Increase in Bronchial Responsiveness to Methacholine and Late Asthmatic Response after the Inhalation of Ultrasonically Nebulized Distilled Water*

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We studied ten subjects who had an asthmatic response after the inhalation of ultrasonically nebulized distilled water and did not show any refractory period to repeated challenge with such water. The change in responsiveness to methacholine after inhalation of distilled water and the occurrence of any water-induced late asthmatic response were investigated on separate days. All of the tested subjects showed a significant increase in bronchial responsiveness to methacholine after prior stimulation with ultrasonically nebulized distilled water, which waned within two hours in eight of them. The other two subjects showed a progressive increase in responsiveness to methacholine, and they also had a further reduction in the caliber of the airways three to four hours after inhalation of distilled water. The late responses were less severe than the initial responses and lasted four to five hours. After the spontaneous recovery, no significant increase in responsiveness to methacholine was detected. Our results confirm previous observations on hyperresponsive ness induced by ultrasonically nebulized distilled water and demonstrate the occurrence of late reactions after inhalation of such water.

Bronchoconstriction elicited by the inhalation of ultrasonically nebulized distilled water is presumed to be at least partly provoked by a nonimmunologic release of mediators from mast cells in the airways. Recent evidence has suggested this hypothesis.

In fact, circulating concentrations of the mast cell-associated mediators, histamine and neutrophilic chemotactic factor, increase in asthmatic subjects after inhalation of ultrasonically nebulized distilled water. This accompanies the development of distilled water-induced asthma in a way analogous to the increase in both mediators observed after antigenic challenge.

Disodium cromoglycate, a mast-cell stabilizer, can modify the asthmatic response to inhaled ultrasonically nebulized distilled water as it modifies the early reaction to inhalation of allergen.

Finally, the hypo-osmolarity of the aerosol seems to be an important determinant of bronchoconstriction induced by ultrasonically nebulized distilled water and it has been demonstrated that mast cells in vitro can release histamine in hypotonic solutions. Transient changes in the osmolarity of the fluid surrounding mast cells in the airways may be all that is required to induce release of mediators and contraction of smooth muscles, either directly or via the vagus nerve.

Black et al have recently demonstrated that inhalation of ultrasonically nebulized distilled water can induce an increase in nonspecific bronchial responsiveness as allergenic exposure does. This might provide further evidence that there are some common mechanisms in asthma induced by distilled water and in asthma induced by inhalation of allergens.

Nevertheless, some questions arise about this point. In fact, it is well known that the increase in bronchial responsiveness elicited by allergenic exposure is generally associated with the occurrence of late reactions, and although the factors provoking these two phenomena remain unknown at present, inflammation and the release of mediators from pulmonary cells or from cells migrated during the inflammatory response seem to be involved. By contrast, no late asthmatic response to inhaled ultrasonically nebulized distilled water has been reported in asthmatic subjects, and at present, there is no evidence demonstrating that the time course of such a water-induced increase in bronchial hyperresponsiveness is similar to that observed after inhalation of allergens.

We have recently noticed that some of the subjects who were taking part in a separate study of asthma induced by ultrasonically nebulized distilled water complained of wheezing several hours after the inhalation. In the present study, we therefore investigated the occurrence of any late asthmatic response after
Table 1—Characteristics of Subjects

<table>
<thead>
<tr>
<th>Subject, Sex, Age (yr)</th>
<th>FEV₁, percent of predicted</th>
<th>Atopic Status*</th>
<th>Regular Treatment†</th>
</tr>
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<tbody>
<tr>
<td>1, M, 21</td>
<td>92</td>
<td>-</td>
<td>A, not daily</td>
</tr>
<tr>
<td>2, F, 12</td>
<td>88</td>
<td>+</td>
<td>A, not daily</td>
</tr>
<tr>
<td>3, F, 39</td>
<td>62</td>
<td>+</td>
<td>F(800); I(320)</td>
</tr>
<tr>
<td>4, M, 39</td>
<td>112</td>
<td>+</td>
<td>A(800)</td>
</tr>
<tr>
<td>5, F, 8</td>
<td>99</td>
<td>-</td>
<td>F, not daily</td>
</tr>
<tr>
<td>6, F, 14</td>
<td>101</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>7, M, 25</td>
<td>99</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>8, M, 8</td>
<td>108</td>
<td>+</td>
<td>A(400)</td>
</tr>
<tr>
<td>9, M, 42</td>
<td>96</td>
<td>-</td>
<td>A(800)</td>
</tr>
<tr>
<td>10, M, 30</td>
<td>72</td>
<td>+</td>
<td>A(800)</td>
</tr>
</tbody>
</table>

