Circadian Variation of Bronchial Caliber and Antigen-induced Late Asthmatic Response*

Shinya Kondo, M.D., F.C.C.P.

To study whether circadian variation of bronchial caliber participates in causing late asthmatic response (LAR), house-dust inhalation was made at 10 AM and 6 PM on separate days in 6 house-dust–sensitive asthmatic children aged 8 to 13 years. Bronchial obstruction was assessed through measurements of FEV1 at 4-h intervals from 24 h before to 24 h after the inhalation. The LAR, which is a 15 percent or greater decrease in FEV1, from the value at the same hour of the previous day, occurred 4 h or later after the inhalation in all challenges. The mean (± SD) time to the occurrence of the lowest FEV1 (maximum LAR) following the morning inhalation was 14.7 ± 2.1 h versus 10.0 ± 2.2 h following the evening inhalation (p<0.05). Regardless of the hour of inhalation, FEV1 after the inhalation was lowest or near-lowest at 2 AM in all. Therefore, the maximum LAR was indistinguishable from the trough of further amplified circadian variation in FEV1, following the inhalation. These findings suggest that the downward arm of circadian variation may partially participate in causing the LAR.

(Chest 1993; 104:01-05)

CV = coefficient of variation; IAR = immediate asthmatic response; LAR = late asthmatic response

Both the late asthmatic response (LAR) and nocturnal bronchial obstruction are based on airway inflammation,1-3 however, definite airway inflammation following antigen inhalation does not necessarily cause LAR in anesthetized animals with acquired sensitization,4 and airway inflammation alone cannot explain the mechanism of LAR. In asthmatic patients who have LAR following bronchial provocation, a further increase in bronchial responsiveness is associated with LAR,5 and it already starts to increase around 2 h after the provocation,6,7 obviously preceding actual bronchial obstruction of the LAR. The increase in bronchial responsiveness also affects circadian variation of bronchial caliber. Generally, an upward arm of circadian variation of bronchial caliber in asthmatic patients continues until early afternoon.8 Its downward arm starts from late afternoon and terminates between late night and early morning.9 The magnitude of this circadian variation increases further as bronchial responsiveness increases.8,10 Even a single exposure to antigen increases the maximum bronchoconstrictive response, as well as bronchial responsiveness,11 and amplifies the circadian variation of bronchial caliber with nocturnal exacerbation.12 Furthermore, the Denver group demonstrated that the LAR following morning bronchial provocation occurs later than the one following evening provocation.13 Taken together, these raise the possibility that further transiently amplified circadian variation of bronchial caliber following bronchial provocation may have a relationship to the LAR, and the downward arm of such variation may participate in causing the LAR in asthmatic patients.

If that is true, maximum bronchial obstruction (maximum LAR) may range between late night and early morning, when bronchial obstruction based on the circadian variation usually becomes most severe,8 regardless of when the bronchial provocation is made and also regardless of when the bronchial obstruction starts. To confirm the possibility, antigen inhalation was made at 10 AM and 6 PM on separate days in 6 children with stable asthma. The hour of maximum bronchial obstruction (maximum LAR) was assessed through measurements of FEV1 at 4-h intervals from 24 h before to 24 h after the inhalation to see its consistency with the trough of nocturnal bronchial obstruction.

Patients and Methods

The group studied consisted of 6 children with stable asthma (4 boys and 2 girls) aged 8 to 13 years (Table 1). They all met the criteria of the American Thoracic Society for the diagnosis of asthma* and had an intradermal reaction of 10 mm or more of wheal to 0.02 ml of either a 1:100,000 or 1:10,000 (weight/volume)

