly via stimulation of vagal reflex, we wondered if muscarinic receptor stimulation in vitro would cause a suppression of airway effects of histamine. Thus, the aim of this study was to determine whether airway muscarinic receptor stimulation with aerosolized methacholine would influence the subsequent bronchoconstrictor responses to histamine. Since airway hyperresponsiveness to methacholine and histamine is a hallmark of bronchial asthma, we also studied any possible differences in methacholine-histamine interaction between normal and asthmatic subjects.

**Materials and Methods**

**Subjects**

Fifteen nonsmoker subjects (ten men and five women) without a recent history of upper respiratory tract infection were included in the study. Six subjects had a history of mild asthma; their ages ranged from 25 to 52 years (mean 37). The remaining nine subjects had no personal or family history of atopy. Their ages ranged from 22 to 38 years (mean 30). The asthmatic subjects were asymptomatic at the time of the study, and had not received inhaled beta agonists for at least 12 hours prior to each test. The protocol was approved.
by the institutional ethics and research committees. Informed consent was obtained from each subject.

**Measurements**

Baseline pulmonary function tests consisted of spirometry and measurements of airway resistance and functional residual capacity (FRC) by body plethysmography. Specific airway conductance (SGaw) was calculated by dividing the reciprocal of airway resistance by the thoracic gas volume at which airway resistance was measured.

**Bronchial Provocation**

Both methacholine and histamine provocation tests were performed in an identical manner. Agonists were delivered to the lungs through a DeVilbiss No. 42 nebulizer by a modified technique of Chai et al. The nebulizer was attached to a dosimeter, which consisted of a breath-activated solenoid valve and a source of compressed air (20 psi). The solenoid valve was set to remain open for 0.6 s during inhalation to allow the compressed air to flow through the nebulizer, dispersing an average of 0.023 ml of the solution with each breath. The aerosolized material was delivered from FRC position through the course of a submaximal inspiratory effort. After obtaining the baseline measurements of specific airway conductance, the subjects inhaled five breaths of saline diluent, and the measurements were repeated after a 2-min interval. Dose-response curves for each agent were then established by having the subjects take five inhalations from each of the increasing concentrations of the agonist, at intervals of 5 min. The first concentration was 0.075 mg/ml, while the concentrations of subsequent doses increased in an alternating two-fold manner. For both agonists, the test was stopped when the SGaw had fallen by at least 50 percent from the post-diluent value or the maximal concentration of 5 mg/ml had been reached. The SGaw was then plotted against the cumulative agonist dose, expressed in breath units. One breath unit was defined as one inhalation of a 1 mg/ml concentration of the agonist. The results were expressed as the cumulative provocative dose of the agonist causing a fall in the SGaw by 50 percent (PD_{50}). At the end of each experiment day, the subjects took two inhalations of albuterol to reverse the bronchoconstriction.

We used SGaw as an indicator of airway narrowing during bronchial provocation to avoid the potential problems associated with deep inspiration and also because methacholine may cause changes in SGaw and minimal changes in forced expired volume in one second (FEV_{1}).

![Histogram](image)

**FIGURE 1.** Individual dose response curves to histamine and methacholine in a normal subject. Postmethacholine there is a rightward shift in the histamine dose-response curve (panel A).

<p>| Table 1 — Baseline Pulmonary Function Tests of Normal and Asthmatic Subjects |
|-----------------------------|---------------------|---------------------|------------------|----------------|---------|</p>
<table>
<thead>
<tr>
<th>TLC</th>
<th>FRC</th>
<th>FVC</th>
<th>FEV_{1}</th>
<th>FEV_{1}/FVC</th>
<th>Raw</th>
<th>SGaw</th>
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<tbody>
<tr>
<td>Normals</td>
<td></td>
<td></td>
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<tr>
<td>Subjects</td>
<td>6.7</td>
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<tr>
<td>SE</td>
<td>0.7</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
<td>1.9</td>
<td>0.2</td>
</tr>
<tr>
<td>(103)</td>
<td>(99)</td>
<td>(108)</td>
<td>(105)</td>
<td></td>
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</tr>
<tr>
<td>Asthmatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Patients</td>
<td>6</td>
<td>4.1</td>
<td>3.4*</td>
<td>2.5*</td>
<td>72%</td>
<td>2.4</td>
</tr>
<tr>
<td>SE</td>
<td>1.2</td>
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<td>0.6</td>
<td>0.4</td>
<td>4.8</td>
<td>0.3</td>
</tr>
<tr>
<td>(116)</td>
<td>(130)</td>
<td>(78)</td>
<td>(70)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from normals, p<.05.

