Respiratory mechanics and maximal expiratory flow in the anesthetized mouse

Y.-L. LAI, AND H.-C. CHOU
Department of Physiology, National Taiwan University College of Medicine, Taipei, Taiwan

Lai, Y.-L., and H.-C. Chou. Respiratory mechanics and maximal expiratory flow in the anesthetized mouse. J. Appl. Physiol. 88: 939–943, 2000.—Mice have been widely used in immunologic research and other research to study the influence of different diseases on the lungs. However, the respiratory mechanical properties of the mouse are not clear. This study extended the methodology of measuring respiratory mechanics of anesthetized rats and guinea pigs and applied it to the mouse. First, we performed static pressure-volume and maximal expiratory flow-volume curves in 10 anesthetized paralyzed C57BL/6 mice. Second, in 10 mice, we measured dynamic respiratory compliance, forced expiratory volume in 0.1 s, and maximal expiratory flow before and after methacholine challenge. Averaged total lung capacity and functional residual capacity were 1.05 ± 0.04 and 0.25 ± 0.01 ml, respectively, in 20 mice weighing 22.2 ± 0.4 g. The chest wall was very compliant. In terms of vital capacity (VC) per second, maximal expiratory flow values were 13.5, 8.0, and 2.8 VC/s at 75, 50, and 25% VC, respectively. Maximal flow-static pressure curves were relatively linear up to pressure equal to 9 cmH2O. In addition, methacholine challenge caused significant decreases in respiratory compliance, forced expiratory volume in 0.1 s, and maximal expiratory flow, indicating marked airway constriction. We conclude that respiratory mechanical parameters of mice (after normalization with body weight) are similar to those of guinea pigs and rats and that forced expiratory maneuver is a useful technique to detect airway constriction in this species.

Static compliance; dynamic compliance; forced expiratory maneuver; airway reactivity

MICE HAVE BEEN WIDELY USED in immunologic research to investigate the influence of different diseases on the lungs. However, functional analysis of the airway in mice is often limited by their small dimensions. The pressure-volume (P-V) (7, 13) and maximal expiratory flow-volume (MEFV) curves (13) were rarely evaluated in the mouse. Therefore, the first aim of this study was to establish a method to perform the P-V and MEFV curves.

In addition to resistance and dynamic compliance (2, 5, 8, 14, 18), values of forced expiratory volume (FEV) and maximal expiratory flow (V_max) are useful in estimation of airway constriction (10, 22). The second aim of this study was to compare values of FEV and V_max with dynamic compliance before and after a bronchoconstrictor-induced airway constriction.

