Lung function and ventilation inhomogeneity in rat lungs after allergen challenge

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1Laboratorio de Fisiopatología Respiratoria Experimental, Servicio de Neumología, Fundación Jiménez Díaz, Universidad Autónoma, 28040 Madrid, Spain; 2Biomedical Physics Laboratory, Université Libre de Bruxelles, 1070 Brussels; and 3Akademisch Ziekenhuis, Vrije Universiteit Brussel, 1090 Brussels, Belgium

Sánchez-Cifuentes, M. Victoria, Maria L. Rubio, Mercedes Ortega, German Peces-Barba, Manuel Paiva, Sylvia Verbanck, and Nicolás González Mangado. Lung function and ventilation inhomogeneity in rat lungs after allergen challenge. J. Appl. Physiol. 88: 821–826, 2000.—We studied the early response to ovalbumin challenge in sensitized Brown-Norway rats through its effect on N2, He, and SF6 phase III slopes of the single-breath washout and on indexes of lung function. Sensitized rats showed varying degrees of response in terms of pulmonary pressure (Ptl), with increases ranging between 125 and 225% of baseline. The sensitized rats presented decreased quasistatic compliance, forced vital capacity, and end-expiratory flow, with all three lung function indexes showing a significant negative correlation with corresponding Ptl values. They also showed significant positive correlations of Ptl with the N2, He, and SF6 phase III slopes, reflecting diffusion-convection-dependent inhomogeneities generated by conformation changes throughout the entire rat lung. In addition, the rats showing the most marked Ptl increases (>150% baseline Ptl) also revealed a reversal of the SF6-He slope difference because of a more marked SF6 than He slope increase. This latter finding suggests that the degree of structural heterogeneity during early response is even more marked in the most peripheral rat lung generations.

Brown-Norway rats; early response; diffusion-convection-dependent inhomogeneity

It has been suggested theoretically (9) and experimentally (2) that, in human lungs, a large portion of the phase III slope in the single-breath washout (SBW) is caused by a mechanism of diffusion-convection-dependent ventilation inhomogeneity (DCDI) in the lung periphery. Theoretically, DCDI generates concentration differences between lung units that are asymmetric by their different volume and/or unequal airway narrowing when units subtend from branch points situated along the so-called diffusion front (9). In human subjects, DCDI is thought to occur at the level of the acinus, and the DCDI component of the phase III slope is expected to reflect intra-acinar structure. In addition, phase III slopes obtained from gases with differing diffusivities (He, SF6) can be used to assess conformation changes at the level of their respective diffusion front (the diffusion front for SF6 being situated more peripherally than that for He).

Recent experimental studies of DCDI with the use of single- and multiple-breath washout techniques have shown the potential of detecting acinar airway alteration in both asthmatic and chronic obstructive pulmonary disease patients (10, 20, 21). Whereas these studies clearly indicated that through DCDI it is possible to detect acinar structure abnormalities under a given baseline condition, its potential to detect dynamically change, such as during bronchoprovocation testing, remains obscure. In a previous study on hyperresponsiveness in normal subjects (21), for instance, no significant changes in acinar ventilation inhomogeneity were observed during histamine challenge, despite earlier reports of a possibility that histamine could also affect the peripheral airways. This observation suggested either that histamine really did not affect the acinar airways or that it was impossible to pick up any such alteration through the DCDI mechanism.

It was the aim of this study to investigate quantitatively whether DCDI can be affected by bronchoprovocation at all. For this purpose we chose the rat lung as the model for DCDI and tested its response to allergic bronchoconstriction. In rat lungs, DCDI is indeed the predominant mechanism of ventilation distribution (5, 17, 22). Experimental phase III slopes can be quantitatively reproduced by simulations of diffusion and convection in a lung geometry based on the detailed morphometric description of rat lung structure down to the alveolar end (13). These simulations indicated that DCDI is operational over most of the rat lung, with the He diffusion front extending from generations 3–4 out into the periphery and the SF6 front starting off approximately eight generations more peripherally. In particular, the simulations mimicked the experiments by reproducing the larger He than SF6 phase III slopes (i.e., negative SF6-He slope difference). In the absence of lung disease, negative SF6-He slope difference in rats contrasts with observations in any other species, includ-
ing humans. This is a direct consequence of the respective lung structures in which the DCDI mechanism is operational, i.e., the whole rat lung or the human lung acinus. For a detailed description of the different characteristics, such as acinar distribution along the bronchial tree and volume asymmetry in subsequent lung generations, which actually lead to He and SF₆ phase III slope inversion between rat and human lungs, we refer to the simulation study in which DCDI in both species are compared (22).

