Direct Graphical Recordings of the Cumulative Dose-Response Curves of the Airway to Methacholine in Normal, Bronchitic and Asthmatic Subjects

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We studied bronchial responsiveness to methacholine in 10 normal subjects, in 60 patients with bronchial asthma and in 30 patients with bronchitis using a new device, with which we were able to obtain the dose-response curve of respiratory resistance (Rrs) continuously and graphically by the 3 Hz oscillation method during inhalation of methacholine. All normal subjects were non-responders, while all of the bronchial asthma cases, 63% of the chronic bronchitis cases and 50% of the acute bronchitis cases were responders. Among responders we found a very poor correlation between the initial respiratory conductance (Grs.cont) and the bronchial sensitivity (defined as the reverse of the cumulative dose until Rrs starts to increase); yet we found a good correlation between Grs.cont and the bronchial reactivity (defined as the slope of the decreasing rate of Grs). Metaproterenol and atropine decreased the bronchial sensitivity and reactivity in 28 asthmatic patients. We concluded that for clinical purposes the new method was very useful for assessing bronchial responsiveness to inhalation challenge because of its simplicity of operation as well as the quantitative differentiation between bronchial sensitivity and reactivity. Our results suggest that the bronchial response system to methacholine is explainable by a model of multiple dose-response curves without any parallel shift.

Bronchial responsiveness to various stimuli is used as one of the diagnostic criteria for bronchial asthma (American Thoracic Society 1962), and hyper-responsibility is also involved in chronic bronchitis or acute infectious respiratory disease (Parker et al. 1965; Simonsson et al. 1967; Empey et al. 1976; Little et al. 1978).

Received for publication, December 8, 1980.

A preliminary report of this investigation was presented at XIII World Congress on Diseases of the Chest in July, 1978 in Kyoto, and at the Annual Meeting of the American Lung Association, the American Thoracic Society, and the Congress of Lung Association Staff in May, 1979 in Las Vegas, Nevada, USA.
Since the study of Tiffeneau and Beauvallet (1945), the bronchial provocation test for estimating the hyperresponsibility of the airway has been generally performed by spirographic measurement after the inhalation of a bronchoconstrictor. In this procedure, subjects have to expire periodically with maximum efforts after each inhalation of the increasing doses of a bronchoconstrictor in order to obtain the threshold of responsiveness, i.e. a 20% decrease in the forced expiratory volume in 1 sec (FEV$_{1.0}$). Then, the following problems must be taken into consideration: 1) The test lacks objectivity due to its dependence on the efforts of human subjects. 2) Forced expiration itself may introduce bronchoconstriction (Gimeno et al. 1972) and may modify the degree of bronchoconstriction after a constricting stimuli. 3) This test is time-consuming for both subjects and investigator because of the complex and discontinuous procedures involved. Even though the measurement of airway resistance with a constant volume body plethysmograph (Fish et al. 1976; Rubinfeld and Pain 1977) or that of the maximum expiratory flow volume curve (Bouhuys et al. 1969; Olive and Hyatt 1972) has been used for the evaluation of bronchial responsiveness, the above problems have been not yet solved.

Recently Orehek et al. (1977) proposed not only the threshold of responsiveness (sensitivity) but also the slope of the bronchoconstrictor dose-response curve (reactivity) as an important factor for estimating the hyperresponsibility of the airway. They determined airway resistance by means of body plethysmography and defined bronchial sensitivity as 25% decrease in specific airway conductance. Therefore, this value did not indicate the true onset of the decrease of airway conductance, since it included a factor of bronchial reactivity.

In the present study we examined the characteristics of bronchial sensitivity and reactivity in normal subjects and in patients with acute bronchitis, chronic bronchitis and bronchial asthma using the new device, with which we were able to obtain the true sensitivity and reactivity by monitoring the continuous response curve of respiratory resistance ($R_{rs}$) during the inhalation of methacholine. In addition, we examined the effects of metaproterenol and atropine on the bronchial sensitivity and reactivity.

**Methods**

**Subjects**

In order to examine airway responsiveness to methacholine, we studied 10 normal subjects (all men, 9 nonsmokers and 1 smoker, mean age 29.9 years) without cardiopulmonary disease; 14 patients with acute bronchitis (8 men and 6 women, mean age 43.6 years); 16 patients with chronic bronchitis (8 men and 8 women, mean age 47.8 years); and 60 patients with extrinsic and intrinsic asthma (36 men and 24 women, mean age 41.8 years). For the examination of the effects of metaproterenol and atropine on bronchial responsiveness, we studied 28 asthmatic subjects (17 mean and 11 women, mean age 32.9 years).

Chronic bronchitis was diagnosed on the basis of the clinical history of cough and sputum for at least 3 months of the year over a minimum period of 2 years (American Thoracic Society 1962). Bronchial asthma was diagnosed on the basis of clinical history and evidence that the disease was markedly reversible through the use of bronchodilator drugs.
(American Thoracic Society 1962). Patients with acute bronchitis had a history of symptoms of sputum and cough for a short period only. All were outpatients who avoided all medication for at least 10 hr before the examination. All subjects with bronchial asthma were off-attack upon examination.

