Comparison of responses to methacholine and cold air in patients suspected of having asthma

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In 140 adult patients with FEV₁ ≥ 60 percent predicted referred because of suspected asthma, we compared responses to methacholine and isocapnic cold-air hyperventilation. Most challenges were accomplished on the same day, cold air always being done first. The cold-air test employed a single episode of hyperventilation (target, 30 times the FEV₁,) and subsequent FEV₁ changes were noted, a decrease of 10 percent being defined as a positive test result. For methacholine, subjects inhaled aerosols of increasing concentrations and the dose associated with a 20 percent decline at concentrations ≥ 8 mg/ml. Of the 140 patients, 65 had negative results on both challenges. Twelve patients had positive results on cold-air testing but negative responses to methacholine, and 17 had the opposite result. Among patients with positive results to either test, there was a significant correlation (p < 0.001) between change in FEV₁ with cold air and log PC₉₀, but there was considerable scatter, the results of one test accounting for 25 percent of the variability in the other. Some scatter may have been related to the methods we used, but much was probably due to the patients themselves. Neither test should be used on an exclusive basis to make the diagnosis of asthma. 

*Methacholine and Cold Air in Patients Suspected of Having Asthma*

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In recent years bronchoprovocation tests have become well established in making the diagnosis of asthma.1,2 The most commonly used tests involve the inhalation of aerosols of bronchoconstrictor agents such as methacholine and histamine, but isocapnic cold-air hyperventilation has been suggested as a more physiologic test, since it appears to operate by mechanisms similar to exercise-induced asthma.3 In known asthmatic patients and normal subjects, results of methacholine and cold-air testing have been shown to correlate well.4-7 To our knowledge, methods of bronchoprovocation have not been compared in patients in whom the diagnosis of asthma is suspected but not established, although it is in this setting that the tests are thought to be of greatest value. We therefore sought to make such a comparison.

**MATERIAL AND METHODS**

Patients who were referred to our laboratories to establish or rule out a diagnosis of asthma were studied, provided they had an FEV₁ ≥ 60 percent predicted normal6 and were at least 17 years old and gave informed consent.

Methacholine testing was carried out using the method of Hargreave et al.1 Control and methacholine aerosols were generated by a Wright nebulizer with a gas flow rate of 10 L/min, so that 0.13 ml/min of solution was nebulized. Aerosols were inhaled from the nebulizer via face mask during 2 min of tidal breathing. The aerosols used were a control composed of diluent, and then successively increasing concentrations of methacholine: 0.03, 0.125, 0.5, 2.0, 4.0, 8.0, and 16.0 mg/ml. Using a waterless spirometer that met ATS specifications (model 822, Sensor Medics), the FEV₁ was measured three times before testing and again 0.5 and 1.5 min after exposure to each aerosol. Postexposure values of FEV₁ were compared with the largest preexposure FEV₁. Aerosol administration was discontinued when the FEV₁ fell by 20 percent or more compared with the baseline value. Change in FEV₁ (% FEV₁) was plotted against the log of methacholine concentration, and the methacholine dose associated with a 20 percent change in FEV₁ (PC₉₀) was calculated by linear interpolation between the last two points. A positive methacholine response was defined as PC₉₀ ≥ 8 mg/ml.

Cold-air challenges were done as previously reported from this laboratory,7 using methods developed by Deal et al. A mixture of compressed air and 5 percent CO₂, which was dry, was passed over a cooling coil and supplied to a Hans Rudolph breathing valve. Inspired volume was measured with a dry gas meter and the expired side of the valve sampled for CO₂. End-tidal CO₂ was maintained constant by adding CO₂ to the inspirate when necessary. Inspired gas temperature was −17° to −20°C. After three baseline measurements of FEV₁, the patients hyperventilated cold air for 6 min. The target ventilation was 30 times the FEV₁. The FEV₁ was measured 1, 3, 5, 7, and 9 min after hyperventilation and compared.
These 12 patients did not differ significantly from the after any cold-air-induced changes in FEV1 had returned to control. The decrease was greater than 20 percent.

\[ \text{tMBC} = \text{FEV1} \times 35. \]

In a few subjects the cold-air response was large enough to merit bronchodilators for 6 challenges were carried out at least 30 minutes after the cold-air test and after any cold-air-induced changes in FEV1 had returned to control.