Despite the different baseline condition of He and SF₆ slopes with respect to humans, DCDI theory predicts that any conformation change at the level of a given diffusion front will affect the corresponding phase III slope. In both the human acinus and the whole rat lung, the SF₆ diffusion front is situated several generations more peripherally than the He front, and, therefore, a preferential increase of, for instance, SF₆ phase III slopes reflects a more peripheral alteration in the human acinus or the whole rat lung. Our laboratory previously studied the differential behavior of He and SF₆ phase III slopes in rats with induced panacinar and centroacinar emphysema (4, 14). We now apply the same technique to study allergen challenge in rats that were first sensitized to ovalbumin (OA). During the early response, a combination of functional and ventilation distribution measurements was performed.

**MATERIALS AND METHODS**

Experimental protocol. A total of 28 inbred male Brown-Norway rats was selected for this study when they were 14–16 wk old and weighing 292 ± 9 (SD) g. Twenty rats were submitted to the sensitization procedure, whereas the remaining eight were used as controls. The sensitization consisted of a subcutaneous injection of 1 ml sterile solution containing 1 mg OA (grade V, Sigma Chemical, St. Louis, MO) and 200 mg hydroxide aluminum (Aldrich Chemical, Milwaukee, WI) in saline. In addition, a peritoneal injection of 0.25 ml of Bordetella pertussis vaccine, containing 7.5 × 10⁹ heat-killed bacilli, was given as coadjuvant (1). The control rats also underwent subcutaneous and peritoneal injections but with the use of saline instead of OA. A period of 15 days followed until the study day, when functional and ventilation tests were carried out in both groups.

On the study day, the rats were anesthetized with pentobarbital sodium (50 mg/kg ip), paralyzed with pancuronium bromide (1 mg/kg ip), and tracheotimized in the cervical region. A tubing (40 mm long, 2.3 mm OD, 1.6 mm ID) was used to connect the trachea to a breathing assembly consisting of a differential pressure transducer, a mass spectrometer (Marquette Electronics, Milwaukee, WI), a gas reservoir, and a stopcock valve that allowed communication either to a ventilator (model S-4833, Harvard Apparatus, Edenbridge, UK) or to a syringe. The rat was placed into a 1.6-liter volume displacement plethysmograph, with a pneumotachograph (8 mm of maximum internal diameter) coupled to a differential pressure transducer (MP45–871, ± 2 cmH₂O; Validyne, Northridge, CA). This pneumotachograph was used for all the tests except for the recording of the flow-volume curves. In this case, a second pneumotachograph (11 mm of maximum internal diameter) was used that was more efficient for high flows. Volumes were obtained by hardware integration of flow (FV 156–871, Validyne). Data acquisition of volume and gas concentrations was done at 62 Hz and stored on a personal computer (IBM). For on-line monitoring of flow and volume signals, an oscilloscope was used (model BS-601, Aaron), while pulmonary pressure (PL) was continuously registered on a four-channel recorder (Rikadenki, Kogyo, Japan). Flow-volume curves were performed by using an additional X-Y recorder. The above-described instrumentation was identical to the one used in our laboratory’s previous rat ventilation studies (4, 5, 14), except for the aerosol delivery system. The ventilator was used here to aspire the aerosol from the nebulizer (Updraft II, Hudson, Temecula, CA) and deliver it directly to the trachea in a tidal respiration pattern (4 ml tidal volume; 58 beats/min). The solutions to be nebulized were either OA solution (5% OA in saline) or saline.

Figure 1 depicts the test sequence for all OA-sensitized rats. The control rats underwent exactly the same procedure but with saline substituted for OA during both sensitization and aerosol challenge. The entire test sequence was monitored by the tracheal pressure at end inspiration of the tidal volume breathing. This pressure is also referred to as PL (P₁–P₁₀ in Fig. 1). The sequence started with three inspiratory capacity (IC) inhalations, i.e., up to the volume corresponding to 30 cmH₂O, to standardize volume history in all animals. Then all rats were exposed to a saline aerosol for 3 min after which they reached the so-defined baseline condition (P₂). Functional measurements (i.e., IC, pressure-volume and flow-volume curves) were recorded, after which the OA aerosol (sensitized rats) or saline (control rats) was administered for 5 min. Immediately after the OA or saline challenge, PL corresponded to P₅. After that, when either PL had reached a plateau or 30 min had elapsed, this value was defined as P₆, and a second set of functional measurements was initiated. Over the course of these functional measurements, the average PL was termed P₇. The rats were then killed with N₂ IC expansions immediately followed by another PL measurement (P₈). Within a time span of ~30 min postmortem, two rebreathing and four SBW tests were performed. Because of the large contribution of gas exchange to phase III slope in rats, ventilation distribution tests should be performed postmortem. The exact contribution of gas exchange, depending on the maneuver performed, and the effect of rigor mortis, which necessitates that the SBW tests be done within 1 h postmortem, can be found elsewhere (4). Before the first and immediately after the fourth SBW test, PL was recorded as P₉ and P₁₀ respectively. Before each SBW, the lung was inflated to IC for 30 s to standardize volume history. Before each SBW,