The apparatus of the bronchial provocation test

The principle of the new device is the continuous measurement of the respiratory resistance ($R_{rs}$) by the 3 Hz forced oscillation method during inhalation of aerosolized drugs (Fig. 1). The equipment consisted of three components as follows:

The aerosol delivery system. The aerosol delivery system could produce aerosol of various concentrations of drugs. It contained twelve nebulizers (Vaponephrin, mean particle size 2.5 µm according to the manufacturer's specifications) driven with a constant air flow of 5 liters per min by the air compressor (Nissho, Japan). The nebulizers were labeled No. 1 to No. 12. Each nebulizer was connected to the main tube between the mouthpiece and the flowmeter. The air compressor could be switched in turn from nebulizer No. 1 to No. 12 at constant time intervals. In this way, twelve kinds of aerosol could be sequentially delivered. Each nebulizer delivered approximately 0.15 ml solution/min.

The constant amplitude pressure generator. The constant amplitude pressure generator could produce a fairly constant amplitude pressure of 3 Hz sine wave at the mouth with a loud speaker box system which was similar to the system discussed in the previous report (Grimby et al. 1968). A 32 cm loud speaker mounted in a box was driven by a sine wave generator and power amplifier. The impedance tube, which gave high inertial impedance to the high frequencies but low impedance to the subject's normal breathing, was connected through the inside of the box to the atmosphere. A constant bias flow of 0.4 liter/sec was introduced just beyond the mouthpiece to minimize the dead space. The air flow at the mouth ($V$) was measured with the Fleisch pneumotachometer and a differential pressure transducer (Validyne, Model MP 45–1). The mouth pressure ($P_{ao}$) relative to ambient pressure was measured with the differential pressure transducer (Validyne, Model MP 45–1).

The display of $R_{rs}$. The $R_{rs}$ display consisted of an analog computer and an X-Y recorder. The calculation of $R_{rs}$ was performed by an analog computer according to Hyatt et al. (1970). In order to extract the component of only 3 Hz wave of flow and pressure, $V$ and $P_{ao}$ were passed through a 3 Hz band pass filter the width of which was 0.4 Hz, and the time constant of which was 0.3 sec. The flow was differentiated, and zero points of

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![Fig. 1. Block diagram of the apparatus.](image-url)
differentiated flow were identified by a zero comparator circuit. Moreover, the maximal and minimal flow, corresponding to the zero points of the differentiated flow, were sampled and held. From the maximal and minimal flow, \( \Delta V \) was derived by another subtraction circuit. Furthermore, two samples of pressure corresponding to maximal and minimal flow were subtracted to generate \( \Delta P_{Pao} \). \( R_{rs} \) was computed by a division circuit as \( \Delta P_{Pao}/\Delta V \). The \( R_{rs} \) was measured continuously, and was filtered by a 0.2 Hz low pass filter, the time constant of which was 4.0 sec, in order to avoid rapid fluctuations in \( R_{rs} \). The response time for 90% change of \( R_{rs} \) with this apparatus was 10 sec.

As the bronchial stimulus, we used methacholine hydrochloride solution in isotonic saline because of its stability and longer period of action in comparison to acetylcholine (Goodman and Gilman 1975). Ten dilution increments were used: 49, 98, 195, 390, 1563, 3125, 6250, 12500 and 25000 \( \mu g/ml \). Nebulizer No. 1 contained the 2 ml saline solution, while a 2 ml methacholine solution of each concentration was contained in nebulizers No. 2 to No. 11, respectively. Nebulizer No. 12 contained metaproterenol or atropine as a bronchodilator. When \( R_{rs} \) reached twice the initial \( R_{rs} \), metaproterenol was inhaled for a period of two min; or, when the inhalation of methacholine was performed to its maximum concentration; or, when subjects indicated a sign of dyspnea.

**Experimental protocol**

All subjects were examined during quiet breathing in a sitting position. Both sides of the cheeks were pressed by two balloons containing air, to minimize the oscillation of the cheeks.

In the first experiment, in order to examine the influence of the difference in the duration of the inhalation period for each methacholine concentration, ten asthmatic patients inhaled each concentration of the methacholine solution every minute on the first day and every two minutes on the second day. The second bronchial provocation test was performed within seven days of the first challenge. We compared two response curves of the \( R_{rs} \) in the same patients, whose clinical conditions were not different between the first and second challenges.

In the second experiment, we obtained the response curves of \( R_{rs} \) in 10 normal, 60 asthmatic and 30 bronchitic subjects during the inhalation of the incremental challenge. All subjects inhaled each concentration of the methacholine solution every minute.

In the third experiment, we examined the effects of metaproterenol and atropine on bronchial responsiveness to methacholine. In 16 asthmatic patients, the responsiveness to methacholine before and after administration of metaproterenol or atropine was examined on two separate days within seven days after the first challenge. In 10 of the patients, the methacholine challenge was performed on the first day as described above. On the second day, the methacholine challenge was performed immediately after the administration of 2% metaproterenol, inhaled for a period of 2 min. In 6 of the patients, the same protocol was employed except for the administration of 0.01% atropine inhaled for a period of 0.5 min. We selected this concentration and inhalation period of atropine, because we could find no response to methacholine after the pretreatment of 0.1% atropine at the 0.5 min inhalation period in the preliminary experiment.

In 12 other asthmatic patients, two methacholine challenges were performed consecutively in a single sitting. After the \( R_{rs} \) of the subjects reached twice the initial \( R_{rs} \), metaproterenol aerosol was administered for 2 min period in patients, or atropine (0.05%) aerosol was administered for 0.5 min period in the remaining 6 patients. After the \( R_{rs} \) decreased to its initial level, following the administration of both bronchodilator aerosols, the methacholine challenge was repeated. In this case, we selected a 0.05% concentration of atropine because the 2 min period of inhalation of 0.01% atropine did not restore the \( R_{rs} \) to its baseline, and the 2 min period of inhalation of 0.1% atropine completely blocked the response to the second challenge of methacholine following the restoration of \( R_{rs} \) in the first challenge.

