Ventilation distribution during histamine provocation

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For the study of ventilation distribution in the normal or the diseased lung, various techniques have been used to evaluate the sites and mechanisms of ventilation inhomogeneity. In the area of bronchoconstriction, radioisotope techniques have been applied to identify central and peripheral deposition of a radiolabeled bronchoconstrictor agent and to assess its effect on lung function and gas exchange (19, 21) or, alternatively, to evaluate modifications in distribution of a radiolabeled aerosol or radioactive xenon when inhaled immediately before and after bronchoprovocation (5, 27). In general, the resolution of the gamma camera used for this type of study is limited and only allows differences in ventilation between relatively large lung zones, i.e., comprising several hundreds of acini, to be evaluated. A second group of tests to differentiate between possible gas concentration differences generated in very small units, i.e., at the level of acini, and those generated in much larger lung units, which would also become apparent with the above-mentioned tests.

MATERIAL AND METHODS

Equipment. All lung function parameters, except those related to the MBW test, were obtained by means of standard lung function laboratory equipment (Sensormedics, Biltovhen, The Netherlands) and according to recommended procedures (1). The MBW tests were performed with a dedicated breathing assembly incorporating a set of pneumatic valves enabling communication with a 400-liter bag-in-box system (Fig. 1). Inspiratory and expiratory bags in the box are connected to the subject through a nonrebreathing valve that separates the inhaled and expired air. A third connection between the patient and the box is for air breathing to and from the box. A Fleisch-type pneumotachograph is fitted in the wall of the box to record all volume changes generated by the subjects breathing in and out from either the bags or the box. The Flow signal from the pressure transducer is integrated to give volume. For continuous monitoring of N2 concentration at the mouth, the needle valve from an N2 analyzer (P. K. Morgan, Kent, UK) was fitted in the tubing in front of the subject’s mouth. Finally, the subject was equipped with the rib cage band of a respiratory inductance plethysmograph (model 150, AMI), only as an independent means of monitoring end-tidal lung volume position. Volume, N2 concentration, and rib cage signals were acquired by using a dedicated Labview program (National Instruments, Austin, TX), which also controlled the valves and provided a visual feedback of volume on a monitor in front of the subject.

Procedure and subjects. The MBW test requires a regular breathing pattern with a tidal volume of ~1 liter, starting from functional residual capacity (FRC) by using pure O2 for inspiration. This was handled as follows. During a short period of quiet breathing on the mouthpiece, the subject,
withdrawing the screen, breathed air from the box in and out. When the operator observed on the screen a breathing pattern with stable end-tidal volume, the computer was given a signal to switch the inspiratory path from box (air) to the inspiratory bag (100% O2). The switch occurred during an exhalation so that, starting from the subsequent inspiration, the subject would breathe 100% O2 from the inspiratory bag. With our valve configuration, the dead space volume, i.e., the volume that does not contain 100% O2 before the MBW test, is 50 ml. As soon as the valves were switched to O2, the subject was instructed to watch the screen during each inspiration and fill a tank up to an indicator line and then exhale freely back to his FRC. The tank content was a graphical representation of pneumotachograph-integrated volume, and the line indicator corresponded to a target 1-liter inspiration, starting from the end of the previous exhalation. After 20–25 breathing cycles, the subject was asked to exhale completely down to residual volume. The exact number of breaths was dependent on subject performance and on progressive N2 dilution. The interval between any two subsequent MBW tests was dependent on the rate of return to baseline alveolar N2 concentration in each subject.

The MBW tests were performed by each subject in three stages that are hereafter referred to as baseline, bronchoprovocation, and bronchodilatation. Baseline measurements included single-breath CO-dilution capacity (D1-CO), D2-CO divided by alveolar volume (k2-CO), FEV1, forced vital capacity (FVC), peak expiratory flow (PEF), and forced expiratory flow after exhalation of 75% FVC (FEF75). After baseline lung function testing, three baseline MBW tests were performed, followed by a forced expiration to give another baseline set of FEV1, FVC, PEF, and FEF75 values.

The bronchoprovocation stage started when the subject inhaled successively increasing doses of extract until FEV1 had decreased by >20% compared with baseline, or a cumulative dose of 2 mg histamine had been inhaled (vital capacity breath; dosimeter MB3, MEFAR, Bovezzo, Italy). At this point, the subject performed one forced expiratory maneuver, two MBW tests, and another forced expiration. Finally, the subject was given salbutamol (100 µg Ventolin, 2 puffs), and 10 min later bronchodilatation was assessed by performing a forced expiration, followed by two MBW tests and another forced expiration. Note that, at each stage of the study, a set of two or three MBW tests was preceded and followed by a forced expiratory maneuver before the subject passed on to the next stage. This was mainly done not only to account for any effect of ongoing constriction or dilatation over the course of the period during which two MBW tests were performed (typically, 9 min) but also to verify any possible influence that the MBW pure O2 breathing could have on the forced expiratory maneuver.