Mean ± SE; % predicted in parentheses; RV = residual volume in liters; TLC = total lung capacity in liters; FRC = functional residual capacity in liters; FVC = forced vital capacity in liters; FEV = forced expiratory volume in 1 second in liters; Raw = airway resistance in cm H_{2}O/L/s; SGaw = specific airway conductance, L/s·cm⁻¹. 

Loss of Protective Muscarinic Receptor Mechanism in Asthma? (Ayala, Ahmed)

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Experimental Protocol

Each subject was studied on five occasions separated by at least 72 h. On experiment day 1, after obtaining baseline pulmonary function test results, a control bronchial provocation test with histamine was performed to establish PD_{50} to histamine. Normal subjects in whom PD_{50} was not achieved even with 5 mg/ml concentration of histamine were excluded from the study to avoid problems with statistical analysis. On experiment days 2 to 5 the following combination of histamine and methacholine challenges were performed in a random sequence: methacholine-histamine; histamine-methacholine; methacholine-methacholine and histamine-histamine. After the first challenge, the subject was allowed to recover spontaneously. The second challenge was performed approximately 1 h later when the subject's SGaw level had returned to within 90 percent of the prechallenge baseline. Simultaneous dose-response curves and PD_{50} values for each challenge were obtained for comparative analysis. In five normal subjects, dose-response to histamine was repeated on two additional days, after prior exposure to a lower dose of methacholine (5 breath units, ie, equivalent to methacholine PD_{50} of asthmatic patients), or following pretreatment with inhaled ipratropium bromide (36μg).

Statistical Analysis

Data were expressed as mean±SE. For PD_{50}, the data were analyzed by a nonparametric test, ie, the Wilcoxon signed rank tests for paired samples. Baseline pulmonary function tests between the two groups were compared by an unpaired t-test. The level of significance was accepted at p<.05 (beta = .01, alpha = .05).

RESULTS

Baseline Pulmonary Function

These results are shown in Table 1. Asthmatic subjects had evidence of mild airway obstruction as

![Figure 1: Modification of bronchoconstrictor response to histamine by prior muscarinic stimulation with methacholine in normal subjects (n = 9) and subjects with bronchial asthma (n = 6). Data are expressed as mean ± SE PD_{50} of histamine (cumulative provocative dose of histamine which caused a 50 percent decrease in SGaw). *p<0.05.](image1)

![Figure 2: Individual dose-response curves to histamine and methacholine in an asthmatic subject. Prior muscarinic stimulation did not cause a rightward shift in the histamine dose-response curve.](image2)

![Figure 3: Cumulative breath units](image3)

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shown by significantly lower SGaw, FEV₁, and forced vital capacity. The SGaw was comparable on different experiment days within each group. The mean ± SE post-diluent values of SGaw on experiment days 2-5 were 0.21 ± 0.02, 0.21 ± 0.02, 0.20 ± 0.02, and 0.19 ± 0.01 s cm H₂O⁻¹, respectively, for normal subjects, and 0.11 ± 0.03, 0.13 ± 0.02, 0.11 ± 0.02 and 0.12 ± 0.02 s cm H₂O⁻¹, respectively, for asthmatic patients.