**Analysis of the dose-response curves**

Fig. 2 shows an example of the dose-response curves obtained from the apparatus described above (and shown in Fig. 1). One unit of abscissa equals 1 min, i.e. the duration
Bronchial Sensitivity and Reactivity to Methacholine

Period of the inhalation of methacholine at specified concentrations. The Rrs was kept almost constant by the inhalation of a saline aerosol of 390 µg/ml of methacholine, after which the Rrs increased rapidly as the concentration of methacholine increased. After the inhalation of metaproterenol, the Rrs decreased immediately. To evaluate the dose-response curve, we used several indices as follows:

1. Grs.cont — The control value of the conductance; i.e. the reciprocal of Rrs during the inhalation of the saline aerosol.

2. Dmin — The threshold of responsiveness (Dmin); i.e. the amount of the cumulative dose at which point Rrs starts to increase. We defined the reciprocal of Dmin as bronchial sensitivity. We calculated the cumulative dose according to the standardization of inhalation challenge techniques recommended by Chai and co-workers (1975) (one unit equals 1 min of inhalation of aerosol solution at 1.0 mg/ml).

3. SGrS — The decreasing rate of conductance (Grs). Although we observed the curvilinear increase of Rrs during the methacholine inhalation after Dmin, we obtained an approximately linear dose-response curve by using Grs instead of Rrs. Therefore, we could represent it as the best fitting straight line (drawn by eye). The slope of the straight line, that is, \( S_{GrS} = -\frac{\Delta Grs}{\Delta t} \), was defined as the bronchial reactivity. Moreover, we obtained \( S_{GrS} \) normalized by Grs.cont as the corrected reactivity.

RESULTS

Response patterns of Rrs

Fig. 3 shows the typical response patterns of Rrs in a normal subject (Case 1), a patient with chronic bronchitis (Case 2), and patients with bronchial asthma (Cases 3-6). In the normal subject, the Rrs did not change until the maximum concentration of 25000 µg/ml of methacholine was reached. In chronic bronchitis, the Rrs increased gradually from a methacholine concentration of 3125 µg/ml to 25000 µg/ml, but decreased after the inhalation of metaproterenol. On the other hand, in bronchial asthma after the Rrs remained at an almost constant value for a short period, it increased rapidly and curvilinearly at various concentrations of
methacholine, but decreased rapidly after the inhalation of metaproterenol. The patterns in the cases of bronchial asthma were similar to isosceles triangles. Thus, by inspection of the response patterns of Rrs we could distinguish apparent differences among those of the normal subject, patients with chronic bronchitis and bronchial asthma.

Differences of inhalation duration in the increment challenge

We compared the $D_{min}$ obtained from the 1 min interval inhalation challenge ($D_{min} (1\ min)$) with that of the two minute interval inhalation challenge ($D_{min} (2\ min)$) in ten subjects who had almost the same value of $Rrs_{cont}$ (within 10%) in two response curves of $Rrs$. Fig. 4 shows the relationship between $D_{min} (1\ min)$ and $D_{min} (2\ min)$ in each subject plotted on the logarithmic scale.
The dotted line indicates the identity of $D_{\text{min}} (1 \min)$ and $D_{\text{min}} (2 \min)$. There was a highly significant correlation between $\log D_{\text{min}} (1 \min)$ and $\log D_{\text{min}} (2 \min)$. The regression line was expressed as follows: $\log D_{\text{min}} (2 \min) = 0.83 \log D_{\text{min}} (1 \min) - 0.10$, $r = 0.962$ ($p < 0.01$). From these results, we thought there was no definite difference between $D_{\text{min}} (1 \min)$ and $D_{\text{min}} (2 \min)$, and so far clinical convenience, in the following experiments, we chose the 1 min interval inhalation of a fixed concentration, increasing from 49 to 25000 $\mu g/ml$, in the step-wise incrementation described above.

**Incidence of $D_{\text{min}}, S_{Gr},$ and $S_{Gr}/Grs.cont$**

Table 1 shows the mean values and standard deviation of the initial conductance, the sensitivity ($1/D_{\text{min}}$), the reactivity ($S_{Gr}$) and the corrected reactivity ($S_{Gr}/Grs.\ cont$) in the responders of each group as well as the initial conductance in the non-responders of each group. We found a progressive increase of bronchial sensitivity in acute bronchitis, in chronic bronchitis and in bronchial asthma. There were significant differences in the sensitivity between acute bronchitis and chronic bronchitis ($p < 0.05$) and between chronic bronchitis and bronchial asthma ($p < 0.01$), respectively. The reactivity ($S_{Gr}$) was highest in acute bronchitis, lowest in chronic bronchitis, and medial in bronchial asthma; however, differences among three groups were not significant. On the other hand, the corrected reactivity was lowest in chronic bronchitis, medial in acute bronchitis and highest in bronchial asthma, in that order. We found a significant difference ($p < 0.01$) in $S_{Gr}/Grs.\ cont$ between bronchial asthma and chronic bronchitis, but no significant difference between acute bronchitis and chronic bronchitis, nor, between acute bronchitis and bronchial asthma.