MATERIALS AND METHODS

Determination of respiratory mechanics. Ten 8- to 12-wk-old C57BL/6 mice weighing 20.6 ± 4.2 g were used in this study. After anesthesia with pentobarbital sodium (70 mg/kg ip), each animal's trachea, carotid artery, and jugular vein were cannulated with an 18-gauge needle, PE-10 tube, and PE-10 tube, respectively. After being paralyzed with gallamine triethiodide (1 mg/kg), the animal was artificially ventilated with a tidal volume (VT) of 8–10 ml/kg and frequency of 120 breaths/min by using a small animal ventilator. It has been shown that arterial acid-base balance was maintained in the normal condition under this type of artificial ventilation (8, 14). To ensure that the animal was anesthetized during paralysis, we administered gallamine according to the following plan. 1) Gallamine was only given when its active period (40 min) (17) was within the effective duration of pentobarbital (1–2 h) (17). If it was necessary to administer gallamine beyond this effective period of the anesthetic, supplemental doses of pentobarbital were given before any more gallamine treatment. 2) If an additional dose of gallamine was needed after a single dose, we first examined the level of anesthesia and made sure that the expected anesthesia could be maintained longer than the effective duration of gallamine. The second injection of gallamine was then given to the animal. The anesthetized paralyzed and ventilated animal was placed supine inside a whole body plethysmograph (6 cm ID and 15.25 cm length) for the mouse (Buxco Electronics, Troy, NY). According to Buxco Electronics, the frequency response characteristics of the plethysmograph were flat to >60 Hz. The flow rate was monitored with a Validyne DP45 differential pressure transducer as the pressure dropped across three layers of 325-mesh wire screen in the wall of the plethysmograph. Between a flow rate of 0 and 20 ml/s, we found that there was a linear relationship between the flow rate and pressure signal. Lung volume change was obtained via integration of flow. Airway opening pressure (Pao) was measured with a Validyne DP45 differential pressure transducer as the pressure dropped across three layers of 325-mesh wire screen in the wall of the plethysmograph. At peak volume during the third inflation, the inflation valve was shut off, and immediately another solenoid valve for deflation was automatically turned on. The deflation valve was connected to a 20-liter container with a negative pressure of 40 cmH2O. This negative pressure produced the V_max. The changes in flow, volume, and Pao were traced on a polygraph (model TA11, Gould), and the MEFV plot was also stored on a cathode-ray storage oscilloscope (VC-6025, Hitachi). The flow-volume curve reproduced fairly well for each animal but varied a great deal among animals.
For the static P-V maneuver, Pao was measured as described above, and pleural pressure was estimated from the esophageal pressure (Pes). To ensure that an accurate reading of Pes was obtained, the tip of the fluid-filled esophageal catheter (PE-100 tube) was positioned in the lower one-third of the esophagus where negative pressure is usually greatest. Transpulmonary pressure is the difference between Pao and Pes. In addition, we validated the measurement of Pes by observing no change in transpulmonary pressure when the animal was doing respiratory efforts against an occluded airway before paralysis. The lungs were inflated with air to TLC three times. The third inflation was interrupted at TLC, and then stepwise deflation was carried out. Each deflation of 0.1–0.3 ml was followed by a 2- to 3-s pause. The deflation was continued until Pao reached −10 cmH2O or lower. During artificial ventilation (between the interval of the MEFV or P-V maneuvers), VT and its accompanying Pao difference (ΔPao) were used to calculate dynamic respiratory compliance (Crs = VT/ΔPao). ΔPao was measured between end inspiration and end expiration (i.e., instances of no flow).

Before and after each MEFV or P-V maneuver, functional residual capacity (FRC) (the lung volume at Pao = 0) was determined by using a modified neon dilution method (11). Starting from FRC, the lungs were inflated with a standard neon (0.5%) gas mixture to 50% vital capacity (VC). Gas in the lungs, in the dead space of the instrument, and in the syringe was mixed thoroughly by repeating the injection and withdrawing the gas mixture 10–20 times. The equilibrated gas mixture was withdrawn and analyzed with a Varian gas chromatograph (model 3300). The total volume (including FRC and instrumental dead space) was calculated. The FRC was obtained by subtracting the instrumental dead space from the total volume.

In addition, systemic arterial blood pressure was monitored from the carotid artery with a pressure transducer. Body temperature of the animal was estimated from the temperature detected from the rectum by using a thermistor.

Test of bronchial function before and after methacholine challenge. To induce airway constriction, 10 anesthetized paralyzed animals weighing 23.8 ± 0.4 g were challenged with intravenous injection of methacholine (2 mg/kg). This methacholine dose was relatively high compared with the doses used by Levitt and Mitzner (15) and by Martin et al. (18) in C57L/6 mice. Immediately after the injection, VT decreased and Pao increased gradually. These changes in VT and Pao reached a maximum 40.5 ± 1.8 s after the injection (see results). At the baseline (before methacholine challenge) as well as at the time of the maximal response to methacholine, the MEFV maneuver was performed and the Crs value was calculated in each animal. Values of FEV in 0.1 s (FEV0.1), Vmax at 50% VC (Vmax50%), and Vmax at 30% VC (Vmax30) were obtained from tracings of the MEFV curve.

Statistical analysis. All values are reported as means ± SE. ANOVA was used to establish the statistical significance of differences among groups. If significant differences among groups were obtained by using the ANOVA, then Duncan's multiple-range test was used to differentiate the differences between groups. Differences were considered significant if P < 0.05.