In most patients both challenges were accomplished on the same day, the cold-air challenge always being first. Methacholine challenges were carried out at least 30 min after the cold-air test and after any cold-air-induced changes in FEV1 had returned to control. In a few subjects the cold-air response was large enough to merit bronchodilator therapy, and in these subjects methacholine testing was carried out on a subsequent day. No testing was done unless the subject reported had not taken theophylline for 12 h or inhaled bronchodilators for 6 h.

Statistical analysis was done to determine significance, using unpaired t tests and correlation coefficients.

**RESULTS**

A total of 140 adults with FEV1 \( \geq 60 \) percent predicted were studied (Table 1). Of these, 77 had PC\(_{20} \) >8 mg/ml, i.e., had a negative methacholine challenge, while the remaining 63 demonstrated PC\(_{20} \) \( \leq 8 \) mg/ml. Baseline lung function test results did not differ between the two groups, nor did sex distribution, but those with positive responses were slightly and significantly younger than those with negative responses. Of the patients with negative methacholine responses, 12 responded to cold-air hyperventilation with decreases in FEV1 that exceeded 10 percent, and in four patients the decrease was greater than 20 percent. These 12 patients did not differ significantly from the others in terms of age, sex, or baseline lung function values. Further, the level of ventilation achieved during cold-air testing did not differ significantly (Table 2), whether expressed in absolute terms or as a fraction of predicted MBC, calculated as FEV1 \( \times 35. \)

Of patients with positive methacholine responses, 17 had negative cold-air responses, 20 showed a decrement in FEV1 from 10 to 19 percent with cold air, and 26 showed decreases of FEV1 that exceeded 20 percent (Table 2). These subgroups did not differ significantly in terms of age, sex, or baseline FEV1, and also did not differ significantly in terms of the ventilation achieved during cold-air testing, although there was a tendency for those with the greatest cold-air responses to have achieved the greatest levels of ventilation.

Among patients who responded to methacholine, PC\(_{20} \) differed according to cold-air sensitivity. Patients with negative cold-air tests had a mean PC\(_{20} \) of 4.76 mg/ml (SEM = 0.48), those with cold-air responses of 10 to 19 percent of control FEV1 had a mean PC\(_{20} \) of 3.45 mg/ml (SEM = 0.50), and those with cold-air responses of at least 20 percent of baseline FEV1 had a mean PC\(_{20} \) of 1.55 mg/ml (SEM = 0.29). The last figure was significantly different (p < 0.05, unpaired t test) from either of the other two, which were not significantly different. There was a significant correlation between log PC\(_{20} \) and change in FEV1 after

<table>
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<tr>
<th>Dose</th>
<th>No.</th>
<th>% Female</th>
<th>Age, yr</th>
<th>FEV1, % Pred</th>
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<tr>
<td>PC(_{20} ) &gt; 8 mg/ml</td>
<td>17</td>
<td>38.1</td>
<td>96.4</td>
<td>60.3</td>
</tr>
<tr>
<td>Cold &gt; 20%</td>
<td>26</td>
<td>34.6</td>
<td>97.3</td>
<td>64.7</td>
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<tr>
<td>Cold 10%-20%</td>
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<td>39.6</td>
<td>102.3</td>
<td>57.5</td>
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<td>Cold &lt; 10%</td>
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<td>43.8</td>
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<tr>
<td>Cold ( \leq 8 ) mg/ml</td>
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<td>41</td>
<td>37.3</td>
<td>96.7</td>
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<table>
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<th>N</th>
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<td>PC(_{20} ) &gt; 8 mg/ml</td>
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<td>47</td>
<td>42.2**</td>
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<tr>
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<td>42.0</td>
<td>112.4</td>
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<td>26</td>
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<tr>
<td>Cold &lt; 10%</td>
<td>12</td>
<td>43.8</td>
<td>101.6</td>
</tr>
</tbody>
</table>