In both groups, the baseline PD₉₀ values of methacholine and histamine were highly reproducible on two different days (normal subjects: histamine PD₉₀, 13.7 ± 3.1 and 16.7 ± 3.7 breath units; methacholine PD₉₀, 13.9 ± 2.8 and 16.9 ± 3.6 breath units. Asthmatic patients: histamine PD₉₀, 3.6 ± 2.5 and 3.4 ± 2.5 breath units; PD₉₀ methacholine, 4.9 ± 3.3 and 4.3 ± 2.5 breath units). The control values of PD₉₀ of histamine and methacholine were significantly lower in the asthmatic group.

**Effect of Prior Muscarinic Receptor Stimulation on Histamine and Methacholine-induced Bronchoconstriction**

In the normal group, prior muscarinic receptor stimulation with methacholine caused a rightward shift in the histamine dose-response curve in most of the subjects and a significant increase in histamine PD₉₀ (Fig 1 and 2). The mean ± SE histamine PD₉₀ on a control day was 13.7 ± 3.1 breath units; the histamine PD₉₀ increased to 28.4 ± 7.2 breath units when histamine challenge was performed after muscarinic stimulation (p<0.05) (Fig 2). In the asthmatic group, muscarinic receptor stimulation failed to change the PD₉₀ of histamine (Fig 2 and 3). Mean ± SE PD₉₀ of histamine on control day was 3.6 ± 2.5 breath units, which was not different from PD₉₀ of 4.1 ± 3.5 breath units, when histamine challenge was performed after muscarinic stimulation (p = NS) (Fig 2). The suppression of histamine-induced bronchoconstriction by prior muscarinic stimulation in normal subjects was unrelated to the dose of methacholine used; as prior exposure to a lower dose of methacholine (5 breath units vs 19 breath units) in five subjects caused an equivalent increase in PD₉₀ of histamine (Fig 4).

Prior methacholine inhalation did not cause any change in the bronchoconstrictor response to a second methacholine challenge in both normal subjects and asthmatic groups. In the normal group, mean ± SE PD₉₀ values of methacholine were 13.9 ± 2.8 breath units and 13.8 ± 2.4 breath units for the first and second methacholine challenges, respectively, (p = NS) (Fig 1 and Table 2). In the asthmatic group PD₉₀ values of methacholine were 4.9 ± 3.3 breath units and 2.5 ± 1.9 breath units for the first and second methacholine challenges, respectively, (p = NS) (Fig 3 and Table 2).

**Effect of Prior Histamine-Receptor Stimulation on Methacholine or Histamine-induced Bronchoconstriction**

Prior challenge with aerosolized histamine did not

<table>
<thead>
<tr>
<th>Table 2—Effects of Prior Histamine Stimulation (A) and Muscarinic Stimulation (B) on Bronchoconstrictor Responses to Methacholine in Normal (n=9) and Asthmatic (n=6) Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) Methacholine PD₉₀</strong></td>
</tr>
<tr>
<td>Methacholine (control)</td>
</tr>
<tr>
<td>Normal Subjects</td>
</tr>
<tr>
<td>Asthmatic Patients</td>
</tr>
<tr>
<td>Methacholine (1st Challenge)</td>
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<tr>
<td>Methacholine (2nd Challenge)</td>
</tr>
</tbody>
</table>

Data shown as mean ± SE cumulative provocative dose of methacholine in breath units which caused a 50% decrease in SGaw (PD₉₀).

Loss of Protective Muscarinic Receptor Mechanism in Asthma? (Ayala, Ahmed)
modify the bronchoconstrictor responses to methacholine in normal subjects or in asthmatic subjects. In the normal group, mean ± SE PD_{50} to methacholine was 16.9 ± 3.6 breath units on a control day and 14.6 ± 2.8 breath units after histamine stimulation (p = NS) (Fig 1 and Table 2). Similarly, PD_{50} values of methacholine in the asthmatic group were 4.3 ± 2.5 breath units and 4.1 ± 3.3 breath units without and with prior histamine exposure, respectively, (p = NS) (Fig 3 and Table 2).

