Cholinergic Bronchomotor Tone in COPD*
Estimates of Its Amount in Comparison with That in Normal Subjects
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The use of anticholinergic bronchodilators in COPD is based on the reversal of cholinergic bronchomotor tone. There is little information about the magnitude of cholinergic tone in patients with COPD as compared with normal subjects. As an index of the amount of cholinergic tone we measured the maximum increase in FEV₁, following administration of an optimal dose of the anticholinergic agent atropine methonitrate. The study included nine normal nonsmoking subjects, ten normal smokers and 22 subjects with mild to moderately severe COPD. We found that normal nonsmokers had smallest increases in FEV₁ following atropine methonitrate administration. Responses of subjects with airway disease were progressively greater. Greatest responses occurred in the group of subjects with prebronchodilator FEV₁ values less than 55 percent of predicted. The most plausible explanation for this is that cholinergic tone in COPD is increased in proportion to the severity of airway disease. Other explanations are possible.

(Chest 1989; 96:984-87)

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\text{COPD} = \text{chronic obstructive pulmonary disease}; \text{FEV}_1 = \text{forced expiratory volume in 1 s}; \text{SGaw} = \text{specific airway conductance}; \text{ANOVA} = \text{analysis of variance}; \text{TLC} = \text{total lung capacity}
\]

In patients with COPD, there are several mechanisms of airflow limitation. Many of these are related to destructive and fibrotic changes in the lungs which narrow or obliterate the airways. These structural changes are not reversible by pharmacologic means. However, most patients with COPD are capable of some bronchodilatation, and most if not all of the bronchodilatation of which they are capable can be achieved by means of an anticholinergic bronchodilator. Patients with COPD thus differ from patients with asthma, who nearly always respond better to an adrenergic bronchodilator. We have suggested that one explanation why patients with COPD respond equally well to anticholinergic and adrenergic agents is that, unlike asthmatic subjects, the major reversible component of their airways narrowing is that due to cholinergic bronchomotor tone. The question arises: how much bronchomotor tone is present in subjects with COPD?

Although it is known from the work of Widdicombe and Nadel, and others that cholinergic elements of the parasympathetic system exert an important degree of control over airway caliber, the amount of cholinergic tone cannot be directly measured in man. However, because atropine-like agents are specific antagonists of the muscarinic effects of acetylcholine, we reason that it might be possible to make indirect, semiquantitative estimates of cholinergic tone by measuring the increase in airflow when cholinergic tone is abolished with atropine-like agents. Using this method we have compared the amount of bronchodilatation resulting from administration of an anticholinergic agent in patients with COPD with that in normal subjects. The results are consistent with the hypothesis that cholinergic tone is increased in proportion to the severity of airway disease.

**Subjects and Methods**

All subjects were recruited from Hines VA Hospital. Those with COPD were outpatients who had chronic bronchitis and/or emphysema as defined by recently revised criteria of the American Thoracic Society. All had airflow limitation (FEV₁ less than 70 percent of the predicted value) and a history of cigarette smoking equivalent to at least one pack per day for ten years. Patients with any features of asthma, allergies, peripheral eosinophilia or elevated serum IgE levels were excluded. None was receiving cromolyn sodium or oral corticosteroids. A response to bronchodilators was not a condition of entry. There were 22 patients in this group.

Normal subjects were recruited from the staff and volunteers of the hospital. We included only subjects between 50 and 75 years of age in order to match the age of subjects with COPD and excluded subjects with any features of asthma or allergies. In this group there were nine subjects who had never smoked and ten subjects who were current cigarette smokers and had smoked one or more packs per day for ten years.

Each of the 41 subjects was studied on three occasions separated by at least three days. Those receiving bronchodilators withheld their medications for at least 12 h before the start of each study day, 24 h in the case of methylxanthines, and current smokers were asked not to smoke on each day of the study. All studies commenced between 7 and 9 AM; after a brief rest period spirometry was performed in duplicate (baseline). The subject then inhaled a nebulized solution of atropine methonitrate, 1.5 mg dissolved in 3 ml isotonic saline solution delivered by a DeVilbiss 645 nebulizer powered by a flow of air at approximately 10 L/min over approximately 5 min. Spirometry was repeated in duplicate at intervals of

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Supported in part by Veterans Administration Research Service, Hines VA Hospital.