Table 1 — Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient, Sex, Age (yr)</th>
<th>Height, cm</th>
<th>Dose of Inhaled House-dust Solution, ml</th>
<th>Maintenance Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, M, 11</td>
<td>139</td>
<td>0.3</td>
<td>Ketotifen</td>
</tr>
<tr>
<td>2, M, 12</td>
<td>147</td>
<td>0.5</td>
<td>Cromolyn sodium</td>
</tr>
<tr>
<td>3, F, 8</td>
<td>121</td>
<td>0.3</td>
<td>Ketotifen</td>
</tr>
<tr>
<td>4, M, 12</td>
<td>140</td>
<td>0.3</td>
<td>Ketotifen</td>
</tr>
<tr>
<td>5, F, 11</td>
<td>150</td>
<td>0.5</td>
<td>Cromolyn sodium</td>
</tr>
<tr>
<td>6, M, 13</td>
<td>145</td>
<td>0.3</td>
<td>Ketotifen</td>
</tr>
</tbody>
</table>

*From the Children's Asthmatic Center, Kawasaki City Ida Hospital, Kawasaki City, Japan. Manuscript received October 19, 1992, revision accepted February 17, 1993. Reprint requests: Dr. Kondo, Children's Asthmatic Center, Kawasaki City Ida Hospital, Kawasaki City, Kanagawa, Japan 211

CV = coefficient of variation; IAR = immediate asthmatic response; LAR = late asthmatic response
house-dust solution (Torii Co, Tokyo, Japan). The children were staying in a residential treatment center because of uncontrolled asthma at their home. They performed spirometry almost every morning except for Sunday morning. The coefficient of variation (CV) of the 5 consecutive morning FEV₁ values before the beginning of each challenge study was less than 5 percent in all patients. No child was steroid-dependent, and none had had a respiratory tract infection for at least 6 weeks before the study. The patients stayed indoors quietly except for a walk around the center throughout the study. Although they went to bed at 8:30 PM and got up at 6 AM, they could lie down for a rest at any time they wanted.

Six other patients were studied; however, they were excluded from the study because three had an immediate asthmatic response (IAR) following the antigen inhalation, and three did not have an IAR after the morning challenge.

Written consent was given by each parent. The protocol was approved by the Clinical Research Committee, Children’s Asthmatic Center, Kawasaki City Ida Hospital.

**Design of Study**

Although some patients received oral bronchodilators as needed (a combination of short-acting salbutamol [2 mg] and aminophylline [100 mg]), and 2 patients received inhaled cromolyn sodium 3 times per day and 4 received oral ketotifen twice per day regularly, all of these medications were withdrawn for at least 36 h before and throughout the study. Each patient had the morning and the evening challenge studies on separate days, and the order of the studies was determined in a single blind manner. The studies were separated by at least 4 weeks and at most 6 weeks. Each challenge study consisted of two consecutive days, the diluent-inhaled control day followed by the antigen challenge day.

**Morning Challenge Study**

Measurement of FEV₁, was started at 10 AM on day 1. Just after the measurement, the same dose of diluent (0.9 percent sodium chloride with 0.5 percent phenol) as that of the antigen solution used for bronchial challenge (see subsequent text) was inhaled. Measurements of FEV₁, were made 10, 30, and 60 min afterward to examine IAR. Then FEV₁, measurements were made every 4 h from 2 PM to 10 AM on day 2. These six FEV₁, values were used as controls. The house-dust solution was inhaled just after the measurement at 10 AM on day 2. House-dust extract was diluted in the diluent to 1:100 (weight/volume) immediately before use. Measurements of FEV₁, were made 10, 30, and 60 min afterward to examine IAR. Then, FEV₁, measurements were made every 4 h from 2 PM to 10 AM on day 3. Measurements of FEV₁, were made by using a hot-wire auto-spirometer (AS-1500, Minato Ikagaku, Osaka, Japan). Values were expressed as percent predicted normal FEV₁, based on the equations of Sumida et al. for Japanese schoolchildren, which are as follows: FEV₁, (ml) in boys = 0.0442 x height (cm) – 2,746; FEV₁, (ml) in girls = 0.0442 x height (cm) – 2,829.

