Histamine receptors in normal human bronchi

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Summary

1. Nine normal subjects inhaled increasing concentrations of histamine aerosol from an aerosol generator attached to a breath-actuated dosimeter. The responses were monitored by measuring specific airways conductance in a body plethysmograph, and the results were expressed as cumulative log dose–response curves. On separate days, histamine challenges were repeated after intravenous injections of sodium chloride solution (placebo), or an H₁-receptor antagonist chlorpheniramine, or an H₂-receptor antagonist cimetidine, or H₁- and H₂-receptor antagonists together. The anticholinergic activity of chlorpheniramine was estimated by comparing the effect of chlorpheniramine and atropine on methacholine challenge.

2. In all subjects the response to histamine was reproducible. Analysis of the variance showed that placebo did not alter the histamine dose–response curve significantly. In contrast, chlorpheniramine produced a large shift in the histamine dose–response curve to the right and cimetidine produced a significant shift of this curve to the right only at the highest dose of histamine. A combination of cimetidine and chlorpheniramine produced a shift not significantly different from that seen with chlorpheniramine alone. Chlorpheniramine showed no significant anticholinergic activity in this study.

3. In the normal subjects histamine-induced bronchoconstriction appeared to be mediated predominantly by the H₁-receptors. The H₂-receptor contributed very little to this bronchoconstriction.

Key words: bronchi, dose–response curve, histamine, H₁-receptor antagonist, H₂-receptor antagonist.

Introduction

In 1929, Weiss, Robb & Blumgart found that histamine was a powerful bronchoconstrictor in asthmatic subjects. Their findings were extended by Curry (1946) and Herxheimer (1951), both of whom advocated the use of existing antihistamines for the treatment of asthma. In contrast, others, including Karlin (1972), concluded that these drugs were of little therapeutic value.

The concept of two distinct populations of histamine receptors, the H₁ and H₂-receptors, was first proposed by Ash & Schild (1966), who suggested that receptors antagonized by conventional antihistamines (e.g. mepyramine) should be designated H₁-receptors. The synthesis of burimamide, by Black, Duncan, Durant, Ganellin & Parsons (1972), and the subsequent development of metiamide and cimetidine has recently allowed definition of the histamine H₂-receptor.

Maegwyne-Davis (1968), Eyre (1969), Krell & Chakrin (1977), Chand & Eyre (1977) and Okpako, Chand & Eyre (1978) have identified H₁ and H₂-receptors in the tracheobronchial tree of the cat, sheep, guinea pig, dog and horse. Dunlop & Smith (1977) have suggested, as a result of studies in vitro, that both types of receptors are present in bronchial smooth muscle in man.

The present study was designed to determine whether both types of receptors could be demonstrated in the normal human bronchus in vivo, and whether, if present, H₂-receptors mediated
bronchodilatation or bronchoconstriction. The effect of both the H₁-receptor antagonist chlorpheniramine and the H₂-receptor antagonist cimetidine on bronchial challenge with histamine, has been studied in non-asthmatic subjects. The results of these challenges were expressed as dose–response curves.

Method

Nine normal subjects (Table 1) were studied. They were all laboratory workers who gave informed consent for these investigations. None had respiratory infections at the time of the study, nor during the preceding month. The response of the airways to the inhaled histamine was monitored by serial measurements of specific airway conductance (sGaw), measured in a constant-volume body plethysmograph by the method of Dubois, Botelho & Comroe (1956). An extra port was constructed in the front of the plethysmograph through which the challenge aerosol was inhaled (Fig. 1). A breath-activated ‘dosimeter’ (Rosenthal, Norman, Summer & Permutt, 1977) delivered a standardized quantity of aerosol to the subject. As the subject breathed in the change in mouth pressure triggered the dosimeter and regulated air flow at a pressure of 23 kg/cm² for 0.6 s, through a solenoid valve to a Hudson nebulizer; the nozzle of the nebulizer was directed through the port and was 36 cm from the mouth. The subject was asked to inhale the aerosol to a similar depth, near to vital capacity, and at the same rate during each challenge.