Manuscript received November 21, 1988; revision accepted March 15.

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Table 1—General Features of 41 Patients

<table>
<thead>
<tr>
<th>COPD</th>
<th>Normal</th>
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<tbody>
<tr>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Age, yr</td>
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</tr>
<tr>
<td>61.1 ± 6.9</td>
<td>60.7 ± 7.5</td>
</tr>
<tr>
<td>Baseline FEV₁ (range)</td>
<td>37.6 ± 12.7</td>
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<tr>
<td>% Predicted</td>
<td></td>
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<tr>
<td>(mean ± SD)</td>
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Mean ± 1 SD
0.5 h for 3 h. Atropine methonitrate is a poorly absorbed quaternary congener of atropine whose effects are confined to the respiratory mucosa when the drug is inhaled.

A subgroup of the subjects with COPD (baseline FEV₁ less than 55 percent predicted) was studied on seven or eight occasions each. The protocol was identical to that described above except that body plethysmography was performed immediately preceding spirometry, from which SGaw was determined.

Spirometry and plethysmography were performed on Morgan and Cardiopulmonary Instruments equipment.

Data used in this report include the baseline FEV₁, the greater of each duplicate before bronchodilator being accepted, and the FEV₁ increment, the difference between the greatest FEV₁ following bronchodilator administration and the baseline FEV₁. All FEV₁ values, both baseline and increment, were converted to percent predicted for that subject to minimize variance due to age, sex and body size. For the subgroup that was studied on seven or eight occasions we also analyzed the baseline and increment of SGaw.

In the first part of this report, which concerns intersubject variation in FEV₁ increment, we calculated the mean baseline FEV₁ and mean FEV₁ increment for each subject from the three sets of data obtained from each subject. The mean baseline and increment of FEV₁ of each subject was entered as a single data point. In the subsequent part, which concerns intrasubject variability of bronchodilator response, we used the individual baseline and increment of FEV₁ or SGaw taken during the seventh or eight visits for each of the subjects. These latter data were analyzed to determine if the correlation between airflow increment on baseline could be calculated.

Standard statistical analyses were performed using commercial software (Statpro, Penton Software Inc, New York). Atropine methonitrate was used under IND permit from the Food and Drug Administration. Each subject gave informed consent and the study was approved by the hospital's human studies subcommittee.

RESULTS

General features of the 41 subjects are present in Table 1. For simplicity they are divided into four groups based on their baseline FEV₁ values. The normal subjects could be divided without overlap of baseline FEV₁ into smokers and nonsmokers, and the patients with COPD were divided into "mild" and "moderate" by virtue of a baseline FEV₁ of above or below 55 percent of predicted. Although none of the subjects in this study had baseline FEV₁ values below 31 percent of predicted, it should be noted that many patients with COPD have baseline FEV₁ values below this level. The four groups shown in Table 1 did not differ significantly with respect to sex ratio, age or predicted FEV₁.

The response in terms of FEV₁ increment following atropine methonitrate is shown in Figure 1. The maximal response of normal nonsmokers was 7.9 ± 0.9

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FIGURE 1. Increment of FEV₁ following atropine methonitrate inhalation in the four groups of subjects. Bars indicate standard errors. Numbers in bars are the number of subjects in each group. The response of groups differed significantly.

(mean ± SE) percent predicted FEV₁; normal smokers, 10.9 ± 1.6 percent; "mild COPD," 13.0 ± 1.6 percent; and "moderate COPD," 14.0 ± 3.0 percent. The difference in response between groups (ANOVA) was significant at p < 0.01. The ANOVA with repeated entry and t test comparisons of pairs of groups showed that the group of normal nonsmokers was significantly different from each of the other groups (p < 0.01), but that there were no significant differences among each of the other three groups.