If provocation with a cumulative dose of 2 mg histamine failed to provoke a 20% decrease in FEV1, the subject was classified as nonhyperresponsive. We studied 20 symptom-free volunteers (11 men, 9 women), with ages ranging from 18 to 42 yr, until we accumulated 10 hyperresponsive and 10 nonhyperresponsive subjects. Subject recruitment was done on a volunteer basis, and a questionnaire was also used that asked for risk factors to better target the number of subjects in each group. In particular, all subjects were nonsmokers, none took any medication, and none suffered from upper airway infection.

Method of analysis. Figure 2 shows volume and N2 concentration tracings, as a function of time, obtained from a typical MBW experiment performed by a subject in the provocation stage. According to traditional MBW analysis, the continuous N2 concentration tracing of Fig. 2 is translated into a so-called “N2 washout curve” obtained by plotting, on a semilogarithmic scale, the progressive decrease of mean expired N2 concentration in each subsequent breath. Figure 3A shows N2 washout curves derived from three baseline MBW tests (closed symbols) and from two provocation MBW tests (open symbols). Mean expired N2 concentration of each breath is expressed as a percentage of the initial N2 concentration in the lungs (IN2), and its logarithm is plotted as a function of lung turnover (TO), i.e., cumulative expired volume divided by the subject’s FRC. FRC was computed from the quantity of cumulatively expired N2 down to the point where 1.5% of [N2] had been reached. Typically, for a tidal volume = 1 liter and FRC = 3 liters, as was the case for the subject with the N2 washout curve in Fig. 3A, it takes about three breaths to reach one lung TO. The reason for using lung TO instead of breath number on the abcissa in Fig. 3A is that it allows for better comparison of subjects with different lung volumes and dilution (6).

The MBW tests were also analyzed according to a method first proposed in a theoretical work by Paiva (16) and subsequently applied experimentally by Crawford et al. (9). Basically, this consists of treating each expiration as a single-breath N2 washout and determining breath by breath the alveolar slope, by linear regression of N2 concentration vs. expired volume in the alveolar phase III. We used a linear regression between 0.65 liter and the end of expiration (nominally, 1 liter), with a possibility for readjustment of slope limits to avoid possible disturbance of, e.g., cardiogenic oscillations, especially in the baseline phase MBW tests. For each breath, alveolar slope is then divided by mean expired N2 concentration of that breath, to give a normalized alveolar slope (S). The inset of Fig. 2 illustrates a large increase in the normalized phase III slope between breaths 1 and 20 in the case of a provocation MBW test performed by a hyperresponsive subject. Figure 3B is a graphical representation of all S values as a function of TO, obtained in the same subject, where closed and open symbols represent the average of three baseline MBW tests and two provocation MBW tests, respectively.
In Fig. 3, A and B, the provocation curves (open symbols) are derived to illustrate the alveolar and acinar slopes of the MBW (see Theoretical background). The magnitude of $S_{\text{alin}}$ and $S_{\text{cond}}$ is determined by linear regression in that part of the MBW where only conductive airways are known to contribute to the rate of rise of $S$, i.e., between TO = 1.5 and TO = 6 (see Theoretical background). $S_{\text{alin}}$ is determined by subtracting that part attributable to the conductive airways from the slope of the first breath, i.e., $S_{\text{cond}}$ multiplied by the TO value of the first breath (~0.3 in the case of Fig. 3B). In the example in Fig. 3B, the baseline MBW leads to $S_{\text{cond}} = 0.02$ liter$^{-1}$ and $S_{\text{alin}} = 0.15$ liter$^{-1}$, and the provocation MBW leads to $S_{\text{cond}} = 0.12$ liter$^{-1}$ and $S_{\text{alin}} = 0.15$ liter$^{-1}$. In fact, a sixfold rate of increase in $S_{\text{cond}}$ per unit TO, and a smaller value for $S_{\text{alin}}$ is reported here to relate to indexes that have been used in the clinical context before. For this same reason we also computed the alveolar and acinar slopes and their difference per unit TO, and the provocation MBW leads to a positive $S_{\text{alin}}$ slope. One of the factors that has been hypothesized to contribute to the alveolar $S_{\text{lin}}$ slope is gravity-dependent flow sequencing between upper and lower lung units. Although the lung units involved need a priori not be as large as, e.g., entire lung regions, they need to be subtended by conductive airways proximal to the diffusion front to be solely convection dependent. The second mechanism, also referred to as diffusion-convection-dependent inhomogeneity, reflects a far more complex diffusion-convection interaction process without necessity for convective flow sequencing during expiration to produce a positive $S_{\text{alin}}$ slope. For this mechanism to apply, two
conditions need to be fulfilled: 1) comparable magnitude of convective and diffusive transport and 2) asymmetry of the lung structure where diffusion and convection interaction can develop. Asymmetry may be due to unequal narrowing of parallel airways or differences in volume subtended by two daughter branches. Even in normal healthy subjects, these two conditions are met in the lung periphery, more specifically at the acinar level of the bronchial tree where the diffusion front stands. In the case of abnormal lung behavior such as airway inflammation or emphysematous lesions, asymmetry may be increased, leading to an increased N2 slope.