Histamine dose–response challenges were performed as follows: after four measurements of sGaw had been made the subject inhaled histamine acid phosphate (five breaths, each of 2 g/l), 2 min later, sGaw was measured three to four times. Every 2½–3 min the subject inhaled double the previous doses of histamine, up to a final concentration of 64 g/l. The nebulizer was washed and dried between each dose. The duration of the challenge was between 15 and 20 min. Any subject wheezy at the end of the challenge was given salbutamol aerosol (100 µg).

On separate days the histamine challenges were repeated, either after no premedication or 10 min after intravenous injections of sodium chloride solution (150 mmol/l: saline placebo), chlorpheniramine (20 mg), cimetidine (200 mg), cimetidine (400 mg) or chlorpheniramine (20 mg) with cimetidine (200 mg). Each subject had seven histamine challenges in random order. This was a single blind study, i.e. the subjects were ignorant of which compound was being injected intravenously.

The plan of the study was approved by the Charing Cross Hospital Ethical Committee.

H₁-receptor antagonists are known to have some anticholinergic activity (Wilson & Schild, 1968). The extent of this activity on the bronchi was estimated by comparing the effect of 20 mg of chlorpheniramine given intravenously with that of 1.5 mg of atropine methonitrate aerosol on a methacholine challenge in all subjects. Methacholine challenges were performed in duplicate in all subjects and then repeated 10 min after chlorpheniramine and 30 min after atropine on separate days. Methacholine challenges were performed in a similar manner to the histamine challenges, with serial dilutions of methacholine between 1 and 100 g/l. However, as there was

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Smoker</th>
<th>sGaw (s⁻¹ kPa⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>28</td>
<td>No</td>
<td>1.33 ± 0.07</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>20</td>
<td>No</td>
<td>2.22 ± 0.19</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>24</td>
<td>No</td>
<td>2.47 ± 0.31</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>19</td>
<td>Yes</td>
<td>1.51 ± 0.23</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>32</td>
<td>Yes</td>
<td>1.69 ± 0.39</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>29</td>
<td>No</td>
<td>1.39 ± 0.21</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>29</td>
<td>No</td>
<td>1.50 ± 0.31</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>31</td>
<td>No</td>
<td>1.51 ± 0.14</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>31</td>
<td>No</td>
<td>1.92 ± 0.18</td>
</tr>
</tbody>
</table>
greater inter-subject variation in response to methacholine than histamine, a trial challenge was performed to find a more suitable starting dose for each subject. Every 3 min a further dose, double the previous concentration, was given until sGaw fell to about 50% of the baseline value.

**Aerosol characteristics**

The extra port of the plethysmograph represented a relatively large dead-space separating the nebulizer outside and the subjects' mouth inside the plethysmograph. In order to establish how much aerosol was lost within the port during inhalation, and whether that loss was constant, the nebulizer containing water was triggered 50, 100 and 250 times during simulated quiet breathing. The aerosol was absorbed by anhydrous calcium chloride in a receptacle at the mouthpiece. Absorption of aerosol within the port was negligible (Fig. 2a). The frequency-distribution of the aerosol-droplet size was determined by measuring the craters made by the aerosol on a slide coated with magnesium oxide held at the mouthpiece (Sellick & Widdicombe, 1971; Jain, 1975). Of droplets counted 89% were between 6 and 15 μm in diameter, with a peak at 8 μm (Fig. 2b).

**Data analysis and statistical validation**

The response to histamine challenge was expressed as a dose–response curve. Preliminary studies had shown that the maximum effect of a single dose of histamine occurred after 1 min and was maintained for 20 min. Since the duration of the challenge was 15–20 min, cumulative dose–response curves were drawn. The responses to both histamine and methacholine challenge were expressed in absolute values of sGaw. In addition the response to histamine was expressed as a percentage of baseline since there was relatively little variation in baseline sGaw between subjects. Analysis of variance was used to compare the effects of the antihistamines on subsequent challenges.