A more powerful test of the relationship between FEV₁ increment and baseline is provided by analysis of linear regression of individual subject FEV₁ increment on FEV₁ baseline. The linear regression of data for all 41 patients had a regression coefficient of -0.10 ± 0.05, and the correlation coefficient (r = -0.46) was significant at p < 0.01. These data suggest that the response to an anticholinergic agent increases progressively as a function of disease severity from normal subjects through subjects with baseline FEV₁ values between 31 and 55 percent of predicted.

The preceding analysis was performed on the means of the three sets of data obtained from each subject. However, within subjects there was some variation in both baseline and increment of FEV₁ from visit to visit, as one would expect. For an individual subject the FEV₁ increment tended to be greatest on the occasions when the subject's baseline FEV₁ was lowest. This was the case for subjects in each of the four categories of disease severity, including the normal subjects (data not shown). This tendency was explored in a subset of six subjects with "moderate COPD" who were each studied on seven or eight additional occasions during which both FEV₁ and SGaw were measured.

In each individual subject we found that the increment in FEV₁ or SGaw correlated significantly (and negatively) with the baseline FEV₁ or SGaw. Data from all six subjects (46 visits) are shown in Figure 2
which shows the FEV\textsubscript{1} increment as a function of baseline FEV\textsubscript{1}, for each visit in these subjects. The overall regression equation was:

\[
\text{FEV}_1 \text{ increment} = (-0.81 \times \text{baseline FEV}_1) + 45.2,
\]

both FEV\textsubscript{1} increment and baseline being expressed as percent predicted. The correlation coefficient was \(r=-0.82, p<0.0001\); the standard error of the regression coefficient was \(\pm 0.12\); and 67 percent of the variation in the FEV\textsubscript{1} increment could be explained by variation in the baseline FEV\textsubscript{1}.

For the same six patients the increment of SGaw on baseline is shown in Figure 3. The regression equation for SGaw was:

\[
\text{SGaw increment} = (-0.72 \times \text{baseline SGaw}) + 0.069,
\]

the units of SGaw being cm \(H_2O^{-1}s^{-1}\). The correlation coefficient was \(r=-0.77, p<0.0001\); the standard error of the regression coefficient was \(\pm 0.10\); and 59 percent of the variation in SGaw increment could be explained by variation in the baseline SGaw.

These results show that the anticholinergic-induced increment in airflow experienced by a patient with COPD on any occasion is strongly dependent on his baseline airflow on that occasion being greater when baseline airflow is worse.

**Discussion**

The principal aim of this study was to estimate cholinergic tone in patients with COPD in comparison with normal subjects. We estimated cholinergic tone by measuring the increment in FEV\textsubscript{1} when cholinergic tone is abolished by an anticholinergic agent, atropine methonitrate. We consider first some factors which might confound the premise that the FEV\textsubscript{1} increment following anticholinergic administration can be equated with cholinergic tone.

First, performance of an FEV\textsubscript{1} requires an inspiration to TLC, which itself may alter vagal tone.

Although this has been shown for asthmatic subjects, airway conductance in normal subjects is unaffected by a deep inspiration.\textsuperscript{9,10} It is not known what effect a deep inspiration has on vagal tone in subjects with COPD. However, we found that data for SGaw, which was performed before spirometry and which does not require a deep inspiration, matched those of FEV\textsubscript{1}. This makes it unlikely that conclusions predicated on FEV\textsubscript{1} data were vitiated by changes in cholinergic tone due to the deep inspiration required to perform spirometry.

Second, to abolish cholinergic tone a maximal dose of an anticholinergic agent is needed. Previous studies suggest that a maximal FEV\textsubscript{1} response is obtained with 1.5 mg of nebulized atropine methonitrate.\textsuperscript{11} The dose used in the present experiment, 1.5 mg, therefore is optimal.