RESULTS

In 20 anesthetized paralyzed mice weighing 22.2 ± 0.4 g, averaged blood pressure and respiratory parameters were as follows: mean systemic blood pressure, 74.4 ± 2.0 mmHg; body temperature, 29.4 ± 0.4°C; FRC, 0.25 ± 0.01 ml; TLC, 1.05 ± 0.04 ml; VC, 0.95 ± 0.03 ml; static lung compliance, 0.075 ± 0.004 ml/cmH2O; and Crs, 0.021 ± 0.001 ml/cmH2O. Therefore, FRC occurred around 24% TLC.

Respiratory mechanics. Mean static P-V curves of the lung, chest wall, and total respiratory system are illustrated in Fig. 1. For volume changes between 100 and 10% TLC, mean pressure changes in the lung, chest wall, and total respiratory system were 30.1, 4.9, and 35 cmH2O, respectively. The curve of the chest wall was fairly steep, and its total pressure range was small, indicating a compliant chest wall. Because of the compliant chest wall, the curve of the total system was nearly similar to that of the lungs.

An averaged MEFV curve is shown in Fig. 2. The peak flow was 16.0 ± 0.7 ml/s and occurred at 86.0% VC. After the peak Vmax, Vmax decreased gradually until it reached residual volume (RV) at ~10% TLC. Vmax values were 13.5, 8.0, and 2.8 VC/s at 75, 50, and 25% VC, respectively. Combining both MEFV and static P-V curves of the lung, the maximal flow-static pressure curves were plotted in Fig. 3. The fitted correlation curves were relatively linear, and all slopes were significantly different from zero.

Bronchial function before and after methacholine challenge. Intravenous injection of methacholine induced changes in flow, volume, and Pao (Fig. 4). Both flow and volume decreased, whereas Pao increased soon after the challenge. The change in Pao reached a maximum around 40 s after the challenge. In addition, methacholine challenge caused a marked change in the flow-volume curve (Fig. 5). Averaged values of Crs, FEV0.1, Vmax50%, and Vmax30 before and after methacholine challenge are listed in Table 1. Methacholine challenge caused significant decreases in all values, indicating methacholine-induced marked airway constriction.

![Fig. 1. Mean static pressure-volume (P-V) curves of the lung, chest wall, and total respiratory system. TLC, total lung capacity; Transpulmonary pressure (Pl) reflects lung P-V curve, esophageal pressure (Pes) reflects chest wall P-V curve, and airway opening pressure (Pao) reflects total system P-V curve.](http://jap.physiology.org/)
DISCUSSION

Averaged TLC and FRC were 1.05 ± 0.04 and 0.25 ± 0.01 ml, respectively, in anesthetized paralyzed mice. The static P-V curve of the chest wall was fairly compliant and the FRC-to-TLC ratio (FRC/TLC) was relatively low in the mouse. The peak $V_{\text{max}}$ was 16.0 ± 0.7 ml/s and occurred at 86.0% VC. $V_{\text{max}}$ values were 13.5, 8.0, and 2.8 VC/s at 75, 50, and 25% VC, respectively. In addition, methacholine challenge caused significant decreases in Crs, FEV$_{0.1}$, $V_{\text{max}50}$, and $V_{\text{max}30}$. Several features of these results will be discussed below.

Respiratory mechanical properties of the mouse. The peak Pao used for the mean static P-V curve was 25 cmH$_2$O (Fig. 1). The lung volume at this static peak Pao should be fairly close to the peak volume at dynamic peak Pao of 30 cmH$_2$O during the MEFV maneuver because of the following three reasons. 1) There is a resistive pressure loss during the dynamic condition. This resistive pressure loss is required to overcome the airway and lung tissue resistance but cannot help to expand the lung. 2) There is a stress relaxation immediately after the transition from the dynamic to the static condition or flow interruption. 3) The P-V curve is fairly flat at the volume close to TLC. A difference in pressure of 1–2 cmH$_2$O should produce very little change in volume. Accordingly, we believed that TLCs during the static P-V and dynamic MEFV maneuvers should occur at about the same volume.