Fig. 5 shows the incidence of $D_{\text{min}}, S_{Gr},$ and $S_{Gr}/Grs.\ cont$ in normal subjects and in three other groups of patients with pulmonary diseases. The classification of the grade of each parameter was performed according to the lower panel. That is, subjects who had a $D_{\text{min}}$ over the maximum 50 units ($S_{Gr}$ of zero liter/sec/cmH$_2$O/min or $S_{Gr}/Grs.\ cont$ of zero 1/min) were defined as non-responders; subjects who had a $D_{\text{min}}$ from 2 to 50 units ($S_{Gr}$ from zero to 0.025 liter/sec/cmH$_2$O/min or $S_{Gr}/Grs.\ cont$ from zero to 0.15 1/min) were defined as low responders; and subjects who had a $D_{\text{min}}$ below 2 units ($S_{Gr}$ over 0.025 liter/sec/cmH$_2$O/min or $S_{Gr}/Grs.\ cont$ over 0.15 1/min) were defined as high responders. 2 units of $D_{\text{min}}$ was the approximate upper limit of mean±standard deviation (s.d.) of $D_{\text{min}}$ for patients with bronchial asthma; while, 0.025 liter/sec/cmH$_2$O/min and 0.15 1/min were the approximate lower limits of mean±s.d. of $S_{Gr}/Grs.\ cont$ for the same patients. In our normal subjects we could not recognize the increase of $Rrs$ at all in the range of the methacholine concentration that was used. On the other hand, we recognized responders in 50% of the patients with acute bronchitis, 63% of those with chronic bronchitis and 100% of those with bronchial asthma. High responders ($D_{\text{min}}$ below 2 units) were found in 7% of the patients with acute bronchitis, in 31% of the patients with chronic bronchitis and in 80% of the patients with bronchial asthma.
High responders with $S_{Gr}$ over 0.025 liter/sec/cmH$_2$O/min were found in 50% of the patients with acute bronchitis, in 50% of the patients with chronic bronchitis and in 80% of the patients with bronchial asthma. Moreover, high responders with $S_{Gr}/Grs\cdot cont$ over 0.15 l/min were found in 29% of acute bronchitis cases, in 12.5% of chronic bronchitis cases and in 85% of bronchial asthma cases.

**Relationship between Grs.cont and Dmin or S$_{Gr}$**

Next we examined how the level of the initial state of the narrowing airway, that is, the control value of $Gr$, could modify the dose-response curves. Fig. 6A shows the relationship between $Grs\cdot cont$ and $Dmin$ in all responders. There was a

<table>
<thead>
<tr>
<th>Cases</th>
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<th>$Grs\cdot cont$ (liter/sec/cmH$_2$O)</th>
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<tr>
<td>Normal</td>
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<td>Non-responder 10</td>
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<td>Responder 0</td>
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<td>Acute bronchitis</td>
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<td>Non-responder 7</td>
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<td></td>
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<td>Responder 7</td>
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<td>Chronic bronchitis</td>
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<td>Non-responder 6</td>
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<td></td>
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<td>Responder 10</td>
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<td>Bronchial asthma</td>
<td>60</td>
<td>Non-responder 0</td>
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<td></td>
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<td>Responder 60</td>
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$Grs\cdot cont$: Initial respiratory conductance.

$Dmin$: Accumulated threshold of inhalation dose expressed by unit.

One unit was defined as 1 min inhalation of methacholine concentration of 1.0 mg/ml.

![Table 1. Mean values and standard deviations of initial conductance, and asthmatic subjects](image)

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$Grs\cdot cont$: Initial respiratory conductance.

$Dmin$: Accumulated threshold of inhalation dose expressed by unit.

One unit was defined as 1 min inhalation of methacholine concentration of 1.0 mg/ml.

Fig. 5. Incidence of responders to metacholine in normal subjects and in patients with acute bronchitis, chronic bronchitis and bronchial asthma. $Dmin$, $S_{Gr}$, and $S_{Gr}/Grs\cdot cont$ were classified in three degrees as shown in the lower panel.

High responders with $S_{Gr}$ over 0.025 liter/sec/cmH$_2$O/min were found in 50% of the patients with acute bronchitis, in 50% of the patients with chronic bronchitis and in 80% of the patients with bronchial asthma. Moreover, high responders with $S_{Gr}/Grs\cdot cont$ over 0.15 l/min were found in 29% of acute bronchitis cases, in 12.5% of chronic bronchitis cases and in 85% of bronchial asthma cases.

**Relationship between Grs.cont and Dmin or S$_{Gr}$**

Next we examined how the level of the initial state of the narrowing airway, that is, the control value of $Gr$, could modify the dose-response curves. Fig. 6A shows the relationship between $Grs\cdot cont$ and $Dmin$ in all responders. There was a
very poor correlation between both parameters ($r=0.381$, $p<0.01$). On the other hand, as shown in Fig. 6B, we found a good correlation between $Grs.cont$ and $S_{Grs}$ ($r=0.577$, $p<0.01$). The regression lines in the three groups were indicated as follows: $S_{Grs}=0.004+0.210 \; Grs.cont$ ($r=0.697$, $p<0.01$) in bronchial asthma; $S_{Grs}=0.006+0.102 \; Grs.cont$ ($r=0.788$, $p<0.01$) in chronic bronchitis; and $S_{Grs}=0.017+0.117 \; Grs.cont$ ($r=0.495$, N.S.) in acute bronchitis. We found a particularly significant correlation between $Grs. \; coat$ and $S_{Grs}$ (as shown in Fig. 7) in asthmatic subjects whose log $D_{min}$ was in the narrow range ($\pm 1/2$ s.d. of the mean log $D_{min}$ in all asthmatic patients). The regression line in these cases was indicated as $S_{Grs}=-0.003+0.229 \; Grs.cont$ ($r=0.780$, $p<0.01$).