Prior histamine inhalation did not cause a significant change in the bronchoconstrictor response to a second histamine challenge in either the normal or the asthmatic group; however, mild tachyphylaxis to histamine was observed in five normal and three asthmatic subjects. In the normal group, mean ± SE PD_{50} values of histamine were 16.7 ± 3.7 breath units and 19.9 ± 4.8 breath units for the first and second histamine challenges (p = NS) (Fig 1 and Table 3). In the asthmatic group, PD_{50} values of histamine were 3.4 ± 2.5 breath units and 4.7 ± 3.7 breath units for the first and second histamine challenges, respectively, (p = NS) (Fig 3 and Table 3).

**Effect of Ipratropium Bromide on Histamine-Induced Bronchoconstriction**

Pretreatment with inhaled ipratropium bromide (36μg) in five normal subjects shifted the histamine dose-response curve to the right and increased the PD_{50} of histamine (Fig 5). Mean ± SE values of histamine PD_{50} on control and post-ipratropium bromide days were 10.3 ± 1.2 and 28.3 ± 4.6 breath units, respectively (p<.05). This suggested that in normal subjects bronchoconstrictor effects of histamine, in part, are vagally mediated.

**DISCUSSION**

The results of this study demonstrate that prior stimulation of airway muscarinic receptors by inhaled methacholine causes a suppression of bronchoconstrictor response to histamine. Suppression of bronchoconstrictor response to histamine by prior muscarinic receptor stimulation was only observed in normals and not in subjects with bronchial asthma. Our study also demonstrates that suppression of histamine responses was specific and only observed after prior muscarinic receptor stimulation. This was demonstrated by the absence of any change in the bronchoconstrictor response to methacholine by prior exposure to methacholine or histamine.

Our in vitro results are consistent with the in vitro findings of Ishii and Kato who observed suppression of histamine-induced contraction of guinea pig ileum by prior exposure of the tissues to methacholine. They also observed that the suppressive action of methacholine on subsequent histamine-induced contraction was specific, as neither methacholine nor histamine exposure had any effect on subsequent contractile response to methacholine. Furthermore, prior histamine exposure, per se, failed to alter the subsequent contractile response to histamine. Thus, our in vitro findings in normal human airways further extend and confirm the in vitro findings of Ishii and Kato.

Our results are different from a recently reported study by Manning and O'Byrne in human subjects.
with bronchial asthma. Since they did not study normal subjects, we are able to compare the results of our asthmatic group only to those of Manning and O'Byrne. In our asthmatic subjects, prior inhalation of histamine or methacholine caused no suppression of subsequent bronchoconstrictor responses to either agonist. In contrast, Manning and O'Byrne observed a suppression of bronchoconstrictor responses to histamine and acetylcholine by prior inhalation of histamine, and not by prior inhalation of acetylcholine. The reason for these differences is not clear. These differences may be related to variance in the study population, or use of methacholine vs acetylcholine in our study. Although five normal and three asthmatic subjects showed mild tachyphylaxis to histamine, for each group there was no significant tachyphylaxis to histamine. Absence of significant histamine tachyphylaxis in our study may also be related to the fact that airway responses were evaluated by measurements of SGaw and not FEV1. Since histamine tachyphylaxis is a concentration-dependent event, it is possible that concentration of inhaled histamine required to change SGaw is different from that needed to change FEV1, and not enough to produce significant tachyphylaxis. It is also possible that histamine tachyphylaxis may only be exhibited in changes of FEV1 and not SGaw. However, these variances in methodology do not explain the differences between normal and asthmatic subjects observed in our study.

Our results demonstrate differences between normal and asthmatic subjects in terms of agonist-agonist interaction. Whereas in normal subjects prior muscarinic stimulation suppressed the histamine-induced bronchoconstriction, no such suppression was observed in subjects with asthma. It is possible that lack of suppressive action of methacholine on histamine-induced bronchoconstriction in asthmatic subjects may be related to two factors: (a) lower SGaw, and (b) lower PD20. It is unlikely that a lower baseline SGaw in asthmatic subjects would have altered the deposition of methacholine in such a way as to affect the subsequent airway responsiveness to histamine. Because of their sensitive airways, asthmatic subjects required a markedly lower PD20 of methacholine, resulting in a smaller total dose of methacholine deposited in the airways, which could be responsible for lack of suppressive action. In vitro data of Ishii and Kato do not support this concept, as methacholine could suppress the histamine-induced contraction of guinea pig ileum at a concentration as low as 10-4 M or lower, indicating that a high concentration of methacholine was not needed for the suppressive action. This is further supported by our observations in five normal subjects, in whom prior exposure to higher or lower doses of methacholine (19 vs 5 breath units) caused an equivalent suppression of subsequent histamine-induced bronchoconstriction (Fig 4). Thus, lack of suppressive effect of methacholine on subsequent histamine-induced bronchoconstriction in asthmatic subjects is probably related to the disease state.