![Fig. 1. Protocol of study. IC, inspiratory capacity; OA, ovalbumin aerosol challenge; FRC, functional residual capacity; RV, residual volume; SBW, single-breath washout test; P₁–P₁₀, pulmonary pressures (PL) 1–10 (see text for details); P₁, PL after IC; P₂, PL after saline inhalation (baseline); P₅, PL after functional measurements; P₆, PL just before OA inhalation; P₇, PL at end of OA inhalation; P₈, maximal PL during early response (within 30 min); P₉, PL during lung function; P₁₀, PL after death; P₆ and P₁₀, PL measured before and after, respectively, the 4 SBW tests.](http://jap.physiology.org/10.22033.1.on September 10, 2017)
test, the breathing assembly was flushed with the washout gas mixture to limit the instrumental dead space to 0.15 ml.

Lung function and ventilation distribution. Pressure-volume curves were obtained by manually inflating the lung with air up to 30 cmH2O, after which the mass spectrometer emptied the lung down to residual volume (RV) at a constant flow of 1.2 ml/s. Lung compliance was calculated as the maximal slope in the deflation limb of the pressure-volume curve.

Forced expiration maneuvers were carried out by inflating the lung to 30 cmH2O and rapidly deflating to RV by using the vacuum reservoir with a negative pressure of -40 cmH2O. Forced vital capacity (FVC), expiratory flow after 75% exhalation of FVC (F75), and specific F75 (F75/FVC) were derived directly from the expiration flow-volume curves.

Static lung volumes, i.e., functional residual capacity (FRC) and expiratory reserve volume (ERV), were determined immediately after death with the use of a rebreathing test in which a N2-free gas mixture was rebreathed for 30 s by a tidal airway pressure change between 0 and 20 cmH2O and a final expiration to -20 cmH2O. The relation between initial N2 and final N2 concentration was used to determine the initial lung volume at 0 cmH2O, i.e., FRC. The difference between the last rebreathing expiration and inspiration volume was computed to represent ERV, and RV was calculated by subtracting ERV from FRC.

SBW tests were performed by slowly inflating the lung (~1 ml/s), starting from FRC with 4 ml of a gas mixture containing 5% He, 5% SF6, and 90% O2, and emptying the lung down to RV by using the mass spectrometer (1.2 ml/s). In the SBW tests, phase III slopes were computed by linear regression over the portion of the curve between 40 and 80% of expired volume. The negative slopes resulting from phase III of the inspired gases (He, SF6) were considered in absolute value. Finally, phase III slopes were normalized by dividing the N2 slope by mean expired N2 concentration and by dividing He or SF6 phase III slopes by inspired minus mean expired He or SF6 concentration (5).

Statistical analysis. Most comparisons in this study involved three groups, and analyses of variance were used to check for differences among them. Within each group, we checked for differences between pre- and post-OA challenge (or pre- and post-saline) by means of a nonparametric pairwise comparison (Wilcoxon). Spearman rank correlations were evaluated among PL, phase III slopes, and functional parameters. All statistical analyses were performed by using Stata Graphics Plus software (Manugistics, Rockville, MD), and statistical significance was accepted at the P = 0.05 level.

RESULTS

Figure 2 shows the PL throughout the test sequence in the control group and in two subgroups of the OA-sensitized rats classified as nonresponders (NR) and responders (R) as follows. Early response to OA was quantified in terms of change in PL between P2 (baseline condition) and P6 (maximum 30-min post-OA challenge), as indicated by the arrows in Fig. 2. A significant early response to OA was confirmed when P6 was ≥150% P2, analogous to the 150% baseline pulmonary resistance cutoff previously used by others (1). According to this criterion, the sensitized rats were separated into 8 NR and 12 R rats.

After the three initial IC expansions and saline challenge, P2 (in absolute value) was similar in control [7.91 ± 0.70 (SD) cmH2O], NR (8.10 ± 1.95 cmH2O), and R groups (8.41 ± 1.51 cmH2O). Over the 5-min period of OA challenge, both groups showed significant PL increases (P4-P5), and, in the 30-min interval after OA challenge, both NR and R groups separated from the control group in terms of P6. This maximal PL value for P6 appeared in a significantly shorter time interval after OA challenge in the R group (7 ± 2 min) than in the NR group (18 ± 4 min) (P < 0.05).