Compared with the rat (12), the mouse has a similar FRC/TLC and P-V curve characteristics. Compared with normal awake humans, however, the anesthetized paralyzed mouse has a smaller FRC and RV expressed as %TLC. Although anesthesia and/or paralysis might alter respiratory mechanics, the lower FRC and RV may be related mainly to the compliant chest wall. Let us compare compliance in the VT range for the mouse and humans. In the horizontal posture, the mouse’s chest wall is much more compliant (23.6 ml·cmH$_2$O$^{-1}$·kg body wt$^{-1}$) than that of humans (2.9 ml·cmH$_2$O$^{-1}$·kg body wt$^{-1}$) (21). The P-V curve of the mouse’s chest wall, compared with that of humans, is shifted to the right below midlung volumes. This shift moves the point of resting balance of forces between the lung and the chest wall to a lower volume and results in a lower FRC. Contrary to chest wall compliance, the value of lung compliance is closer between humans (3.7 ml·cmH$_2$O$^{-1}$·kg body wt$^{-1}$) (21) and the mouse (3.6 ml·cmH$_2$O$^{-1}$·kg body wt$^{-1}$ obtained from Fig. 1) within the VT range. Gillespie (6) reviewed cross-species data showing the negative correlation trend between chest wall compliance/TLC and FRC/TLC. In addition, Leith (13) suggests that the mouse’s chest wall is so compli-

Fig. 2. Averaged maximal expiratory flow ($V_{\text{max}}$)-volume curve.

Fig. 3. $V_{\text{max}}$-static recoil pressure curves of the lung in 10 mice.

Fig. 4. Example of methacholine challenge-induced changes in flow, volume, and Pao in an anesthetized paralyzed and ventilated mouse. Methacholine was intravenously injected at time 0. Arrow, maximum change in Pao.
ant that mice set their FRC with constant activation of the inspiratory muscles. Functionally, FRC acts as a buffer for pulmonary gas exchange (1). To compensate for its lower FRC, the mouse has a much higher breathing frequency than do humans so that fluctuation in alveolar gas concentration is correspondingly reduced.

\[
V_{\text{max}} \left( \frac{\text{ml}}{\text{s}} \right) = 16.0
\]

Peak \( V_{\text{max}} \) was 16.0 ml/s and occurred at 86.0% VC (or 88.5%, TLC) in the mouse. The occurrence of the mice’s peak \( V_{\text{max}} \), expressed as %TLC, was similar to that of guinea pigs at 84% TLC (11) and hamsters at 70–95% TLC (16). After peak \( V_{\text{max}} \), \( V_{\text{max}} \) decreased gradually with reducing lung volume. No apparent plateau on the MEFV curve was detected. This may be related to the negative pressure employed to perform the MEFV curve in this study. In our previous study of \( V_{\text{max}} \) in guinea pigs, a plateau on the MEFV curve was usually found when a vacuum pressure of –10 to –20 cmH\(_2\)O was used but not when a vacuum pressure of –40 cmH\(_2\)O or higher was used (11).

In terms of VC per second, peak \( V_{\text{max}} \) of the mouse (13.2 VC/s) is similar to that of the rat (18.0 VC/s) (3) and the guinea pig (17.9 VC/s) (11). However, the mouse’s peak \( V_{\text{max}} \) is much higher than that of humans with 1.8 VC/s (9). Similarly, \( V_{\text{max}} \) values (in VC/s) at other lung volumes are much higher than those of humans (Table 2). This higher \( V_{\text{max}} \) would indicate a lower airway resistance and could help to reduce the work of breathing in this species with high breathing frequency mentioned in Respiratory mechanical properties of the mouse.

Table 1. Values of \( \text{C}_{\text{rs}}, \text{FEV}_{0.1}, V_{\text{max}50}, \text{and } V_{\text{max}30} \) before and after methacholine challenge