The mechanism of the suppressive action of muscarinic stimulation on histamine-induced bronchoconstriction is unclear. It is unlikely to be related to heterologous desensitization, because prolonged treatment with methacholine was not required and methacholine specifically suppressed histamine effects and not those of methacholine. It is now widely accepted that muscarinic receptors can be further divided into at least three subtypes, M1, M2 and M3 receptors. Ishii and Kato observed in vitro that methacholine-induced suppression of contractile action of histamine was probably mediated by M3-muscarinic receptors, as pirenzepine, a M1-receptor antagonist, failed to modify the suppressive action of methacholine. This concept is further supported by recent findings of Minette and Barnes in human and guinea pig airway smooth muscle. Those investigators observed that the muscarinic agonist, pilocarpine, inhibited the contractile response to electrical field stimulation, which was reversed by prejunctional M2-receptor antagonist, gallamine, and not by the M1-receptor antagonist, pirenzepine. Based upon in vitro observations in human and guinea pig airway smooth muscle, existence of inhibitory muscarinic receptors ("auto-receptors") has been suggested in the parasympathetic nerves. Minette and Barnes also demonstrated that gallamine enhanced the contractile response to electrical field stimulation, thus confirming the functional existence of prejunctional inhibitory M2-receptors.

Contractile actions of histamine are mediated directly via stimulation of H1-histamine receptors on airway smooth muscle and, in part, indirectly via stimulation of vagal reflex causing release of acetylcholine at the parasympathetic nerve endings. Inhibition of histamine-induced bronchoconstriction by the anticholinergic agent, ipratropium bromide, in our normal subjects is consistent with this concept. Thus, it is possible that suppression of histamine-induced bronchoconstriction by prior methacholine exposure in normal subjects may be mediated via prejunctional inhibitory muscarinic receptors. It is interesting to note that muscarinic receptor blockade with ipratropium and muscarinic receptor stimulation with methacholine caused an equivalent suppression of histamine-induced bronchoconstriction. The inhibitory action of prior methacholine stimulation on histamine-induced bronchoconstriction was observed after the bronchoconstrictor action of methacholine had dissipated, suggesting that compared to bronchoconstrictor muscarinic receptors (possibly M1 and M3 subtypes) the duration of stimulation of inhibitory
muscarinic receptors (possibly M4-subtype) is of a much longer period.

Our results are consistent with the recent observations of Minette et al who studied the effects of prior muscarinic receptor stimulation with pilocarpine on subsequent bronchoconstrictor responses to SO2, an agent known to cause bronchoconstriction via cholinergic reflex. In normal subjects, pretreatment with pilocarpine and not histamine inhibited the SO2-induced bronchoconstriction, whereas in the asthmatic group pilocarpine failed to inhibit the bronchoconstrictor effects of SO2. Based upon their data, these authors also concluded the presence of feed-back inhibitory muscarinic receptors in normal airway and postulated a defect of these receptors in asthmatic airways.

The clinical significance of this finding is not clear. In normal subjects, it may represent a protective role of the cholinergic system at the level of inhibitory muscarinic receptors, which may modulate local regulation of airway smooth muscle tone. A lack of this suppressive action of muscarinic stimulation in asthmatic subjects may represent the absence of this protective mechanism, which leads us to hypothesize a loss of the inhibitory muscarinic receptor ("auto-receptor") mechanism in bronchial asthma.

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