Each value of sGaw in this study was the mean of four measurements. For each value the SD and the coefficient of variation were calculated. As an expression of reproducibility of these measurements in the two unpremedicated histamine challenges, the mean and the ranges of these SD values and coefficients of variation for all nine subjects were obtained in the resting state and after 300 and 1260 μg of histamine (Table 2). The intra-subject

![Graph](image)

**Fig. 2.** (a) Frequency distribution of particle size as the aerosol emerged at the mouth. (b) Relationship between number of nebulizer puffs (x axis) and weight (g) (y axis) of either the nebulizer (●) or the calcium chloride receptacle (□).

**Table 2. Reproducibility of the measurement of sGaw during the two unpremedicated histamine challenges in nine normal subjects**

<table>
<thead>
<tr>
<th></th>
<th>Mean sGaw (s⁻¹kPa⁻¹)</th>
<th>Range of SD of measurements (s⁻¹kPa⁻¹)</th>
<th>Mean SD of measurements (s⁻¹kPa⁻¹)</th>
<th>Range of coefficients of variation (%)</th>
<th>Mean coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting value</td>
<td>1.83</td>
<td>0.08-0.30</td>
<td>0.16</td>
<td>5-16</td>
<td>9</td>
</tr>
<tr>
<td>300 μg of histamine</td>
<td>1.42</td>
<td>0.02-0.21</td>
<td>0.08</td>
<td>0-12</td>
<td>6</td>
</tr>
<tr>
<td>1260 μg of histamine</td>
<td>0.74</td>
<td>0-0.15</td>
<td>0.05</td>
<td>1-11</td>
<td>6</td>
</tr>
</tbody>
</table>
reproducibility of the unpremedicated histamine challenges was determined by calculating a correlation coefficient and a paired t-test in each subject for results from the responses to the two challenges with the seven doses of histamine (0–1260 μg) (Table 3). Mean curves were derived from the data in all nine subjects to give overall group dose–response curves for each challenge. The two group curves were also compared by using a correlation coefficient and a paired t-test.

There was considerably more inter-subject variation in response to methacholine than to histamine. In order to use the method of analysis of variance to compare the effect of the blocking drugs on the methacholine response, the effective dose was normalized in the following way. From each subject’s mean methacholine dose–response curve, the dose of methacholine needed to reduce baseline sGaw by 20% was found and designated ‘cumulative unit 1’. At multiples of 2 and 4 of this cumulative unit, sGaw was read off the mean methacholine curve. sGaw was then read off the methacholine challenges premedicated with chlorpheniramine and atropine at cumulative units 1, 2 and 4. This process was repeated for each subject. Since the derived units were independent of the dose given, inter-subject comparison was possible.

Results

Reproducibility

The reproducibility of the measurements of sGaw is shown in Table 2. The overall coefficient of variation, the mean of the three shown, was 7%. Table 3 shows the reproducibility of the two histamine challenges. With the exception of subject no. 4, there was a highly significant correlation between the two unpremedicated histamine challenges, showing that the shapes of the two challenge curves were similar. The correlation of the mean dose–response curves was also high. The results of the paired t-tests indicated that there was no significant difference between the two mean histamine responses, but the individual subject’s t-tests showed that subjects nos. 6 and 9 had a difference in response to the two challenges. The significant correlations in these subjects, however, indicated that the shapes of the challenge curves remained similar despite this shift.

Baseline sGaw and the effect of antihistamines

The mean baseline sGaw values for all challenges in each subject are given in Table 1. Though there was relatively little intra-subject variability the inter-subject variation was large; in these subjects mean baseline sGaw values ranged between 1-33 and 2-47 s⁻¹ kPa⁻¹. Neither the antihistamines nor the saline placebo injection produced significant changes in the mean baseline sGaw values (Fig. 3a).