**Fig. 6.** Relationship between $Grs.cont$ and $D_{min}$ (A); and $Grs.cont$ and $S_{Grs}$ (B). The $D_{min}$ is plotted on a logarithmic scale. — o, bronchial asthma; --- o, acute bronchitis; — x, chronic bronchitis.
Sensitivity ($1/D_{min}$) and reactivity ($S_{Grs}$)

The reactivity ($S_{Grs}$) correlated strongly with the initial state as shown in Fig. 6B. Therefore, in order to avoid the effect due to the difference from the initial state on $S_{Grs}$, we obtained the corrected reactivity ($S_{Grs}$ divided by $Grs_{.cont}$), and examined the relationship between the sensitivity and the corrected reactivity. We could not find any correlation between both parameters as shown in Fig. 8. Moreover, we obtained a relationship between the sensitivity and reactivity in asthmatic patients whose $Grs_{.cont}$ were within ±1/2 s.d. of the mean $Grs_{.cont}$ in all asthmatic patients as shown in Fig. 9, but we could not find a significant correlation between both parameters.

Effect of metaproterenol and atropine on responsiveness to methacholine

Fig. 10 shows typical cases of the dose-response curve from the inhalation...
Bronchial Sensitivity and Reactivity to Methacholine

Fig. 9. Relationship between bronchial sensitivity \( (1/D_{min}) \) and reactivity \( (S_{Gr}) \) in asthmatic patient with the narrow range of Grs.cont (±1/2 standard deviation of mean Grs.cont in all asthmatic subjects).

Fig. 10. Dose-response curves for methacholine before (1) and after (2) the administration of metaproterenol (A) and atropine (B) measured on separate days. After the administration of both metaproterenol and atropine, the threshold of response and the slope of the dose-response curve decreased in comparison to the control dose-response curve.

challenges administered on separate days. In Fig. 10A, the curve (1) shows the dose-response relationship before treatment of metaproterenol in the first challenge. The threshold of increase of \( Rrs \) corresponded to a methacholine concentration of 390 \( \mu g/ml \); and the bronchial sensitivity was 0.73 units (log \( D_{min}=-0.14 \)); the bronchial reactivity was 0.110 liter/sec/cmH\(_2\)O/min. The curve (2) shows the
dose-response curve after pretreatment of metaproterenol inhalation for 2 min. In comparison to the first challenge, the threshold was 2.45 units (log $D_{\text{min}}$=0.39) higher and the bronchial reactivity was 0.050 liter/sec/cmH$_2$O/min lower than that of the first challenge. Fig. 10B shows the dose-response curve before and after 0.01% atropine aerosol inhalation for 0.5 min. Before atropine inhalation, the threshold of response was 0.34 unit (log $D_{\text{min}}$=-0.47), and the bronchial reactivity was 0.120 liter/sec/cmH$_2$O/min. After atropine inhalation, the dose-response curve shifted to the right, and the slope also showed a definite decrease. That is, the threshold was 5.16 units (log $D_{\text{min}}$=0.71) higher than the value before the pretreatment of atropine inhalation; and the bronchial reactivity was 0.095 liter/sec/cmH$_2$O/min lower than the value before pretreatment.

Fig. 11 shows typical dose-response curves in the double methacholine challenge. Fig. 11A shows two dose-response curves in which metaproterenol was inhaled for 2 min as a bronchodilator. Fig. 11B shows the two dose-response curves in which 0.05% atropine was inhaled for 2 min as a bronchodilator. Fig. 11B shows the two dose-response curves in which 0.05% atropine was inhaled for 2 min. In both cases, after the inhalation of bronchodilators, we found that the second dose-response curve shifted to the right and that the threshold of the response was higher; while the reactivity was lower than those of the first challenge.

Fig. 12A shows the change of the sensitivity and corrected reactivity to methacholine before and after pretreatment of metaproterenol or atropine. The before and after values are connected by a line for each patient for each separate challenge. In both bronchodilators, the sensitivity and corrected reactivity were reduced significantly after the bronchodilator inhalation (paired t-test, $p<0.001$). We observed three types of differences in the effects of the drugs independent of the

![Graph showing dose-response curves](image)

Fig. 11. Dose-response curves of a double methacholine challenge before and after the administration of metaproterenol aerosol (A) and atropine (B). After the administration of metaproterenol or atropine, the threshold of response and the slope of the dose-response curves of the second challenge decreased in comparison to those of the dose-response curves of the first challenge.
kind of drug we used (metaproterenol or atropine): First, a large reduction of
the sensitivity with only a slight reduction of the reactivity; second, a slight
reduction of the sensitivity with a large reduction of the reactivity; and third, a
large reduction of both parameters.

Fig. 12B shows the correlation of the sensitivity and corrected reactivity to
methacholine before and after pretreatment of metaproterenol or atropine. The
before and after values are connected by a line for each patient for each double
challenge. Both the sensitivity and corrected reactivity were reduced significantly
after the bronchodilator inhalation (paired t-test, p<0.001). As in the results of
the separate challenge, we also observed three types of differences in the effects of
the drugs, independent of the kind of drug we used.