The inset of Fig. 3B shows theoretical predictions of S generated by the two mechanisms described above (solid lines), the sum of which typically corresponds to a smoothed version of the experimental provocation S curve (open symbols). The diffusion-convection interaction produces an initial S value that only slightly increases and very rapidly reaches a horizontal asymptote (x). This S asymptote corresponds to an equilibrium state of convection and diffusion, in which relative concentration differences remain constant throughout most of the MBW. The convective sequential emptying produces a steady increase of S (asterisk), reflecting the fact that concentration differences relative to the mean alveolar concentration increase progressively because the best-ventilated lung units get better ventilated at every subsequent inspiration. Moreover, distances between these relatively large units are too large to be covered by diffusive transport, i.e., diffusive homogenization of the concentration differences is negligible. In fact, with this mechanism, S can only eventually reach a horizontal asymptote if one of the large lung units gets washed out completely.

With respect to the experimental S curves, we can summarize here that the S value for the first breath of the MBW is predominantly generated by diffusion-convection-dependent ventilation inhomogeneity in the peripheral acinar lung units. The actual acinar contribution to ventilation inhomogeneity can be characterized by S_{acinar} by subtracting the estimated convection-dependent contribution from the slope of the first breath. The convection-dependent ventilation inhomogeneity, which is generated by unequal inspired concentration and flow sequencing between larger lung units, becomes more apparent as the MBW progresses. Because these large units roughly correspond to lung units subtended by branch points in the conductive airway zone, we refer to S_{cond} for this large-scale ventilation inhomogeneity.

Given these definitions of S_{acinar} and S_{cond}, their baseline values should be considered as two independent indexes of ventilation inhomogeneity in the lungs: S_{acinar} reflects ventilation inhomogeneity resulting from a normal peripheral lung structure with a given asymmetry, and S_{cond} results from a given difference in ventilation between any two diffusion-independent lung units. Whenever S_{acinar} undergoes important changes with respect to baseline, this is due to an important alteration in the peripheral lung structure. Whenever S_{cond} is increased, there has been a change in the conductive airways or the pressure-volume characteristics of the lung units subtended by these conductive airways.

Statistical analysis. All values are given as means ± SE. Using a software package ( Primer of Biostatistics, McGraw-Hill), we performed a repeated-measures analysis of variance followed by a post hoc Bonferroni test for pairwise comparisons. Paired and unpaired comparisons were made with a t-test. For all statistical analyses, P < 0.05 was considered significant.
Table 1. Baseline values of lung function and MBW parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NBHR Group</th>
<th>BHR Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1, %pred</td>
<td>121 ± 3</td>
<td>110 ± 2*</td>
</tr>
<tr>
<td>FEV1/FVC, %pred</td>
<td>86 ± 1</td>
<td>80 ± 3*</td>
</tr>
<tr>
<td>PEF, %pred</td>
<td>118 ± 4</td>
<td>104 ± 4*</td>
</tr>
<tr>
<td>PEF75, %pred</td>
<td>111 ± 9</td>
<td>84 ± 9*</td>
</tr>
<tr>
<td>DlCO, %pred</td>
<td>98 ± 7</td>
<td>105 ± 6</td>
</tr>
<tr>
<td>KCO, %pred</td>
<td>86 ± 7</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>VDatan, ml</td>
<td>175 ± 12</td>
<td>166 ± 9</td>
</tr>
<tr>
<td>VDphys, ml</td>
<td>204 ± 13</td>
<td>201 ± 9</td>
</tr>
<tr>
<td>VDphys - VDatan, ml</td>
<td>29 ± 5</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>FRC, ml</td>
<td>3.139 ± 400</td>
<td>3.139 ± 233</td>
</tr>
<tr>
<td>Curv</td>
<td>0.839 ± 0.018</td>
<td>0.847 ± 0.026</td>
</tr>
<tr>
<td>log[N2]6TO</td>
<td>0.395 ± 0.022</td>
<td>0.376 ± 0.034</td>
</tr>
<tr>
<td>Sadin, liter⁻¹</td>
<td>0.075 ± 0.007</td>
<td>0.107 ± 0.008*</td>
</tr>
<tr>
<td>Scond, liter⁻¹</td>
<td>0.033 ± 0.003</td>
<td>0.023 ± 0.002</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 subjects/group. MBW, multiple-breath washout; pred, predicted; FEV1, forced expired volume in 1 s; FEV1/FVC, FEV1 per unit forced vital capacity; PEF, peak expiratory flow; PEF75, forced expiratory flow after expiration of 75% FVC; DlCO, carbon monoxide-diffusing capacity; KCO, DlCO per unit alveolar volume; VDatan and VDphys, anatomic and physiological dead space derived from the 1st breath of MBW, respectively; FRC, functional residual capacity derived from MBW; Curv, log[N2]6TO, Sadin, and Scond: curvilinearity of N2 washout curve, value of curve for 6 turnover, and measurement of ventilation in homogeneity in conductive and acinar airways, respectively, derived from MBW (see text for details). *Significant difference between nonbronchohyperresponsive (NBHR) and bronchohyperresponsive (BHR) groups, P < 0.05.