Effect of antihistamines on histamine response

The mean results for all nine subjects are shown in Fig. 3 with cumulative dose of histamine (μg) plotted on log scale against sGaw (s⁻¹ kPa⁻¹) in Fig. 3(a) and as a percentage of baseline sGaw value in Fig. 3(b). As seen in Fig. 3 the mean histamine dose–response curve in the normal subjects had a threshold at about 140 μg of

### Table 3. Reproducibility of two histamine challenges in nine normal subjects

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>r</th>
<th>P_r</th>
<th>t</th>
<th>P_t</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.86</td>
<td>&lt;0.01</td>
<td>0.3523</td>
<td>N.S.</td>
</tr>
<tr>
<td>2</td>
<td>0.99</td>
<td>&lt;0.001</td>
<td>-0.4766</td>
<td>N.S.</td>
</tr>
<tr>
<td>3</td>
<td>0.85</td>
<td>&lt;0.01</td>
<td>0.6579</td>
<td>N.S.</td>
</tr>
<tr>
<td>4</td>
<td>0.56</td>
<td>N.S.</td>
<td>-1.896</td>
<td>N.S.</td>
</tr>
<tr>
<td>5</td>
<td>0.86</td>
<td>&lt;0.01</td>
<td>1.0378</td>
<td>N.S.</td>
</tr>
<tr>
<td>6</td>
<td>0.85</td>
<td>&lt;0.01</td>
<td>3.6300</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>7</td>
<td>0.84</td>
<td>&lt;0.01</td>
<td>-1.0229</td>
<td>N.S.</td>
</tr>
<tr>
<td>8</td>
<td>0.89</td>
<td>&lt;0.01</td>
<td>-0.6369</td>
<td>N.S.</td>
</tr>
<tr>
<td>9</td>
<td>0.99</td>
<td>&lt;0.001</td>
<td>4.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean curve</td>
<td>0.99</td>
<td>&lt;0.001</td>
<td>1.253</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Histamine receptors in normal bronchi

Histamine challenge with no premedication; ▲——▲, challenge after saline placebo; ●——●, challenge after cimetidine (200 mg); ●——○, challenge after cimetidine (400 mg); ○——○, challenge after chlorpheniramine (20 mg); ○——○, challenge after chlorpheniramine (20 mg) with cimetidine (200 mg). The least significant differences (vertical bars) at the 5 and 1% levels were calculated from an analysis of variance.

Fig. 3. Mean histamine dose–response curves in all nine normal subjects. Cumulative dose of histamine is plotted on a log scale vs sGaw (s⁻¹ kPa⁻¹) in (a) and vs sGaw expressed as percentage of base line (b).

Some subjects felt wheezy and dyspnoeic after the final dose of histamine. From an analysis of variance the least significant difference at the $P = 0.05$ and $P = 0.01$ level was found for the mean of results calculated in absolute and percentage values. Differences greater than these indicated a significant shift of the dose–response curve. Saline placebo did not significantly alter the histamine dose–response curve. There was a very small shift to the right with cimetidine (200 and 400 mg), but this attained statistical significance ($P < 0.05$, Fig. 3a; $P < 0.01$, Fig. 3b) with cimetidine (400 mg) only at the highest dose of histamine. However, chlorpheniramine, both alone and in combination with cimetidine, produced much larger shifts to the right of the histamine dose–response curve. This was statistically significant at doses of histamine as low as 300 μg. The addition of cimetidine did not appear to alter the effect of chlorpheniramine on the histamine response. The same pattern of response was seen when the dose–response curves were plotted with sGaw in absolute units, or as a percentage of the baseline value.