**Discussion**

*Usefulness of our method*

The bronchial provocation test is extremely important not only for examin-
ing the bronchial responsiveness in various pulmonary diseases, but also for diagnos-
ing bronchial asthma. However, as the method of examination has not yet been
established, each investigator has been forced to proceed by his own method.
Therefore, the comparison of data among different laboratories has been difficult.
Recently Chai et al. (1975) tried to standardize the bronchial inhalation challenge
procedures due to antigen, methacholine and histamine. Using their method, to
obtain the dose-response curve, subjects must inhale aerosol from the residual
volume to the total lung capacity, and perform multiple forced expiratory ma-
nevers or pant many times after every inhalation of the different concentrations of
bronchostimulators. We felt that these procedures themselves might modify the degree of bronchoconstriction (Gimeno et al. 1972), and furthermore that the method was too complex to be used for a screening test. Our present apparatus, however, is able to solve these problems.

Using our method, we are able to measure the continuous change of \( R_{rs} \) during quiet breathing. In addition, the test is over in a short time (about 10–15 min for one subject) and is quite safe, because the measurement could be performed monitoring the change of \( R_{rs} \) and finished without inducing an asthma attack. Therefore, our method is useful for the clinical and screening test of bronchial hyperresponsibility.

**Analysis of the dose-response curves**

We found differences in response patterns among normal subjects, patients with acute or chronic bronchitis and patients with bronchial asthma (Fig. 3). An analysis by inspection itself, however, is not sufficient either for a comparison of various pulmonary diseases or for the purpose of a comprehensive survey. Therefore, we examined some indices which characterize the individual curves. Among them, \( G_{rs, cont} \), that is, the reciprocal of \( R_{rs} \) during the inhalation of saline aerosol, was readily obtained. The sensitivity, that is, the reverse of \( D_{min} \), was also easily obtained by inspection as the point at which \( R_{rs} \) started to increase, or \( G_{rs} \) started to decrease. For estimating the reactivity, we used the slope of the dose-response curves of \( G_{rs} \) obtained from the original dose-response curves of \( R_{rs} \) because of the following reasons: (1) We found the curvilinear increase of \( R_{rs} \) during methacholine inhalation over \( D_{min} \) in the original dose-response curve, so we thought that the slope as the change from 125% \( R_{rs} \) of \( R_{rs, cont} \) to 160% \( R_{rs} \) of \( R_{rs, cont} \) according to Orehek et al. (1977), might reflect only a portion of the whole slope. (2) On the other hand, we recognized the linear relationship in the dose-response curves of \( G_{rs} \) (at least from 100% \( G_{rs, cont} \) to 50% \( G_{rs, cont} \)), so we thought that the slope of the dose-response curves of the \( G_{rs} \) might reflect the whole slope of the original dose-response curve. (3) We could obtain this slope easily. (4) In addition, according to the dose-response curve generally used for the expression of pharmacological response (Van Rossum 1963), we reconstructed the relationship between the cumulative dose on the semilogarithmic scale and \( G_{rs} \), from the original dose-response curve of \( R_{rs} \), in 25 cases of bronchial asthma. From this dose-response curve, we obtained the change of \( G_{rs} \) from 80% \( G_{rs, cont} \) to 50% \( G_{rs, cont} \) (\( \Delta G_{rs} \)) and the change of the logarithmic values of the cumulative doses from 80% \( G_{rs, cont} \) to 50% \( G_{rs, cont} \) (\( \Delta \log \text{Cumulative dose} \)), and calculated the slope from the two changes as \( \frac{\Delta G_{rs}}{\Delta \log \text{Cumulative dose}} \). A shown in Fig. 13, we compared this slope with the slope previously defined. We found that both slopes resulted in a good correlation (\( r=0.887, p<0.01 \)). For all of these reasons, we thought that the slope expressed as \( S_{Gr, r} (\frac{-\Delta G_{rs}}{\Delta t}) \) was clinically convenient for estimating the reactivity.

In this study, in order to express the amount of the inhalation dose, we used a
cumulative dose of which 1 unit was defined as 1 min inhalation of 1 mg/ml according to the recommendation by Chai et al. (1975). In the first study, we found that the \textit{Dmin} of the 1 min interval inhalation challenge corresponded to the \textit{Dmin} of the 2 min interval inhalation challenge for the same subjects (Fig. 4). This fact suggested that for the expression of \textit{Dmin}, the use of the cumulative dose was reasonable, and that the onset of bronchial contraction might be dependent on the amount of aerosol deposition in the airway. On the basis of this result, we also used the incremental inhalation challenge of every minute for the standardized examination. However, when we inhaled 6250 \( \mu \text{g/ml} \) of methacholine by the step method for some bronchial asthma patients, we could not always recognize the correspondence between \textit{Dmin} of the inhalation challenge every minute and the step inhalation challenge (unpublished data). Therefore, we could not say strictly that \textit{Dmin} would be dependent on the amount of aerosol deposition in the airway. Since the amount of aerosol deposition might be affected by the differences of respiratory rate, ventilatory volume, kinds of nebulizers or the amount of bias flow, the dose-response curve in this study are probably quite dependent on the apparatus used. In the future, when we determine the standard technique for the bronchial provocation test, we will have to consider these factors.

\textit{Bronchial responsiveness of normal subjects and patients with bronchial asthma or bronchitis}

There are some studies reporting that not only asthmatic patients but also bronchitic patients have obtained increased responsiveness to the bronchoconstrictor. Parker et al. (1965) showed an increased responsiveness by a reduction in \( \text{FEV}_{1.0} \) (15\%-30\% decrease) after 5 breaths of 2.5\% methacholine in 8 of 14 subjects (57\%) with chronic bronchitis; and in 4 of 8 normal subjects (50\%) with acute upper respiratory diseases. Then, Empey et al. (1976) also recognized increases
(greater than 100 per cent) in airway resistance after inhalation of 1.6% histamine (10 breaths) in 12 of 16 subjects (75%) with upper respiratory tract infections. Though the method of the challenge was different, we also found responders in 63% of the patients with chronic bronchitis and in 50% of the patients with acute bronchitis. Our results confirmed their findings, and we thought an increase of responsiveness might be an important factor in the deterioration not only in the clinical course of bronchial asthma but also in the condition of bronchitic patients when they suffer from viral or bacterial respiratory tract infection.