*Plus indicates one or more wheal-and-flare responses to ten common allergenic extracts; minus indicates no response.
†Minimum treatment required in last four weeks. A, Albuterol (salbutamol) (micrograms daily); F, fenoterol (micrograms daily); and I, ipratropium bromide (micrograms daily).

inhaled ultrasonically nebulized distilled water and the time course of any change in responsiveness to methacholine induced by this stimulus.

This might have clinical relevance because the inhalation of distilled water seems to stimulate a naturally occurring stimulus (foggy weather) in asthmatic patients,1,18 and challenge with ultrasonically nebulized distilled water has been proposed as an alternative method to measure bronchial responsiveness.1,4,13,18

Materials and Methods

Subjects

We studied ten subjects who satisfied the criteria for asthma of the American Thoracic Society36 and who had shown asthmatic responses after inhalation of ultrasonically nebulized distilled water. Five were atopics, as assessed by skin prick testing. No subject had suffered from respiratory infection for at least one month, and no subject had received corticosteroids or disodium cromoglycate in the last two months.

Symptoms of asthma were controlled with minimal medication (Table 1), and the baseline value for the forced expiratory volume in one second (FEV₁) was 72 percent or more of the predicted value36 (Table 1). Subjects demonstrated a range of responsiveness to methacholine from borderline to severely increased.37 Any medication was withheld for 24 hours before each day of study. The study was approved by the university committee on human research, and a written informed consent was obtained from each adult and from the children's parents.

Preliminary Study

To detect the occurrence of any refractory period4 to repeated inhalation of ultrasonically nebulized distilled water, one week before the time of the present investigation, all subjects had three successive challenges with such distilled water, with the second and third tests beginning soon after the recovery from previous bronchoconstriction. Since we have previously observed that refractory patients show no change or even a reduction in responsiveness to methacholine in the two hour period following challenge with ultrasonically nebulized distilled water,36 in this manner, we could relate any observed change in responsiveness to methacholine after prior inhalation of distilled water to the presence or the absence of a refractory response to this stimulus.

Study Design

Each subject attended the laboratory on five separate occasions three days apart. On each day, three baseline measurements of FEV₁ were made at intervals of one minute with a Fleisch pneumotachograph connected to a 47804 Pulmonary Calculator System (Hewlett-Packard 47904).

On the first day, measurements of FEV₁ were repeated every hour for eight hours to know the spontaneous variability of this parameter. On the second and third days, baseline measurements of FEV₁ were followed by a methacholine inhalation test to determine the variations in bronchial responsiveness for each patient.

On the fourth day a challenge with ultrasonically nebulized distilled water was followed by a methacholine inhalation test soon after the return of FEV₁, to within 10 percent of the baseline value. Challenge with methacholine was repeated two hours after the recovery from the bronchoconstriction induced by the distilled water.

On the fifth day a challenge with ultrasonically nebulized distilled water was followed by measurements of FEV₁ every hour for eight hours in order to detect any change in this parameter (greater than the spontaneous variability) occurring after the recovery from the immediate response. If there was a further significant reduction in FEV₁, a methacholine inhalation test was performed soon after the return of FEV₁ to within 10 percent of the baseline value.

Generation and Inhalation of Aerosols

The aerosols of methacholine were generated by a Wright nebulizer (mean output, 0.15 ml/min). The aerosols were passed directly into a face mask held loosely over the mouth and clipped nose and were inhaled by tidal breathing. The aerosol of distilled water was generated by an electronic nebulizer (Mistogen EN145) and was passed into a face mask attached to the side of the airflow tubing from the nebulizer. The nebulizer was equipped with a fan producing a continuous airflow of 14 L/min and was calibrated to deliver five volume outputs by adjusting the amplitude of oscillation.4 The five volume outputs (mean ± SD) were 0.5 ± 0.03, 1.0 ± 0.06, 1.8 ± 0.10, 3.6 ± 0.09, and 5.6 ± 0.20 ml/min (each output was measured volumetrically on five occasions).