In the OA-sensitized rats, the second set of functional measurements (after the OA challenge) induced a transient PL decrease (P8-P9). However, by the time SBW testing was initiated, PL had increased again (P9 was even slightly above the P8 level) and stabilized over the course of SBW testing (no significant changes between P9 and P10). The fact that the significant PL increase between P8 and P9 in NR and R groups was also observed in the control group indicates that at least the portion of P9 increase above the P6 level was due to the animal death itself.

Lung function and ventilation distribution. Table 1 lists the static lung function parameters obtained in the three groups pre- and post-OA challenge (NR and R groups) and pre- and post-saline (control group). Before OA challenge, the only lung function parameter that differed between R and NR groups was F75. With the OA challenge, lung compliance, FVC, F75, and F75/FVC values significantly decreased in both OA-challenged groups, and all these decreases were greater in the R group. LC only decreased significantly after OA challenge in the R group.

Figure 3 shows the phase III slopes derived from the postmortem SBW maneuvers (4-ml inspiration from FRC and exhalation to RV) in all three groups. In the NR group, the slightly increased N2, He, and SF6 slopes and SF6-He slope difference with respect to the control group did not reach statistical significance. By contrast, in the R group, N2, He, and SF6 phase III slopes were significantly different with respect to NR and control groups (all P < 0.01). In addition to the larger slopes in the R group, the SF6-He slope difference actually
changed signs. This reversal of SF$_6$-He slope difference was due to the larger increase of SF$_6$ phase III slope than of He phase III slope. Lung volumes corresponding to these SBW tests showed significant differences in terms of ERV between R (0.81 ± 0.58 ml) and NR groups (1.51 ± 0.55 ml) and between R and control groups (1.22 ± 0.58 ml). FRC values were not significantly different among groups (control: 3.79 ± 0.37 ml; NR: 4.41 ± 0.35 ml; R: 4.39 ± 0.84 ml).

Table 2 shows potential correlations among functional parameters and corresponding PL (i.e., PL = P$_7$) and between phase III slopes or lung volumes and corresponding PL (in this case P$_{9}$ and P$_{10}$). All of these correlations correspond to measurements during early response (before or after death), including only data from NR and R groups. The correlation between lung function and PL is highly significant, but for F$_{75}$ this correlation disappears when it is normalized to FVC. The good correlation between PL and phase III slopes contrasts with the absence of correlation with the SF$_6$-He slope difference. Of the lung volumes, ERV is the only one correlating with PL. In fact, ERV also correlated significantly with phase III slopes of all three gases (N$_2$: P = 0.0001; He: P = 0.0002; SF$_6$: P = 0.0002) but not with the SF$_6$-He slope difference (P = 0.1; not shown in Table 2). The relation between ERV and N$_2$ phase III slope is illustrated in Fig. 4, where, in addition to NR and R groups, data from the control group are shown for comparison.

DISCUSSION

This study clearly shows that it is possible to detect alterations in response to antigen challenge at a level of the lung at which the mechanism of DCDI predominates, as is thought to be the case in the whole rat lung. Inherent to the way the DCDI mechanism generates phase III slopes, a conformation change at any given lung depth will be most reflected in the phase III slope of the gas that has its diffusion front located at this particular lung depth. In the case of the rat lung, the SF$_6$ diffusion front is located approximately eight generations more peripherally than is the He front. In this respect, the fact that early response to OA (in terms of increased airway pressure) induced marked increases in N$_2$, He, and SF$_6$ phase III slopes (Fig. 3) suggests lung conformation changes throughout the entire rat lung. In addition, the reversal of the SF$_6$-He slope difference, which in this case is mainly due to the SF$_6$ slope increasing more than the He slope, suggests an even more marked response to OA in the more peripheral rat lung generations.

It has been suggested that, in rat lungs, metacholine can elicit a response in both airways and tissue mechan-
corresponding pulmonary pressures during early response (using R and NR groups).