The mechanism of increased responsiveness in the infections of the respiratory system was not clear. Recently, however, some attractive papers have reported that airway epithelial damage by exposure to the infection and sensitization of the rapidly adapting airway receptors to inhaled irritants may cause increased bronchoconstriction via a vagal reflex. Little et al. (1978) recognized that bronchial responsiveness to carbachol aerosol was markedly increased in subjects with nonpneumonitic influenza A viral infection, and that airway hyperreactivity gradually diminished during a 7-week period. Moreover, Lee et al. (1977) and Golden et al. (1978) demonstrated that ozone exposure caused a marked increase in responsiveness to histamine aerosol in healthy dogs (Lee et al. 1977) and in normal subjects (Golden et al. 1978). This response was prevented by atropine or by cooling of the vagus nerves. In these reports they supposed that the increased responsiveness was caused by epithelial damage to viral infection or ozone exposure, i.e. the sensitization of the exposed rapidly adapting airway receptors and the subsequent exaggeration of bronchoconstriction via cholinergic postganglionic $G_r$ pathways.

**Determining factors of $D_{min}$ and $S_{Grs}$**

Although the correlation between the initial $G_{rs}$ and $D_{min}$ was very poor, we found a good correlation between $G_{rs, cont}$ and $G_r$, especially in bronchial asthma cases with approximately identical $D_{min}$. These results indicate that the initial conductance before the methacholine challenge might have an influence on the reactivity described here; that is, the smaller the initial airway dimension before the methacholine challenge, the higher the reactivity. In addition, we recognized that at the same $G_{rs, cont}$, the reactivity was highest in bronchial asthma cases, lowest in chronic bronchitis cases and medial in the acute bronchitis cases as shown in the three regression lines of Fig. 6.

We can explain the relation between initial bronchial dimension and the reactivity as follows: If we assume the airway as a single cylindrical tube as shown in Fig. 14, and consider that conductance to laminar flow through a tube is proportional to the fourth power of its radius (Hagen-Poiseuille’s law), the relation between the radius and the conductance of the tube could be approximately expressed as a straight line from the initial conductance to the point which is less than or equal to 50% decrease of the initial conductance (Fig. 14A). This means that the relationship between the initial conductance and the ratio of the change
of conductance over the change of the radius \( \Delta G/\Delta \text{Radius} \) is approximately linear (Fig. 14B). Therefore, we can speculate that when the change of radius for a given dose \( \Delta \text{Radius}/\Delta \text{Dose} \) is equal, that is, the rate of the contraction of the smooth muscles for a given dose, the cases of high initial conductance show high \( \Delta G/\Delta \text{Radius} \) or hyper-reactivity. Furthermore, when the initial conductance is equal, and \( \Delta \text{Radius}/\Delta \text{Dose} \) is high, we have hyper-reactivity; in the opposite instance, when it is low, we have hyporeactivity (Fig. 14C). Therefore, we can assume that the relationship between \( S_{G,s}(\Delta G_{s}/\Delta \text{Dose}) \) and \( G_{s,.cont} \) is linear and that the group with hyper-reactivity in our study is presented by the regression line with the greater slope as shown in Fig. 14D.

As such, the differences of the reactivity among bronchial asthma, acute bronchitis and chronic bronchitis could be explained by the differences of the change of radius for a given dose, that is, the rate of the smooth muscle contraction for the same amount of methacholine. Therefore, the change of the radius in bronchial asthma due to methacholine inhalation would be larger (hyper-reactivity) than that in the other two groups at the same \( G_{s,.cont} \), which explains the fact that the slope of the regression line in bronchial asthma is greater than that of the other two groups. For these reasons we thought that the reactivity in our study should be standardized by the initial \( G_{s} \) in order to compare the reactivity of various dose-response curves with different \( G_{s,.cont} \).

Rubinfeld and Pain (1977) suggested that as the absolute rate of decrease of specific conductance after methacholine inhalation was affected by the initial state of conductance, the decreasing rate should be standardized by the initial conductance. Our results confirm their findings. Recently Benson and Graf (1977) examined the interaction between inhaled histamine and vagal stimulation on the airway of anesthetized and vagotomized dogs. They found that the bronchoconstrictor response to a combination of electrical stimulation of the vagus nerves and inhaled histamine produced a reduction in airway caliber which was significantly greater than the additive results of the two stimuli applied separately; and that the effect of vagal stimulation was to produce a shift in the dose-response curve of inhaled histamine. From these results they concluded that
an increase in the resting bronchomotor tone may contribute to the increase of responsiveness observed in patients with asthma. In their report, they explained the mechanism of the increase of responsiveness by the model of a single dose-response curve of the bronchial smooth muscles to a constrictor stimuli. The relationship between $R_{rs, cont}$ and $S_{Gr}$ in our studies might be explained by their model.

As shown in Fig. 8, we could not find a significant correlation between sensitivity and corrected reactivity. This may mean that both parameters are independent of each other, and that different mechanisms would determine sensitivity and reactivity. Furthermore, the mechanism of bronchial response to the bronchoconstrictors could not be explained by a model of the single dose-response curve indicated by Benson and Graf (1977), but rather by a model with multiple dose-response curves without any parallel shift.