Inhalation Tests

The methacholine inhalation test was performed according to the method described by Juniper and co-workers,23 as in our previous study.34 Aerosols were inhaled for two minutes. Phosphate-buffered saline solution was inhaled first, followed at five-minute intervals by twofold increasing concentrations of methacholine (0.03 to 64 mg/ml). The response was measured by the change in FEV₁ (from the lowest value after saline solution) at 0.5, 1.5, and 3 minutes after each inhalation. The inhalations were stopped when the FEV₁ had fallen by 20 percent or more. Bronchial responsiveness was quantitated by constructing log concentration-response curves, and the provocative concentration of methacholine producing a 20 percent fall in FEV₁ (PC20M) was obtained by linear interpolation of the last two points.35,37

Challenge with ultrasonically nebulized distilled water was performed according to the method described by Sheppard et al.,4 slightly modified. Aerosols of distilled water at each output setting were inhaled for three minutes with clipped nose by tidal breathing; the subjects inhaled increasing volumes of distilled water at five-minute intervals. The response was measured by the change in FEV₁ (from the lowest baseline value) at 0.5, 1.5, and 3 minutes after each inhalation. The inhalations were stopped when the FEV₁ had fallen by 20 percent or more. Bronchoconstriction was analyzed by constructing stimulus-response curves with FEV₁ on the ordinate as percentage of the lowest baseline value and with the output of the nebulizer expressed logarithmically on the abscissa. The provocative

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output at which inhalation of ultrasonically nebulized distilled water produced a 20 percent fall in FEV₁ (PO₉UndW) was obtained by linear interpolation of the last two points on the stimulus-response curve.

Statistical Analysis

Log transformations were applied to all values for PO₉UndW and PC₉M before analysis. Reproducibility of the values for PC₉M obtained on the second and third days was examined by calculating the intraclass correlation coefficient. These values were averaged to reduce intrasubject variance and were then compared with the values for PC₉M measured after prior stimulation with distilled water by analysis of variance and Dunnett's method. Significance was accepted when p<0.05.

RESULTS

Preliminary Study

The mean values for PO₉UndW (± SD in log scale) observed during three successive challenges with ultrasonically nebulized distilled water were 2.24 ± 0.80, 2.15 ± 0.69, and 2.04 ± 0.62 ml/min. These values were not significantly different by analysis of variance (p>0.1). No subject showed any progressive shifting to the right of the output-response curve indicative of refractoriness (Fig 1).

Spontaneous Variability of FEV₁ and PC₉M

On the first day, no subject had a change in FEV₁ greater than 10 percent from the initial baseline value over the eight-hour period of study; thus, any fall in FEV₁ greater than 10 percent occurring after the recovery from the immediate response to inhalation of

<table>
<thead>
<tr>
<th>Subject</th>
<th>Second Day</th>
<th>Third Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.52</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>0.41</td>
<td>0.34</td>
</tr>
<tr>
<td>3</td>
<td>2.20</td>
<td>3.61</td>
</tr>
<tr>
<td>4</td>
<td>0.79</td>
<td>1.35</td>
</tr>
<tr>
<td>5</td>
<td>1.11</td>
<td>1.41</td>
</tr>
<tr>
<td>6</td>
<td>11.20</td>
<td>11.40</td>
</tr>
<tr>
<td>7</td>
<td>16.90</td>
<td>12.70</td>
</tr>
<tr>
<td>8</td>
<td>0.49</td>
<td>0.50</td>
</tr>
<tr>
<td>9</td>
<td>0.18</td>
<td>0.15</td>
</tr>
<tr>
<td>10</td>
<td>2.35</td>
<td>2.28</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>1.33</td>
<td>1.35</td>
</tr>
<tr>
<td>SD†</td>
<td>1.46</td>
<td>1.50</td>
</tr>
<tr>
<td>R‡</td>
<td></td>
<td>0.99</td>
</tr>
</tbody>
</table>

*PC₉M, Concentration of methacholine provoking 20 percent fall in FEV₁.
†Tabulated in log scale.
‡R, Intraclass correlation coefficient.
distilled water was regarded as indicative of a late response. On the second and third days the response to inhaled methacholine was reproducible (Table 2). In all of the tested subjects, PC_{20}M varied by less than twofold (Table 2); a decrease in this value greater than twofold was therefore regarded as indicative of increased hyperresponsiveness.