![Figure 1. Time course of FEV₁, after inhalation of diluent, (0.9 percent sodium chloride with 0.5 percent phenol) (m and e in the morning [at 10 AM] and evening [at 6 PM] challenges, respectively) and after inhalation of house-dust solution (M and E in the morning [at 10 AM] and evening [at 6 PM] challenges, respectively) in 6 children with stable asthma over 2 consecutive days. Open circles indicate FEV₁, as percent of predicted in the morning challenge study; closed circles indicate FEV₁, as percent of predicted in evening challenge study.](http://journal.publications.chest.net/pdffileaccess.ashx?url=/data/journals/chest/20382/ on 06/03/2017)
study is given in Figure 1 and Table 2. The FEV₁ immediately before the house-dust inhalation was within 10 percent of the value at the beginning of the study, at 10 AM in the morning challenge, and at 6 PM in the evening challenge in each patient. The mean (±SD) CV of six FEV₁ values on the control day in the morning challenge was 4.2±3.4 percent versus 5.9±5.2 percent in the evening challenge. The difference was insignificant. The mean (±SD) FEV₁ as percent of predicted immediately before the house-dust inhalation in the morning challenge was 91.7±9.5 versus 94.2±8.3 in the evening challenge. The difference was insignificant.

No IAR occurred following inhalation of either the diluent or the house-dust solution in all challenges. A LAR following the house-dust inhalation occurred in all challenges. The mean (±SD) maximum decrease in FEV₁ in the morning challenge was −35.5±13.1 percent versus −32.0±13.0 percent in the evening challenge. The difference was insignificant. The mean (±SD) time to onset of the LAR following the house-dust inhalation in the morning challenge was 11.3±3.0 h versus 6.0±2.2 h in the evening challenge (p<0.05). The lowest FEV₁ (maximum LAR) was at 10 PM in 2 patients and at 2 AM in 4 in the morning challenge and at 2 AM in 3 and at 6 AM in 3 in the evening challenge. The mean (±SD) clock hour of the lowest FEV₁ in the morning challenge was 00:40±02:06 AM versus 04:00±02:12 AM in the evening challenge (p<0.05). The FEV₁ at 2 AM was lower or within 10 percent of the lowest value in all challenges. The mean (±SD) time to occurrence of the lowest FEV₁ (maximum LAR) following the house-dust inhalation in the morning challenge was 14.7±2.1 h versus 10.0±2.2 h in the evening challenge (p<0.05).

The FEV₁ at 24 h after the house-dust inhalation recovered to within 10 percent of the baseline value in 2 patients in each challenge study (patients 1 and 5 and patients 5 and 6 in the morning and evening challenge studies, respectively) but did not in the other 4 patients.

**DISCUSSION**

In the present study the times to the onset of the LAR and the occurrence of maximum bronchial obstruction (maximum LAR) following the morning challenge were significantly later than those following the evening challenge, despite the same magnitude of obstruction, which is agreement with the results reported by Mohiuddin and Martin.13 Mechanisms related to an upward arm of the circadian variation of the bronchial caliber until early afternoon might delay the development of bronchial obstruction based on inflammation following the morning challenge. Furthermore, the maximum bronchial obstruction following antigen inhalation occurred between 10 PM and 6 AM.

**Table 2—Comparison of Results From Morning and Evening Challenge Studies**

<table>
<thead>
<tr>
<th>Data</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV of FEV₁, (%) of predicted</td>
<td>8.9</td>
<td>0.9</td>
<td>1.7</td>
<td>4.0</td>
<td>2.0</td>
<td>7.7</td>
</tr>
<tr>
<td>on control day, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>11.0</td>
<td>3.5</td>
<td>2.1</td>
<td>3.3</td>
<td>1.7</td>
<td>13.9</td>
</tr>
<tr>
<td>Magnitude of LAR, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>−26</td>
<td>−39</td>
<td>−44</td>
<td>−24</td>
<td>−24</td>
<td>−56</td>
</tr>
<tr>
<td>Evening</td>
<td>−29</td>
<td>−28</td>
<td>−34</td>
<td>−31</td>
<td>−15</td>
<td>−55</td>
</tr>
<tr>
<td>Onset of LAR after inhalation, h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>16</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Evening</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Clock hour of lowest FEV₁ as % of predicted*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning</td>
<td>2:20</td>
<td>0:00</td>
<td>0:00</td>
<td>0:20</td>
<td>2:20</td>
<td>0:20</td>
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<tr>
<td>Evening</td>
<td>0:00</td>
<td>0:06</td>
<td>0:00</td>
<td>0:20</td>
<td>0:00</td>
<td>0:20</td>
</tr>
</tbody>
</table>