Orehek et al. (1977) obtained the dose-response curve for aerosolized carbachol in normal subjects and patients with bronchial asthma using changes in specific airway conductance ($Gaw$). They showed the wide variations in bronchial sensitivity among subjects, defining sensitivity as the dose of carbachol needed to cause a 25% decrease in specific $Gaw$ ($D_{25}$) and the reactivity as the slope of the curve between 25% decrease and 60% decrease in specific $Gaw$. They did not recognize a significant correlation between the 2 parameters which was similar to the findings about the relationship between bronchial sensitivity and corrected reactivity in our study. They also suggested that the bronchial response system to bronchoconstrictors might not be explainable by a single dose-response curve. Again, our results confirm theirs, however, there were some differences between our definitions and theirs concerning bronchial sensitivity and reactivity. For example, $D_{25}$ did not take into account the amount of the dose at the onset of the decrease of specific $Gaw$, and might already have included a factor of reactivity. Moreover, as the reactivity according to Orehek et al. (1977) was obtained as the correlation coefficients calculated by the method of least squares between the % decrease of the specific $Gaw$ and the cumulative dose, it would not be suitable for a comprehensive survey because of the difficult calculations involved.

**Effect of metaproterenol and atropine on responsiveness to methacholine**

It is still not apparent how the bronchodilators can affect airway responsiveness. Casterline et al. (1976) studied the effect of atropine and albuterol aerosol on bronchial response to histamine in human subjects. They reported that although both bronchodilators blocked the response to histamine, the effect of atropine was smaller than that of albuterol. Drazen and Austen (1975) found that the threshold determined from the dose-response curve of pulmonary resistance to intravenous acetylcholine in guinea pigs increased significantly after atropine infusion in comparison to the threshold before atropine infusion. In these studies they did not discuss the effects of the bronchodilator in terms of the sensitivity and reactivity. Therefore, we examined the effects of metaproterenol, a $\beta$
adrenergic stimulant; and atropine, an anticholinergic agent, on the responsiveness to methacholine using our apparatus.

Generally speaking metaproterenol is thought to have a non-competitive agonist-antagonist relationship to methacholine, and atropine is thought to have a competitive agonist-antagonist relationship to methacholine (Van Rossum 1963). If this concept is true, we can anticipate the difference between the effects of metaproterenol and atropine in the following ways: Metaproterenol will not cause a shift of the dose-response curve in parallel with the response axis, that is, metaproterenol can reduce both the sensitivity and reactivity; atropine will shift the dose-response curve in parallel which means a reduction of sensitivity only. Contrary to our expectations, however, we discovered that atropine reduced both parameters as if it were a $\beta$ stimulant. The effect of metaproterenol may be explained by the pharmacological non-competitive agonist-antagonist relationship between metaproterenol and methacholine. On the other hand, we cannot explain the effect of atropine solely by the competitive agonist-antagonist relation between atropine and methacholine.

From our results we could not prove whether the change after atropine inhalation was caused by the atropine effect itself or by other factors. However, in regard to an explanation of the $\beta$ stimulant-like effect of atropine, we can make some speculations. Cabezas et al. (1971) found in dog experiments that the stimulation of the sympathetic nerves inhibited vagal bronchoconstriction but did not block it completely. That is, they indicated that the vagal constrictor pathway was more dominant than the sympathetic pathway. From their result, we can suppose that if the efferent vagal nerve is blocked completely by atropine inhalation, the sympathetic nerve system may be activated, which would result in reducing the bronchial reactivity to methacholine. On the other hand, Mills et al. (1969) have reported that airway contraction itself stimulates the airway irritant receptors, and Orehek et al. (1977) supposed that this phenomenon could create a positive vagal feedback loop and increase the reactivity. If atropine blocks this feedback loop, then it may reduce the reactivity.

As another possibility to explain the modification of the effect of atropine, there may be participation of the adrenal gland during the methacholine provocation test as supposed in a report that histamine-mediated bronchoconstriction may be modified by catecholamine released from the adrenal gland (Drazen 1978). Moreover, since it has been demonstrated that the contraction of the guinea pig trachea provokes the synthesis of prostaglandins (Orehek et al. 1975), increase of prostaglandin could also alter the reactivity to methacholine.

In our study, we found that dose-response curve to the bronchoconstrictor in the human airway was different from that observed in the in vitro experiments (Van Rossum 1963). To explain this discrepancy, we must also consider the physiological factors involved in the measurement of $Rrs$ in addition to the effects of the drug itself.

According to Weibel (1963), the total airway cross section area will get larger
in the peripheral airways than in the central airways. Therefore, the effect of a bronchoconstrictor on airway resistance becomes smaller as the airway increases its generation. Moreover, the $R_{rs}$ eventually reflects the over-all airway resistance, including in itself the resistances of both lung tissue and chest wall. However, we have not yet obtained information on the effects of bronchoconstrictors on these particular resistances.

In addition, the change of $R_{rs}$ may not directly reflect the change of the airway smooth muscle tone. Sasaki et al. (1978) and Takishima et al. (1975) indicated that the bronchomotor tone was counter-balanced by the lung parenchyma. Therefore, bronchial smooth muscle tone could be greatly modified by the lung parenchyma, the elastic recoil of which may also be affected by the bronchoconstrictor.

Thus various factors could modify the change of $R_{rs}$, so it is not surprising to see differences between our results and that of the in vitro experiments in terms of the correlation between the response and the amount of dose.

References

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