**Methacholine Challenge After Stimulation with Distilled Water**

The subjects had a range of responsiveness to ultrasonically nebulized distilled water (Table 3). All showed a significant increase in responsiveness to methacholine soon after the recovery from bronchoconstriction induced by distilled water (p<0.01) (Table 4 and Fig 2).

After two hours the mean PC_{20}M was still significantly lower than mean baseline value (p<0.05) (Table 4); at this time the reduction in PC_{20}M waned in eight of the tested subjects, and PC_{20}M returned to within a twofold concentration difference from the baseline PC_{20}M in five of them (Table 4). On the contrary, subjects 3 and 9 had a further increase in hyperresponsiveness to methacholine (Table 4 and Fig 2).

The change in responsiveness to methacholine was not due to differences in the FEV₁ before methacholine because resting values for FEV₁ (mean percent predicted ± SD) before the first (90.9±11.2) and the second challenge (94.5±11.9) with methacholine were not significantly different from the values obtained on the second and third days averaged (93.6±10.5; p>0.1 for both comparisons).

**Late Response to Distilled Water**

On the fifth day the values for PO_{20}UNDW did not significantly differ from the values obtained on the fourth day (p>0.1) (Table 3). All patients recovered spontaneously from the early asthmatic reactions induced by inhalation of ultrasonically nebulized distilled water. Only the two subjects who had shown the greatest increase in responsiveness to methacholine during the second challenge with methacholine on the fourth day (subjects 3 and 9) experienced a further fall in FEV₁ (Fig 3). The reduction in FEV₁ was well beyond the spontaneous variability measured on the first day; it began three hours after inhalation of distilled water and persisted for four to five hours (Fig 3). These late responses were both less severe than the initial responses.

On both the fourth and fifth days, subject 9 had symptoms of chest tightness and coughing between four and six hours after inhalation of ultrasonically nebulized distilled water. During this period of time, he also showed the maximal delayed decrease in FEV₁ on the fifth day (Fig 3). After the spontaneous recovery from the late-phase reaction, both of the two subjects had an increase in responsiveness to methacholine, but the reduction in PC_{20}M was not greater than twofold from the mean baseline value (Fig 3).

**Discussion**

The present study demonstrates that in asthmatic subjects, bronchial sensitivity to methacholine can increase after the inhalation of ultrasonically nebulized distilled water. The increase in bronchial responsiveness can persist for more than two hours after the recovery from the bronchospasm induced by the dis-
tilled water.

This confirms previous results obtained by Black and co-workers; however, those authors studied the change in responsiveness to methacholine only 40 to 60 minutes after challenge with ultrasonically nebulized distilled water, and their findings have more statistical than physiologic meaning. In fact, the provocative doses of methacholine recorded after challenge with distilled water were within the accepted reproducibility of the test. In our study the changes in PC_{20}M soon after the recovery from bronchoconstriction induced by distilled water were beyond the variability of the test in all of the tested subjects.

The increase in responsiveness to methacholine in our patients was associated with a lack of refractoriness to repeated stimulation with ultrasonically nebulized water.