*Maximum LAR.

The FEV₁ just before the inhalation was used as a baseline value in each patient to assess the magnitudes of IAR and LAR. An IAR was regarded as positive when there was 15 percent or more decrease in FEV₁, from the baseline value among 3 values at 10, 30, and 60 min after the inhalation. The LAR was regarded as positive when there was at least 1 FEV₁ value among 6 values from 4 to 24 h after the antigen inhalation which decreased by 15 percent or more from the value at the same hour on the control day. The magnitude of the LAR was expressed as the decrease in FEV₁ from the baseline value.

The diluent and the house-dust solution were nebulized with a glass jet nebulizer (Nishishiki, Nihon Shoji, Osaka, Japan) with an airflow of 8 l/min. The aerosol particle size from the nebulizer was 1 to 10 μm, and output of distilled water by the nebulizer was 0.20 ml/min. The inhaled dose of the house-dust solution was determined through a preliminary study in each patient so as not to cause an IAR, since bronchial constriction at different clock hours during the morning and the evening challenges might affect the magnitude of the LAR (Table 1). The same nebulizer was used in both challenge studies in each patient. The aerosol was inhaled by tidal breathing.

**Evening Challenge Study**

Measurement of FEV₁ was started at 6 PM on day 1. The diluent and the house-dust solution were inhaled just after the FEV₁ measurement at 6 PM on days 1 and 2, respectively. Following these inhalations, FEV₁ measurements were made at the same interval as in the morning challenge study. Six FEV₁ values from 10 PM to 6 AM on the next day were regarded as the values of each day.

**Analysis**

Standard methods were used to calculate the mean, SD, and CV. Student’s paired t test was used to compare variables. A p value of less than 0.05 was considered statistically significant.

**Results**

Information describing each patient involved in the
AM whenever the bronchial challenge was made. In particular, bronchial obstruction at 2 AM was maximum or near maximum in both challenge studies. Antigen inhalation and the subsequent increase in bronchial responsiveness often amplify the circadian variation,\(^1,18\) and a downward arm of the circadian variation begins in the late afternoon and terminates between 10 PM and 6 AM, generally around 2 AM.\(^9\) Therefore, from the visual inspection of the FEV\(_1\) time course, the maximum LAR was indistinguishable from the trough of further transiently amplified circadian variation following the antigen inhalation at any time. These findings suggest that the amplified circadian variation may have a relationship with the LAR and that the downward arm of that variation may participate in causing the LAR.

Since bronchial smooth muscle relaxant partially ameliorates the LAR,\(^16\) the LAR results from not only submucosal edema and intraluminal mucous retention but also smooth muscle contraction. In an LAR with a large amplitude, smooth muscle contraction may be especially important. In the present study, submucosal edema and mucous retention following provocation might partially explain the progressive bronchial obstruction with late onset; however, they hardly explained the fact that the maximum or near maximum LAR occurred around 2 AM regardless of the provocation hour, since their circadian rhythms were obscure. The phenomenon might be explained by the change in tone of smooth muscle, which was under the control of synergistic circadian rhythms of bronchial responsiveness,\(^17\) autonomic nerve, and endocrine systems.\(^18\)