Anticholinergic effects of chlorpheniramine

During the methacholine studies (Fig. 4), premedication with atropine raised the baseline sGaw value significantly by a mean of 44% and completely abolished the response to methacholine. Chlorpheniramine produced a smaller (22%) but statistically significant rise in baseline sGaw value. However, chlorpheniramine appeared to have no effect on the methacholine dose–response curve; the separation of the two curves after cumulative units 1, 2 and 4 was smaller than the separation of the baseline values of sGaw.

Side-effects of antihistamines

Chlorpheniramine (20 mg) produced varying degrees of drowsiness in all subjects. No side-effects were seen after the injection of cimetidine (200 mg). However, cimetidine (400 mg) and chlorpheniramine (20 mg) with cimetidine (200 mg) caused a transient metallic taste in the mouth of some subjects.
Discussion

Methodology

In this study a quantified reproducible dose of histamine aerosol was given by connecting the dosimeter to a Hudson nebulizer, following the principles for bronchial inhalation-challenge procedures (Chai, Farr, Frochlich, Mathison, McLean, Rosenthal, Sheffer, Spector & Townley, 1975). The particles from the nebulizer were of a suitable size to reach the smaller airways. sGaw was chosen as a measure of airways obstruction because it is effort-independent and does not require full inspiration, which may alter airways calibre (Nadel & Tierney, 1961; Orehek, Gayrard, Grimaud & Charpin, 1975). sGaw is sensitive to small changes in large airways calibre (Lloyd & Wright, 1963; Cohen & Hale, 1965). We found that the measurements of sGaw were reproducible.

Since large doses of chlorpheniramine caused drowsiness in subjects, it was thought unethical for the investigator to be ignorant of the nature of the drugs received by the subjects; the study was undertaken, therefore, in a single-blind fashion.

Dose–response curve

The response to histamine challenge was expressed as a dose–response curve, rather than a single arbitrary end point, since it not only characterizes the effect of the drug more completely (Goldstein, Aronow & Kalman, 1968) but also enables comparisons to be made between responses (Orehek et al., 1975). The use of single dose–response relationships in comparing results is valid only if the dose–response curves are parallel. A parallel shift to the right represents the extent of competitive antagonism. Dose–response curves obtained from strips of muscle in vitro have a threshold, slope and maximum response. In this study in vivo, the shape of the histamine dose–response curve was similar, except that it was impossible to increase the histamine dose sufficiently to reach a maximal response.

At the doses used, chlorpheniramine either alone or with cimetidine produced complete blockade of the histamine response in most subjects and so there was no final slope to the mean histamine response in the presence of chlorpheniramine; this fact precluded distinguishing differences in effect of chlorpheniramine, with or without cimetidine. In order to do this, a final slope of the histamine dose–response curve could have been obtained by either giving much larger doses of histamine or much smaller doses of chlorpheniramine.

Inter-subject comparison is difficult when there is a wide variation of responsiveness. In previous studies, where single dose–response relationships were examined, a ‘provocation dose’ of challenge aerosol, resulting in a predetermined bronchoconstrictor response, was used to make such comparisons. Thus Rosenthal et al. (1977) compared in different subjects the cumulative dose of antigen that produced a 35% fall in sGaw (PD₃₅). In the present methacholine-challenge study, wide variations in sensitivity were found, and a similar technique was used on the whole curve to ‘normalize’ the effective dose.

Statistical analysis

Since the curves were not parallel, it was not possible to calculate the dose-ratios (the horizontal separation of the slopes of the mean histamine curves). Instead analysis of variance was chosen for this study because of its statistical efficiency in dealing simultaneously with both intra- and inter-subject variability (Armitage, 1971).

In this study the small number of subjects, and the relatively large inter-subject variation in baseline, gave large values for the least-significant difference when the analysis of variance was applied to the mean data in absolute values of sGaw. Nevertheless, the blocking action of 400 mg of cimetidine was still statistically significant at the 5% level at the highest dose of histamine. Since the intra-subject variability in baseline was small the data were also calculated in percentage terms to allow for the large inter-subject variation of baseline sGaw; when this was done the effect of 400 mg of cimetidine became statistically significant at the 1% level.