RESULTS

The groups of 10 nonhyperresponsive and 10 hyperresponsive subjects participating in this study will be subsequently referred to as the nonbronchohyperresponsive (NBHR) and bronchohyperresponsive (BHR) groups, respectively. Despite the fact that these subjects were neither trained nor locked into a 1-liter breathing pattern by end-inspiratory valve switching, actual tidal volume obtained from the baseline MBW tests in the NBHR and BHR groups were 1,108 ± 64 and 1,070 ± 37 (SD) ml, respectively. In addition, coefficients of variation of tidal volume within each MBW test averaged 9 ± 2 (NBHR) and 9 ± 1 (SD) % (BHR). Breathing frequency in the baseline phase was 11 ± 3 and 9 ± 2 (SD) breaths/min in the NBHR and BHR groups, respectively. No significant differences in tidal volume, its coefficient of variation, or breathing frequency were found among different phases (baseline, provocation, dilatation) nor between NBHR and BHR groups in any given phase of the study.

Table 1 lists the baseline values (mean ± SE) of all lung function and MBW parameters obtained in NBHR and BHR groups. All spirometry data reported in this section were those obtained by averaging, for each subject, the values recorded before and after the two or three MBW tests performed at a given stage (baseline or after bronchoprovocation and bronchodilatation). FEV1 and PEF75 values recorded before and after the MBW tests showed no significant difference (P > 0.05). The MBW parameters such as Sadin and Scond are derived from an S curve obtained by averaging, breath by breath, two or three alveolar slope curves, each one computed from one MBW test. The same procedure was followed to derive Curv and log[N2]6TO from an average of two or three washout curves. VDpan and VDphys were average values of two or three values of anatomic and physiological dead space, respectively, determined in the first breath of each MBW. Of the lung function parameters, FEV1, FEV1/FVC, and PEF75, and of the MBW parameters, Sadin and Scond, were significantly different between the NBHR and the BHR groups (Table 1).

Table 2 shows how bronchoprovocation and bronchodilatation affect lung function parameters (FEV1, PEF75), dead space volumes (VDpan, VDphys), N2 washout characteristics (Curv, log[N2]6TO), and proximal and peripheral MBW components of ventilation inhomogeneity (Scond, Sacin). We performed a repeated-measures analysis of variance on each of the parameters in Table 2, with a Bonferroni t-test for pairwise comparisons to check the significance of the following: changes from baseline after provocation (between baseline and provocation), reversal of changes after bronchodilatation (between provocation and bronchodilatation), and re-
turn to baseline after bronchodilatation (between bronchodilatation and baseline). The latter was not represented in Table 2, but the result was that, only for FEV$_1$ (in NBHR and BHR groups) and for FEF$_{75}$ (in NBHR group), values were not entirely back to baseline after dilatation. We also checked with an unpaired t-test whether an increase or decrease of a parameter from baseline to provocation was significantly larger in the BHR group than in the NBHR group and found that this was the case only for FEV$_1$, PEF, FEF$_{75}$, Curv, and log(N$_2$)$_{6\text{TO}}$ (Table 2).