The FEV\(_1\) at 24 h after the antigen inhalation did not recover to the level before inhalation in 8 out of 12 challenges, and the clock hour of the maximum LAR following the morning challenge was significantly earlier than that following the evening challenge. Also, the LAR does not always continue more than 24 h.\(^13\) These findings suggest that the amplified circadian variation of the bronchial caliber only partially participates in causing the LAR. Bronchial edema and intraluminal retention of increased secretions may participate in causing the LAR in most cases, as earlier studies reported.\(^1,2,10\) A direct effect of acute bronchial edema on bronchial caliber is not negligible, especially in a child whose bronchial caliber is absolutely small. Bronchial edema in a process of inflammation is supposed to start by 2 to 4 h, reaches a peak at 6 to 12 h, and subsides by 24 h after the bronchial provocation.\(^10,20\) In the present study the peak bronchial obstruction due to edema might range from 4 PM to 10 PM in the morning challenge and from 12 midnight to 6 AM in the evening challenge. If I take the effect of edema on bronchial caliber into consideration, the clock hour of maximum bronchial obstruction (maximum LAR) may be more similar, around 2 AM, in the morning and the evening challenges.

Two patients (patients 1 and 6) had wider circadian variation, with marked decrement at 2 AM, than that seen in the other 4 patients during the control period. Nevertheless, the decrement in the FEV\(_1\) at 2 AM in the 2 patients following both morning and evening provocation seemed less variable than that in the others. It may be explained by the fact that the level of bronchial responsiveness before provocation, expressed as the amplitude of the circadian FEV\(_1\) variation, does not necessarily correlate with the magnitude of the LAR.\(^21\) Also, a partial explanation may be the selection of patients who had maximum bronchial obstruction with similar levels of FEV\(_1\). In the present study to investigate the temporal consistency between the maximum LAR and the trough of the amplified circadian variation, all the significant bronchial obstruction following the provocation had to be clinically tolerable without bronchodilators to avoid artificial influences on the bronchial caliber as much as possible. Smaller amounts of inhaled antigen as the stimulus and different levels of anti-inflammatory mechanisms in each patient could cause most maximum bronchial obstruction to be of similar severity.

Mohiuddin and Martin\(^13\) reported that the LAR following the evening challenge was more severe than that following the morning challenge in their four adult patients who all had a dual asthmatic response. Occurring IARs may depend on the degree of bronchial responsiveness before provocation and the strength of the stimulus.\(^21\) On the other hand, occurring LARs may depend on exacerbation of bronchial inflammation and an increased degree of bronchial responsiveness following the provocation.\(^22\) The asthmatic children in the present study had simply isolated LARs, and the difference in the magnitude of the LAR between the morning and the evening challenges was insignificant. Isolated LARs following antigen inhalation are usual in asthmatic children,\(^23\) although the exact reasons are unclear. The dose of the inhaled antigen might not be enough to cause an IAR but might be enough to cause bronchial inflammation with an increase in bronchial responsiveness and a subsequent LAR. Also, the lack of immediate bronchial constriction in the evening might ameliorate a further increase in bronchial responsiveness and decrease in bronchial caliber related to the clock hour.

Since children's bronchial responsiveness and bronchial caliber often change at short intervals,\(^24\) I made the control and the antigen challenge studies over two consecutive days to minimize the variability of the baseline bronchial caliber. Also, I used mainly the lowest FEV\(_1\) following the bronchial provocation as a marker of the LAR, since the FEV\(_1\) is hardly capable of disclosing an accurate beginning and ending of small
airway obstruction based on inflammation. The hour of the lowest FEV₁ was determined by visual inspection. Cosinor analysis, a standard method to evaluate circadian rhythm, may lose its accuracy in detecting a phase of rhythm, because acute exacerbation of bronchial inflammation and subsequent bronchial obstruction induced by artificial bronchial provocation might impair the genuine pattern of the circadian variation of bronchial caliber. The 4-h interval in consecutive FEV₁ measurements for 48 h, which was tolerable for the children, might also reduce the accuracy of the FEV₁ time course.

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