Dosage and other effects of antihistamines

The dose of chlorpheniramine (20 mg) was chosen since it produced no further effect on the histamine dose–response curve than did 10 mg and so was judged to have blocked the H₁-receptors maximally. Burland, Duncan, Hesselbo, Mills, Sharp, Haggie & Wyllie (1975) have demonstrated 75% inhibition of stimulated gastric secretion, with an intravenous infusion of cimetidine (100 mg/h), but it was not known what bolus dose was needed to antagonize fully any H₂-receptors present in the airways. In this study it appeared that 200 mg of cimetidine did not completely block the bronchial H₂-receptor, since 400 mg had a larger effect on the histamine dose–response curve.

Chlorpheniramine produced no significant change in mean baseline sGaw value before the
histamine challenges but produced a significant rise in this value (mean 20%) before methacholine challenges. In general, the higher the initial baseline sGaw the less was the rise caused by chlorpheniramine. Four subjects had higher pre-chlorpheniramine baseline sGaw values in the histamine-challenge studies than in the methacholine-challenge studies, and this may have contributed to the negligible mean effect of chlorpheniramine on the baseline in the histamine studies. Thus in these four normal subjects chlorpheniramine had no consistent effect on resting bronchial tone. This agrees with the results of Maconochie, Woodings & Richards (1979). In asthmatic subjects there are conflicting reports on the effects of HI-receptor antagonists on bronchial constriction, but that there was no correlation between this and their antihistamine activity. Conversely, bronchodilatation was caused in asthmatic subjects given chlorpheniramine (Popa, 1977), diphenhydramine (Casterline & Evans, 1977) or clemastine (Nogrady, Hartley, Handslip & Hurst, 1978), although Partridge & Saunders (1979) observed no effect with clemastine.

The transient metallic taste in the mouth of some subjects caused by chlorpheniramine and cimetidine together and by the larger dose of the H2-receptor-antagonist cimetidine (400 mg), may indicate the release of some endogenous histamine, since this taste has been described during histamine infusions (Lorenz & Doenicke, 1978). Thermann, Lorenz, Schmal, Schingale, Dormann & Hamelmann (1977) have described endogenous histamine release after administration of H2- and H1-receptor antagonists in dogs. Nevertheless, this effect must have been trivial in our normal subjects since, after injections of antagonists together, sGaw rose in six subjects, was unchanged in two and fell by only 9% in one subject.

The role of the parasympathetic nervous system in the bronchial response to histamine is disputed (Colebatch, Olsen & Nadel, 1966; Sellick & Widdicombe, 1971; Casterline, Evans & Ward, 1976). Results of a study in dogs (Sampson & Vidruk, 1979) suggest that the stimulation of airway rapidly adapting afferent vagal receptors by histamine is mediated by H1-receptors. With our methacholine challenges we have demonstrated that chlorpheniramine produced no significant anticholinergic effects in our subjects. The effect of chlorpheniramine on the histamine response was thus by H1-receptor antagonism. Since this effect was so marked, our results also imply that the H1-receptor is likely to be of primary importance in the histamine response.

The evidence from this study suggests that there may be H1-receptors in normal human airways but that the effect of the H1-receptor is predominant. Both types of histamine receptors appear to mediate bronchoconstriction. Casterline & Evans (1977) have also demonstrated the presence of H1-bronchoconstrictor receptors in asthmatic bronchi in vivo, but did not look for evidence of H2-receptors. However, although the studies in vitro of Dunlop & Smith (1977) on sensitized human bronchi showed both types of histamine receptor in man, their results suggested that H1-receptors were bronchoconstrictors and H2-receptors bronchodilators. Further studies should be undertaken to investigate the status of histamine receptors in asthmatic subjects and their role in antigen challenge.

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References