*Mean values and (SD) are shown.
†MBC = FEV1 \( \times 35. \)

Fig 1. Relationship of methacholine sensitivity to cold-air sensitivity. Ordinate, methacholine PC\(_{20} \) on a logarithmic scale; abscissa, change in FEV1, noted with cold-air challenge. Each point represents a single patient; closed circles, those with PC\(_{20} \) \( \leq 8 \) mg/ml; open circles, those with PC\(_{20} \) >8 mg/ml who have been arbitrarily assigned PC\(_{20} \) = 10 mg/ml.
cold-air testing ($r = -0.53, p<0.001$) among methacholine-positive patients (Fig 1). The relationship remained significant when methacholine-negative, cold-air-positive patients were added by arbitrarily assigning them a $PC_{20}$ of 10 mg/ml (Fig 1, $r = -0.48, p<0.001$).

**Discussion**

The patients we studied were referred to our laboratory to help establish the diagnosis of asthma. Nearly all were referred by the eight pulmonary specialists in our university group for symptoms such as cough and dyspnea, but there was no prospective agreement as to specific indications for inhalation challenge, nor were clinical data such as symptoms and signs collected in a systematic way. Therefore, the fact that 65 patients, about 46 percent of these tested, had negative results on both provocation tests cannot be interpreted beyond stating that there is a relatively high level of suspicion of airways hyperreactivity among physicians in our clinic. It should be noted, however, that our results were very similar to those of Adelroth, et al in that both studies found that 45 percent of patients referred because of suspected asthma had positive results on methacholine challenge. This agreement suggests similar levels of suspicion regarding the diagnosis of asthma in the two clinics. Because we did not collect clinical data in a systematic way, we cannot compare these data with the results of bronchoprovocation tests. Our aim was to compare results of the different provocation tests.

Previous studies have compared responses to cold-air hyperventilation and methacholine challenge in asthma, and most found a distinctly better correlation than we did. Our study differed in several ways. First, it was larger. Second, it examined patients suspected of having asthma as opposed to established asthmatic and normal subjects. Third, our test protocol differed from those employed by most others; we did both tests on the same day in most patients, and we employed only one level of ventilation in our cold-air testing, not constructing dose-response curves by having our subjects breathe cold air at successively increasing levels of ventilation. We will consider these discrepancies, with particular emphasis on whether differences in patient selection and test protocol contributed to the scatter of results.

Of previous studies comparing cold-air and methacholine challenges, three noted age and sex distributions. In two, 40 to 50 percent of the patients were female, as in our study; in the other, 11/13 were female. Our patients were, on average, older than those examined by other workers because we studied a substantial number of middle-aged and elderly patients. Because our lower age limit was 17 years, the large SD of Tables 1 and 2 reflect the inclusion of older patients. Although our methacholine-positive patients were significantly younger than those with $PC_{20} \geq 8$ mg/L, we doubt that this played a major role in our results. There were no significant cold response-related age differences in either group (Table 2), and many older patients had positive responses. Indeed, our oldest patient, a woman of 83, had positive results on both challenges.

Because we examined patients suspected of having asthma, as opposed to those with established asthma, our patients were in general less sensitive to methacholine than those of other comparative studies, which was the case when only patients with positive responses were considered. In all previous studies more than 50 percent of responsive subjects had $PC_{20} < 1.0$ mg/ml, and less than a quarter had $PC_{20}$ of 4 to 8 mg/ml. In our series 17 of 63 methacholine responders had $PC_{20} < 1.0$ mg/ml and 24 had $PC_{20} \geq 4$ mg/ml. Our distribution of methacholine response was similar to that of Adelroth et al, who also studied patients with suspected as opposed to established asthma. The relative insensitivity of our subjects to methacholine did not account for the relatively poor correlation with cold-air challenge, however, since the scatter in the data did not vary with the level of $PC_{20}$. Although the mean response to cold air increased as $PC_{20}$ decreased, the variance was wide at all levels (Fig 1).