**FIGURE 2.** Change in response to inhaled methacholine after prior stimulation with ultrasonically nebulized distilled water (UNDW). Concentration-response curves were obtained soon after recovery from water-induced bronchoconstriction (open squares) and two hours after recovery (solid squares). Baseline curves (circles) were constructed by averaging responses obtained at each concentration of methacholine on second and third days.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>PC_{20} UNDW</th>
<th>PC_{20} BASELINE</th>
<th>PC_{20} POST-UNDW</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.54 ml/min</td>
<td>2.90 mg/ml</td>
<td>1.80 mg/ml</td>
</tr>
<tr>
<td>2</td>
<td>0.90 ml/min</td>
<td>0.17 mg/ml</td>
<td>0.10 mg/ml</td>
</tr>
</tbody>
</table>

**FIGURE 3.** Percentage of fall in FEV_{1} induced by inhalation of ultrasonically nebulized distilled water (UNDW) from time of maximal bronchoconstriction during immediate response up to eight hours after challenge in subjects 3 (solid circles) and 9 (open circles). Shaded area at top indicates spontaneous variability of FEV_{1}, determined on first day. Open arrows mark time at which challenge with methacholine (M) was performed. Concentration of methacholine provoking 20 percent fall in FEV_{1} (PC_{20}M) was compared with baseline values obtained on second and third days, averaged.
distilled water, and we have previously observed no change or even an increase in $PC_{20}$ after such inhalation of distilled water in subjects with a refractory period to this stimulus. These findings, taken together, suggest that there is a range of refractoriness to repeated challenge with ultrasonically nebulized distilled water and a change in responsiveness to methacholine in asthmatic subjects who respond to inhalation of distilled water. This point requires further evaluation.

In the present study the increase in hyperresponsiveness to methacholine waned two hours after the recovery from the bronchoconstriction induced by distilled water in most of our subjects, but at this time, two of them (subjects 3 and 9) had the greatest reduction in $PC_{20}$. On the fifth day, these two subjects showed late responses to ultrasonically nebulized distilled water which persisted for four to five hours. After the spontaneous recovery from the late reactions, a slight reduction in $PC_{20}$ was detected in both subjects, but the change from the baseline value was not greater than twofold.

By contrast, the other patients, who had experienced the maximal increase in hyperresponsiveness to methacholine soon after the recovery from the bronchospasm induced by distilled water, did not show any significant delayed change in $FEV_1$ on the fifth day.

These findings suggest that (1) the occurrence of late asthmatic response to inhalation of ultrasonically nebulized distilled water can be preceded by a progressive increase in hyperresponsiveness to methacholine; and (2) the subjects who do not show any delayed airflow obstruction after distilled water can also have an increase in sensitivity to methacholine which is shorter lasting.

Although the allergen-induced increase in non-specific bronchial responsiveness generally follows late responses and may persist for days to weeks after any alteration in the caliber of the airways has disappeared, recent studies have demonstrated that a significant increase in bronchial responsiveness can also precede the onset of late responses. Furthermore, responsiveness to both histamine and methacholine can be enhanced by an allergenic challenge which does not provoke any change in the caliber of the airways; in this case, increased hyperresponsiveness can appear two to three hours after inhalation of allergens.

These findings suggest that the induced increase in nonspecific bronchial responsiveness may be necessary for, rather than (as previously suggested) consequent upon, the development of a late asthmatic reaction to allergen, and that an early increase in responsiveness can be elicited by inhalation of allergens in the absence of any late response. On the basis of our results, the same seems to be true for hyperresponsiveness induced by ultrasonically nebulized distilled water.

Moreover, from only two subjects, our results cannot exclude the possibility that an increase in bronchial responsiveness may also follow the late response induced by distilled water in other subjects, as it follows allergen-induced late reactions; on the other hand, some late response to inhalation of allergens can indeed occur in the absence of any following increase in responsiveness to methacholine.

All of these arguments and the finding that late reactions induced by distilled water have a time course similar to that seen with inhalation of allergens support the hypothesis that the two stimuli at least partly act through a common mechanism. This restricts the usefulness of challenge with distilled water as an alternative method to measure bronchial hyperresponsiveness.

Further studies are in progress in our laboratory to characterize the late asthmatic response provoked by the inhalation of distilled water and to evaluate the mechanism(s) involved.

**References**

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28 L’Archeveque J, Malo JL, Cartier A. Time-course of the changes in histamine bronchial responsiveness after an antigen challenge which did not cause significant changes in airway caliber (abstract). J Allergy Clin Immunol 1986; 77(suppl):170