Third, the increment in airflow following a given amount of relaxation of airway smooth muscle tone may vary between normal and narrowed airways simply because of the geometric relationship between flow and the radius or circumference of the tube. The question is: will narrowed airways, dilated by a given amount of circumferential increase, permit a greater increase in airflow than normal airways dilated by the same circumferential increase? Although the relationship of airflow to geometry of the airways is enormously complicated,\textsuperscript{12} flow through tubes is directly proportional to some power greater than three of the circumference of the tube depending on flow characteristics.\textsuperscript{13} This relationship predicts that flow will increase less when narrowed airways are dilated by a given amount of circumferential increase than when normal airways are dilated by the same amount, a conclusion that has been carefully explored by Moreno and co-workers.\textsuperscript{14}

We found the reverse (Fig 1). Patients with narrower
airways (lower baseline FEV₁ values) experienced greater increases in airflow (FEV₁ increment) following atropine methonitrate than normal subjects. By the previous reasoning, this result cannot be attributed to narrowing of the airways per se. In fact, one can argue that because patients with narrowed airways experienced greater increments in FEV₁ than normal subjects, the increment in the circumference of their airways must have been even greater than the difference in FEV₁ increments between COPD and normal subjects seen in Figure 1.

It seems reasonable to conclude, therefore, that there is greater smooth muscle relaxation in subjects with baseline airflow limitation than subjects with normal baseline airflow. This could be due to greater cholinergic tone or to greater compliance of the airway wall in subjects with airflow limitation, or a combination of both. We tentatively interpret the increasing FEV₁ increment with decreasing baseline FEV₁ to indicate that cholinergic tone is greater in COPD than in normal subjects, the amount of cholinergic tone correlating directly with the severity of disease.

We did not study any subjects with baseline FEV₁ values less than 31 percent of predicted, and most of our patients had baseline FEV₁ values substantially higher than this. Others have provided evidence that the FEV₁ increment following an adrenergic bronchodilator is less in patients with more severe degrees of airflow disease. Our data only cover moderate degrees of disease severity, so the present results cannot be extrapolated to patients with the most severe degrees of airflow disease.

A secondary finding of this study was that cholinergic tone may vary from day to day. Some day-to-day variation of baseline airflow might be expected when a subject is studied on several occasions. Part of this intrasubject variability is due to error (random variation) in the airflow determination. However, if some of the variability is due to day-to-day variation in cholinergic tone, this should be detectable as a greater airflow increment following cholinergic inhibition when the subject’s baseline airflow is lower, and, conversely, a smaller airflow increment when the subject’s baseline airflow is high. This would yield a pattern of responses in an individual subject such that the FEV₁ or SGaw increment would correlate negatively with the baseline FEV₁ or SGaw. Pooling the data from a group of subjects with similar baseline airflows provided a means of verifying this statistically.

Figure 2 shows that the FEV₁ increment was inversely related to the baseline FEV₁ within subjects. A similar relationship was found for SGaw determinations (Fig 3). Statistically, about two thirds of the variation in airflow increment between occasions could be explained by variation in the baseline airflow. If the airflow increment is an index of cholinergic tone, the implication is that much of the day-to-day variation in baseline airflow was due to variation in cholinergic tone. Why cholinergic tone should vary from day to day is unknown.

In conclusion, we have shown that abolition of cholinergic tone produces more bronchodilatation in subjects with baseline airflow limitation due to COPD than in normal subjects, the amount of bronchodilatation being directly proportional to the severity of baseline airflow limitation. This could be taken to suggest that cholinergic tone is increased in proportion to the severity of airway disease, but other explanations are possible. We also conclude that a major portion of the day-to-day variation in the baseline airflow in patients with COPD can be attributed to variations in cholinergic tone. These findings provide an explanation of the relative efficacy of anticholinergic bronchodilators in COPD and a rationale for their use in patients with COPD.

ACKNOWLEDGMENTS. We thank Donald P. Tashkin, M.D., UCLA School of Medicine, for helpful suggestions and David Smith, Ph.D., for valuable advice on statistical methods and analyses.

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