Figure 4 shows a graphical representation of the FEV$_1$ and FEF$_{75}$ decreases (A), and S$_{\text{acin}}$ and S$_{\text{cond}}$ increases (B). Closed and open symbols are averages in the NBHR group and the BHR group, respectively. For changes in FEV$_1$ with respect to baseline, which is taken as the criterion for hyperresponsiveness, we show the distribution of 20 individual data points (some of which are superimposed), indicating a continuous distribution of hyperresponsive and nonhyperresponsive subjects in terms of FEV$_1$. After bronchoprovocation, average reduction in FEF$_{75}$ (31% in the NBHR group; 49% in the BHR group) was significantly greater than reduction in FEV$_1$ (11% in NBHR; 26% in the BHR group). S$_{\text{cond}}$ showed an average 198% increase in the NBHR group and an average 390% increase in the BHR group on bronchoprovocation, with large standard error bars and with no significant difference between the S$_{\text{cond}}$ increase in the BHR and NBHR groups (P = 0.06). S$_{\text{acin}}$ showed only a 13–20% increase in both groups, which did not reach statistical significance.

Table 2 shows that V$_{\text{Danat}}$ decreased to the same extent, namely, 20 ml, in the NBHR and BHR groups. However, because on average V$_{\text{Danat}}$ was 10 ml lower in the BHR group, the relative change was slightly more important in the BHR group. For V$_{\text{Dphys}}$, no significant changes could be demonstrated in any group. Nevertheless, the so-called “alveolar dead space,” defined as V$_{\text{Dphys}}$ – V$_{\text{Danat}}$, did increase significantly. V$_{\text{Danat}}$ and V$_{\text{Dphys}}$ – V$_{\text{Danat}}$ were back to baseline control values after bronchodilatation. Bronchoprovocation leads to significantly increased curvilinearity of the N$_2$ washout curve (i.e., decreased value of Curv) only in the BHR group. The change in Curv did not reach statistical significance in the NBHR group. Also, the logarithmic value of the mean expired N$_2$ concentration for TO = 6 (i.e., log(N$_2$)$_{6\text{TO}}$) increased significantly on bronchoprovocation only in the BHR group. Both these parameters derived from the N$_2$ washout curve were back to baseline control values after bronchodilatation.

**DISCUSSION**

To evaluate the role of conductive and acinar lung zones in the histamine bronchoprovocation process, an analysis of the normalized slope in the N$_2$ MBW (9) was applied, from which two indexes of ventilation inhomogeneity, i.e., S$_{\text{cond}}$ and S$_{\text{acin}}$, were derived. In the process of extracting a small set of parameters from the MBW normalized slopes for quantitative analysis, we based our choice of S$_{\text{acin}}$ and S$_{\text{cond}}$ on elements from the MBW papers of Crawford et al. (6–9) and subsequent model analysis by our laboratory (26). S$_{\text{acin}}$ was based on the extrapolation method described by Crawford et al. (9), but used linear instead of exponential fitting on the latter part of the MBW and used lung TO instead of breath number as the absissa, as suggested in a subsequent paper (6). The computation of the conductive component S$_{\text{cond}}$ only differed from the method described by Crawford et al. (8), where linear regression was also used, by the choice of lung TO as the absissa. Because the subjects in the series of papers by Crawford et al. (8) are not documented in terms of parameters that are pertinent to our study (e.g., hyperresponsive or nonhyperresponsive, FEF$_{75}$ values, diffusing capacity), direct comparison with our baseline MBW data needs to be handled with caution. However, using the lung volumes reported in Crawford et al. (6) from subjects who also participated in another study by Crawford et al. (7), we used the average normalized slope curve in their Fig. 3 (Ref. 9) to evaluate S$_{\text{acin}}$ and S$_{\text{cond}}$ according to our method, yielding S$_{\text{acin}}$ = 0.070
In subjects, flow rates, equipment, and slope-determination limits are taken into account, these values are in general agreement with our baseline $S_{\text{acin}}$ and $S_{\text{cond}}$ data (Table 1).

Besides the small methodological differences in the computation of convective and diffusion-convective inhomogeneity indexes, the main issue remains whether the exponential extrapolation rather than the linear extrapolation to the slope of the first breath (to obtain $S_{\text{acin}}$) could lead to different results. Model analysis actually predicts that the normalized slope curve vs. lung TO has two components, one fast and one slow (insets, Fig. 3B). That is why we also submitted all our data (including those from provocation and dilatation MBW tests) to a Levenberg-Marquardt routine, which fitted a sum of two exponentials (with boundary conditions on the curvature and asymptotes) to our normalized slope curves as a function of lung TO. Using the statistical method of Bland and Altman (2) to compare $S_{\text{acin}}$ values obtained with two-exponential vs. linear extrapolation, we obtained a mean difference of $0.004 \pm 0.005$ (SD) liter$^{-1}$. Placed against the baseline $S_{\text{acin}}$ values in Table 1, this result led us to conclude that, even in extreme conditions such as provocation, our linear method for $S_{\text{acin}}$ determination is as valid as the more cumbersome exponential method.