For practical reasons, we usually did both cold and methacholine challenges on the same day, the cold air being first. Only when cold-air hyperventilation produced substantial symptomatic bronchoconstriction was the subsequent methacholine challenge done on another day. This raises the question of whether the cold-air challenge influenced the results of the methacholine test. There are contradictory data in the literature regarding the presence of a refractory period after cold-air hyperventilation. Some authors found that successive same-day cold-air challenges are reproducible in asthmatic patients, while others found that this is not the case, the second response being smaller than the first, and they postulated that this is due to relative failure of mediator release with the second challenge. There is much evidence, however, that bronchoconstriction after cold-air hyperventilation is not due to release of mediators. Further, methacholine-induced bronchoconstriction is not thought to be due to endogenous mediators. Finally, studies showing refractoriness after cold air challenge have all used repeated cold-air exposure as the second stimulus. The one study we are aware of that studied responses to methacholine before and after cold-air testing found that they were unaffected. Thus, we believe that it is unlikely that the methacholine responses we observed were altered by the previous cold air exposure.

Our cold-air challenge consisted of a single episode...
of hyperventilation conducted at the highest level of ventilation that the patient could attain. Although one previous study compared this kind of test with methacholine challenge and found close correspondence between the two, most comparative experiments have constructed cold-air dose-response curves by having subjects maintain successively higher levels of ventilation and measuring changes in FEV₁ after each. This approach yields data directly comparable to those with FEY₁ <60 percent predicted, with the result that our patients had, on average, normal FEV₁ (Tables 1 and 2). The initial FEV₁ did not differ between patients with positive and negative methacholine challenges, and in those with PC₂₀ ≤8 mg/ml, the initial FEV₁ did not correlate with PC₂₀. Thus, while our patient group may have included some patients with chronic bronchitis, they did not appear to influence the main trends of our results.

We therefore believe that in patients thought to have asthma, although responses to cold air and methacholine are related, the relationship is by no means perfect, response to one agent explaining something on the order of 25 percent of the variation in response to the other. These results are not entirely unprecedented. In their studies of established asthmatic patients, both Weiss et al and Tessier et al found correlations between methacholine and cold-air challenges that were only slightly better than ours, although their cold-air challenges used the more elaborate dose-response technique, and their results were plotted in a log-log format. Response to one agent is not equivalent to response to the other. This is particularly evident when one considers that in our series 17 patients who had positive methacholine responses had negative cold-air testing, and 12 who had PC₂₀ >8 mg/ml had positive cold-air tests. While it could be argued that in the former group cold-air sensitivity was missed because these patients did not achieve adequate levels of ventilation during the cold-air test, the ventilation that they attained did not differ from those who had positive cold-air tests (Table 2).

Among patients with PC₂₀ <8 mg/ml, we observed negative cold-air responses in one subject who achieved a ventilation of 118 L/min during the test and another who exceeded her predicted MBC. Inadequacy of the ventilatory challenge, on the other hand, may have resulted in underestimation of methacholine-negative, cold-air-positive patients, one of whom showed a 36 percent decline in FEV₁ after a test in which the ventilation was 40 L/min. Patients who had positive cold-air responses and negative methacholine responses did not in general show borderline methacholine responses. Only 4/12 of these patients had PC₂₀ of <16 mg/ml, and only one of these had a cold-air response of greater than 20 percent of the initial FEV₁; the other three patients with such large cold-air responses had PC₂₀ ≥16 mg/ml.

It follows that if these tests are used to diagnose asthma in patients suspected of having the disease, the populations identified will differ according to which test is used. In our hands, there was little difference in test sensitivity; each missed a similar number of patients who had positive responses to the other. We conclude that neither test constitutes a “gold standard” for the diagnosis of asthma.

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