### Table 2. Spearman rank correlations among lung function, static lung volumes, and phase III slopes and corresponding pulmonary pressures during early response (using R and NR groups)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PL</th>
<th>CL</th>
<th>IC</th>
<th>FVC</th>
<th>F75</th>
<th>F75/FVC</th>
<th>N2</th>
<th>He</th>
<th>SF6</th>
<th>SF6-He</th>
<th>FRC</th>
<th>ERV</th>
<th>RV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearman</td>
<td>-0.81</td>
<td>-0.65</td>
<td>-0.66</td>
<td>-0.68</td>
<td>NS</td>
<td>NS</td>
<td>0.63</td>
<td>0.62</td>
<td>0.57</td>
<td>NS</td>
<td>0.61</td>
<td>(P = 0.003)</td>
<td>(P = 0.02)</td>
</tr>
</tbody>
</table>

Pl, pulmonary pressure measured in cmH_2O; N_2, He, and SF_6 phase III slopes and SF_6-He slope difference measured per ml. FRC, functional residual capacity; ERV, expiratory reserve volume; RV, residual volume, all measured in ml. For correlation with lung function (CL, IC, FVC, F75, and F75/FVC), Pl was set to corresponding pressure P_5, and for correlation with phase III slopes and static lung volumes Pl was taken as the average of P_9 and P_10. See text for details.

One effect that appears to be determinant of N_2, He, and SF_6 phase III slopes is ERV (Spearman rank correlations using NR and R groups), bearing in mind, however, that all phase III slopes reported here derive from SBW tests starting inhalation at FRC. In fact, Fig. 4 illustrates that, for a wide range of ERV values in control and NR groups, N_2 slopes are hardly affected. Only in the R group are the generally smaller ERV values accompanied by larger N_2 phase III slopes. This result suggests that only when RV becomes sufficiently close to FRC may some neighboring units develop severe cross-sectional and/or volumetric heterogeneity and, as a result, produce significant phase III slope increases. In addition, the absence of correlation between ERV and SF_6-He slope difference suggests that such a mechanism of structural heterogeneity has no preferential site of action and in fact affects the entire lung. In the case of rat lungs, in which acinar units are widely distributed over most lung generations, one could indeed imagine that such heterogeneity could occur throughout all generations of the rat lung.

In humans, DCDI is only one of the contributors to the phase III slope, because gravity (12) and inhomogeneous volume expansions among gravity-independent lung units may also generate a sloping alveolar plateau. In humans, one way to isolate the DCDI component, i.e., the acinar component, of ventilation inhomogeneity is to consider the SF_6-He slope difference, because, in humans, all effects involving units larger than acini are expected to affect He and SF_6 phase III slopes in the same way (and leave SF_6-He slope difference unaltered) as they operate at branch points proximal to both diffusion fronts. Van Muylem et al. (16) were able to correlate SF_6-He slope differences with indexes of acinar airway inflammation obtained in patients selected for lung resection. Another study by the same group showed a negative SF_6-He slope difference in lung transplant patients only during a rejection phase and a return to a positive SF_6-He slope difference during the recovery, which was mainly attributed to alterations in the first acinar generations (15). A negative SF_6-He slope difference and its modification to zero slope difference after bronchodilatation were observed by Peces-Barba et al. (10) in five asthmatic patients, pointing to severe intra-acinar alterations that were, at least in part, reversible.

Note that, in the absence of lung disease, the SF_6-He slope difference may be positive (as in the human acinus) or negative (as in the whole rat lung), depending on the particular details of each lung structure as indicated by corresponding model simulations (19, 22). Experiments in normal pig lungs have even shown a zero SF_6-He slope difference (6), a result that as yet has not been simulated in a model with realistic lung geometry. Whatever the normal baseline SF_6-He slope difference in a given species, the deviation from this baseline is what actually matters in detecting abnormal ventilation inhomogeneity through the DCDI mech-
anism, as long as it is clear in which part of the lung DCDI is operational. Also, irrespective of the baseline SF₆-He phase III slope difference, a preferential increase of, for example, the He slope (with respect to SF₆) will always reflect a preferential response from the more proximal lung generations (because the He diffusion front is always more proximally located than the SF₆ front).

In conclusion, we have shown that the DCDI mechanism that dominates the phase III slope in the rat lung does respond to the dynamic situation of lung distress such as the early response to OA challenge. The phase III slope increases observed for the different density gases He and SF₆ suggested that conformation changes, involving cross-sectional and/or volumetric heterogeneity, occurred over most of the rat lung generations. In addition, the significantly larger SF₆ than He phase III slope increase after OA challenge suggests that the degree of structural heterogeneity increases toward the lung periphery. Part of the origin of the observed structural heterogeneity throughout the rat lungs may be associated with substantial reductions in ERV during early response to OA.

This study was supported by Fondo de Investigaciones Sanitarias de la Seguridad Social contract 93/0619, by the Belgian Federal Office for Scientific Affairs (program PRODEX), and by the Fund for Scientific Research—Flanders.

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Received 6 October 1998; accepted in final form 25 October 1999.

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