In this study, it was expected that 1) $S_{\text{cond}}$ would increase if large ventilation differences and asynchronous emptying occur, e.g., as a result of inhomogeneous narrowing of parallel conductive airways; and 2) $S_{\text{acin}}$ would increase if any significant alteration occurs at the level of the acinar structure, even in the absence of flow asynchrony. We observed large $S_{\text{cond}}$ increases during histamine-induced airway narrowing in both BHR and NBHR groups, and no significant changes in $S_{\text{acin}}$ in either group. However, prehistamine $S_{\text{acin}}$ was significantly larger in the BHR group. Together with measurements of dead space ($V_{\text{Dnmat}}, V_{\text{Dphys}}$) and lung function parameters (FEV$_1$, FEF$_{75}$), our MBW study suggests that 1) the airways involved during the histamine bronchoprovocation process, part of which are the small airways, are situated proximal to the acinar entrance; 2) between relatively large lung units, i.e., those containing several groups of acini, large differences in inspired gas concentration develop; and 3) the baseline acinar structure is not affected by the provocation process itself but may be related to hyperresponsiveness.

Large-scale inhomogeneities ($S_{\text{cond}}$). The fact that histamine bronchoprovocation generates average $S_{\text{cond}}$ increases on the order of 200 and 400% in the NBHR and BHR groups, respectively (Fig. 4B), indicates not only an average decrease in airway lumen of parallel airways down to a given level or at a given level of the bronchial tree (which reduces FEV$_1$ and FEF$_{75}$) but also an important inhomogeneity in constriction between parallel airways. Indeed, to generate an alveolar slope, parallel differences in ventilation distribution must exist, associated with sequential emptying. Therefore, the present results suggest that the inhomogeneity of airway narrowing that is known to exist in the case of acute asthmatic attack is also present, although to a lesser degree, during bronchoprovocation with a nonspecific agent in asymptomatic subjects. The inequality in response of parallel airways could reflect density differences in muscarinic receptors and/or cholinergic innervation between airways located at a given lung depth (i.e., airways of more or less the same lung generation), in addition to proximal vs. peripheral density differences observed along the bronchial tree (13).

Another category of MBW indexes that can reflect convection-dependent inhomogeneities is that derived from the classic washout curve, Curv and log[$N_2$]$_{\text{ETO}}$ (Fig. 3A). Their modifications after bronchoprovocation did not reach statistical significance in the NBHR group (Table 2). From a theoretical viewpoint, this is surprising because these two parameters should reflect all convective, i.e., large-scale, concentration differences. In particular, the specific ventilation differences between lung units that empty asynchronously during expiration and therefore increase $S_{\text{cond}}$ should also tend to decrease Curv and increase log[$N_2$]$_{\text{ETO}}$. In addition, possible specific ventilation differences generated between lung units that empty synchronously, and therefore do not contribute to $S_{\text{cond}}$, would nevertheless tend to decrease Curv and increase log[$N_2$]$_{\text{ETO}}$ even more. Therefore, the absence of significant change in Curv or log[$N_2$]$_{\text{ETO}}$ and the twofold increase in $S_{\text{cond}}$ (200% baseline) in the NBHR group could indicate that specific ventilation differences are small, whereas flow asynchrony is more apparent during mild bronchoprovocation. In contrast, in the BHR group, both specific ventilation and flow asynchrony become important enough to affect all large-scale MBW parameters (Table 2). Alternatively, it could be argued that any index derived from the classic $N_2$ washout curve, whether it be related to its curvilinearity (such as Curv) or to its value after a number of breaths or lung TO (such as log[$N_2$]$_{\text{ETO}}$), is not sensitive enough to detect the mild bronchoprovocation in the NBHR group.

Small-scale inhomogeneities ($S_{\text{acin}}$). Our results show that, in contrast to $S_{\text{cond}}$, $S_{\text{acin}}$ is not significantly affected by the bronchoprovocation process itself (Fig. 4B). Nevertheless, $S_{\text{acin}}$ is significantly larger in the hyperresponsive subjects (Table 1). Using the alveolar $N_2$ slope of the vital capacity SBW test, Hudgel and Roe (11) found a larger baseline slope for 9 hyperresponsive subjects in a group of 22 coal miners. Inasmuch as the slope of a SBW maneuver and $S_{\text{acin}}$ (i.e., a major part of the slope of the first breath in a MBW) can reflect, at least in part, the same ventilation inhomogeneity, the findings in the group of miners are compatible with our baseline $S_{\text{acin}}$ data. However, Hudgel and Roe could not demonstrate such a baseline $N_2$ slope difference between 8 responders and 33 nonresponders in a group of 41 nonminers. Taylor and Clarke (25) also failed to observe a different baseline SBW alveolar $N_2$ slope in the responder vs. nonresponders to histamine in a group of 21 nonsmoking subjects. One reason could be that, in the case of small changes and for small groups of subjects, the computation of $S_{\text{acin}}$ without the con-
founding effects of large-scale inhomogeneities as would be the case with a SBW alveolar slope, is indeed crucial.