<table>
<thead>
<tr>
<th></th>
<th>Baseline Value</th>
<th>Methacholine (2,000 µg/kg) Value</th>
<th>%Baseline Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{C}_{\text{rs}}, \text{ml/cmH}_2\text{O} )</td>
<td>0.02 ± 0.001</td>
<td>0.01 ± 0.001*</td>
<td>38.2 ± 3.3</td>
</tr>
<tr>
<td>( \text{FEV}_{0.1}, \text{ml} )</td>
<td>0.83 ± 0.02</td>
<td>0.33 ± 0.04*</td>
<td>39.0 ± 2.5</td>
</tr>
<tr>
<td>( V_{\text{max}50}, \text{ml/s} )</td>
<td>10.83 ± 1.12</td>
<td>2.91 ± 0.39*</td>
<td>34.9 ± 2.1</td>
</tr>
<tr>
<td>( V_{\text{max}30}, \text{ml/s} )</td>
<td>6.04 ± 0.68</td>
<td>1.65 ± 0.21*</td>
<td>32.9 ± 1.9</td>
</tr>
</tbody>
</table>

Table 2. Values of maximal expiratory flow in humans and different anesthetized animals

<table>
<thead>
<tr>
<th>Species</th>
<th>( V_{\text{max}}, \text{VC/s} )</th>
<th>( V_{\text{max}50}, \text{VC/s} )</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>1.8</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td>Dog</td>
<td>5.5</td>
<td>4.6</td>
<td>18</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>17.9</td>
<td>8.0</td>
<td>10</td>
</tr>
<tr>
<td>Hamster</td>
<td>14.5</td>
<td>9.2</td>
<td>14</td>
</tr>
<tr>
<td>Rat</td>
<td>18.0</td>
<td>15.6</td>
<td>3</td>
</tr>
<tr>
<td>Mice</td>
<td>13.2</td>
<td>11.5</td>
<td>This study</td>
</tr>
</tbody>
</table>

\( V_{\text{max}}, \text{ maximal expiratory flow; VC, vital capacity.} \)
ance of the lung. Accordingly, changes in Crs, FEV\textsubscript{0.1}, V\textsubscript{max50}, and V\textsubscript{max30} were compared in the mouse in response to methacholine challenge in this study. This methacholine challenge caused 61.8, 61.0, 65.1, and 67.1\% decreases in Crs, FEV\textsubscript{0.1}, V\textsubscript{max50}, and V\textsubscript{max30}, respectively, indicating marked airway constriction. It is possible that the change in dynamic compliance indicates an alteration in the peripheral airway. V\textsubscript{max} is related mainly to the mechanical property of the portion of the airway upstream from the flow-limiting (equal pressure) point (10, 19). Because the flow-limiting point is moving to the peripheral portion of the airway when lung volume is reduced, V\textsubscript{max} should be related mainly to the peripheral airway resistance at low lung volume such as FRC, with the decreased FEV\textsubscript{0.1} value reflecting, perhaps, the more central, and flows at the lower volume the more peripheral, airways. Accordingly, our findings of similar methacholine-induced decreases in Crs, FEV\textsubscript{0.1}, and V\textsubscript{max} may indicate the constriction in all airways, although methacholine has been shown to act mainly in the central airways in humans (23).

Martin et al. (18) reported that methacholine in the dose of 1,950 µg/kg was required to decrease dynamic lung compliance to 65\% of the baseline value. Similarly, we found that a dose of 2,000 µg/kg was needed to decrease Crs to 38.2\% of the baseline value. In addition, methacholine caused a gradual increase in Pao but decreases in both flow and Vt. These respiratory changes reached a maximal value at 40.5 ± 1.8 s after the intravenous injection of methacholine. Subsequently, these changes reduced gradually with time, although they did not recover completely 120 s after the challenge (Fig. 4).

In summary, this study found that the mouse's static respiratory parameters and maximal expiratory values, after normalization, are similar to those of the rat and guinea pig but are higher than those of humans. In addition to resistance and dynamic compliance observed by other investigators, we found that V\textsubscript{max} and FEV\textsubscript{0.1} are also useful indicators of airway constriction in the mouse.

This work was supported by the National Science Council (NSC88–2314-B–002–222 and NSC88–2314-B002–066M41). Address for reprint requests and other correspondence: Y.-L. Lai, Dept. of Physiology, College of Medicine, National Taiwan Univ., No. 1, Sect. 1, J en-Ai Rd., Taipei, Taiwan (E-mail: tiger@ha.mc.ntu.edu.tw).

Received 11 January 1999; accepted in final form 27 October 1999.

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