The larger baseline $S_{\text{acin}}$ value in the BHR group should be interpreted with caution. It merely adds a piece of information to the controversy about the relationship between baseline lung function and hyperresponsiveness (24). The significantly smaller FEV$_1$ and FEF$_{75}$ values in the BHR group (Table 1) are in support of such a dependence in a group of 20 otherwise asymptomatic subjects. Nevertheless, FEV$_1$ and FEF$_{75}$ averages are supranormal or normal in both groups. Because baseline DL$_{\text{CO}}$ and K$_{\text{CO}}$ values are normal and not different between BHR and NBHR groups (Table 1), it is unlikely that intra-acinar alterations reflected in the larger $S_{\text{acin}}$ in the BHR group took place at the level of the alveolar structure. Rather, the larger $S_{\text{acin}}$ points to some degree of intra-acinar airway narrowing, maybe due to inflammation (23). This issue surely needs further investigation in a larger group of subjects with different degrees of hyperresponsiveness, possibly a group that also includes asymptomatic subjects, in whom $S_{\text{acin}}$ can, for instance, be related to PD$_{20}$, the provocative dose necessary to reach a 20% fall in FEV$_1$.

$V_{\text{Danat}}$ and $V_{\text{Dphys}}$. In contrast to the other MBW parameters, $V_{\text{Danat}}$ derived from the first expiration showed a very similar decrease in the BHR and NBHR groups, indicating a similar degree of volumetric reduction of the conductive airways. $V_{\text{Danat}}$ is expected to be less sensitive to airway narrowing than any resistance-related parameter simply because $V_{\text{Danat}}$ is related to the second power of the airway radius, whereas resistance is related to approximately the fourth power of the airway radius. Alternatively, one could argue that a possible increase in lung volume after provocation (20, 27) would tend to oppose a $V_{\text{Danat}}$ decrease. We did not find a significant change in FRC after bronchoprovocation in any of the two groups, in line with FRC measurements by Langley et al. (12) using the same technique. Despite these arguments for a lack of sensitivity of $V_{\text{Danat}}$ to evaluate bronchospasm, the fact that it does not decrease more in the BHR group at all remains surprising. Perhaps it is an indication of upper airway constriction with a limit that is already reached in the NBHR group, where the average FEV$_1$ decrease was 12%. We did not find a correlation between $V_{\text{Danat}}$ and FEV$_1$ decrease in the NBHR group, a correlation that could have confirmed the hypothesis of a progressive $V_{\text{Danat}}$ decrease with FEV$_1$ below the 20% FEV$_1$ threshold. However, the range of changes in $V_{\text{Danat}}$ is probably too small to verify this.

Model analysis predicted that, for the study of ventilation distribution in normal human subjects, $V_{\text{Dphys}}$, or the difference $V_{\text{Dphys}} - V_{\text{Danat}}$, is not very sensitive to evaluate changes in ventilation inhomogeneity (26). Our experimental $V_{\text{Dphys}}$ and $V_{\text{phys}} - V_{\text{Danat}}$ data confirm that the same is true in the case of bronchoprovocation, during which important inhomogeneities are known to occur. In general, our dead space data coincide with the findings of Burke et al. (4), who also found a 20-ml decrease of $V_{\text{Danat}}$ and virtually no effect on $V_{\text{Dphys}}$.

Implication of bronchoprovocation in gas-exchanging units. Despite the small effect of histamine provocation on $V_{\text{phys}}$ and $V_{\text{anat}}$, Burke et al. (4) found a large degree of ventilation-perfusion mismatch, and our data provide an explanation for this gas-exchange impairment. The fact that $S_{\text{cond}}$ increases so dramatically points to large differences in gas concentration between relatively large units, comprising several acini or clusters of acini. The size of these units remains somewhat speculative. In combination with a similar $V_{\text{Danat}}$ decrease in the NBHR and BHR groups, the larger $S_{\text{cond}}$ increase in the BHR with respect to the NBHR group is an indication of the fact that the ventilation differences during bronchoprovocation are generated between units subtended by the more peripheral of the conductive airways. The large decreases in FEF$_{75}$, which, despite its poor reproducibility, is used in clinical practice as a marker of the small airways, provide further support for this (Fig. 4A). The implication of the smaller conductive airways in the bronchoprovocation process is also not surprising in view of the conclusions of a MBW study in normal subjects (7) suggesting that bronchomotor tone of relatively small airways is responsible for a relatively uniform distribution of ventilation. When normal bronchomotor tone is disturbed and large inspired concentration differences occur as a result, gas-exchange impairment is likely to ensue.

Our suggestion that the small conductive airways are the major determinant of gas-exchange impairment during bronchoprovocation is also compatible with bronchoprovocation data in the literature. In the case of the bronchoprovocation study by Oligiati et al. (15), one needs to assume that the peripheral airways that were held responsible for impairment of gas exchange were indeed small airways but nevertheless were situated proximal to the acini, i.e., conductive airways. This is probably also the reason why Schmekel et al. (21) found no difference in impairment of gas exchange, whether methacholine was deposited centrally or peripherally in the lungs. With central vs. peripheral deposition of histamine, Ruffin et al. (19) even found that a lower dose was necessary to decrease FEF$_{75}$ in the case of central deposition. Although these authors concluded that action on central airways was the main determinant of histamine provocation, doubt remained about the dispersion of the histamine dose over the more numerous peripheral airways, leading to submaximal reaction of the very peripheral airways. The same reasoning could lead us to believe that this is why $S_{\text{acin}}$ did not change significantly (Fig. 4B). It is possible that, to elicit the hypothesized peripheral action of histamine, also at the acinar level, intravenous injection of histamine would be more appropriate.

Potential of the MBW method. Provided one corrects for the convective component, as was done here to obtain $S_{\text{acin}}$, the alveolar slope of the first breath of a MBW may be considered as reflecting intra-acinar alterations. The fact that the convection-dependent part, which turns out to be the most important effect during bronchoprovocation, is only poorly reflected in the first breath probably explains the relatively moder-
ate increases in the SBW phase III slope increases seen after provocation (14, 20). Nevertheless, the first breaths of a MBW and the SBW are not strictly comparable because, for the SBW vital capacity maneuver, the conductive and acinar contribution to ventilation inhomogeneity may be quite different (17). In the study by Scano et al. (20), this relative contribution is further complicated by the end-inspiratory breath hold of 5–10 s, which tends to reduce the acinar contribution of the alveolar slope.

The MBW test has been used in association with bronchoprovocation tests before. Langley et al. (12) quantified ventilation distribution in terms of a mixing-efficiency index, derived from the classic N2 washout curve. A MBW was performed before and after methacholine provocation and 81mKr ventilation lung scans were obtained in both phases. From the inspection of the patches on the lung scans, Langley et al. (12) concluded that convection-dependent inhomogeneity alone could not account for the marked decrease in MBW mixing efficiency, and that some diffusion-related mixing inefficiency must be involved in the provocation process. Our interpretation of those data is that important ventilation inhomogeneities exist between the smaller convection-dependent units, which also contribute to decrease the MBW mixing efficiency, but cannot be distinguished on the ventilation scans because of the poor resolution. In fact, the MBW data published by Harris et al. (10) showed that bronchoprovocation had the same effect on He- and sulfur hexafluoride mixing-efficiency curves. Inasmuch as these curves (obtained in only 3 asthmatic subjects) are sensitive enough to make the diffusion-dependent part of mixing efficiency appear, these data also contradict the hypothesis of a diffusion-dependent component in the provocation process. The fact that in our study S_acin did not increase significantly after bronchoprovocation confirms the findings of Harris et al. in a larger group of hyperresponsive, but otherwise asymptomatic, subjects.

Of interest is that bronchoprovocation has been reported to generate a reversal of the apex-to-base ventilation gradient (5, 27). With the gravity-dependent vertical pressure gradient around the lung remaining as it is, the reversal of the apex-to-base ventilation gradient after bronchoprovocation would lead to a negative alveolar slope, or at least the gravity-dependent part of it. This opposing gravity-dependent effect could have contributed to counteract the alveolar slope increase resulting from much smaller lung units. However, human physiological experiments recently performed onboard the Spacelab Life Sciences 1 mission show that the MBW maneuver, involving near-tidal breathing from FRC, and the normalized slopes it generates are not significantly affected by gravity (18). This means that, in the absence of the blurring effect of gravity, the alveolar slope generated in a MBW test can be entirely attributable to intrinsic structural and elastic properties of the lung, a fact that renders this test particularly useful in the clinical context of the lung function laboratory.

In conclusion, our MBW results suggest that, in otherwise asymptomatic subjects with airway hyperresponsiveness to inhaled histamine, airway narrowing occurs predominantly in airways proximal to the acini. These airways are at the point of origin of important inspired gas concentration differences between relatively large units and of a sequential emptying pattern between them. By contrast, the acinar component of ventilation inhomogeneity is not affected by the bronchoprovocation itself, but its baseline value is significantly increased in the BHR group of subjects.

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