End-expiratory and tidal volumes measured in conscious mice using single projection x-ray images

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Lai-Fook SJ, Houtz PK, Lai Y-L. End-expiratory and tidal volumes measured in conscious mice using single projection x-ray images. J Appl Physiol 104: 521–533, 2008. First published September 13, 2007; doi:10.1152/japplphysiol.00729.2007.—The evaluation of airway resistance (Raw) in conscious mice requires both end-expiratory (Ve) and tidal volumes (Vt) (Lai-Fook SJ and Lai YL. J Appl Physiol 98: 2204–2218, 2005). In anesthetized BALB/c mice we measured lung area (AL) from ventral-to-dorsal x-ray images taken at FRC (Ve) and after air inflation with 0.25 and 0.50 ml (ΔVe). Total lung volume (VL) described by equation: VL = ΔVL + VFR = KAL1.5, assumed uniform (isotropic) inflation. Total VFR averaged 0.55 ml, consisting of 0.10 ml tissue, 0.21 ml blood and 0.24 ml air. K averaged 1.84. In conscious mice in a sealed box, we measured the peak-to-peak box pressure excursions (ΔPb) and x-rays during several cycles. K was used to convert measured AL to Ve values. We calculated Vc and Ve from the plot of Vc vs. cos(α − φ). Phase angle α was the minimum point of the Pb cycle to the x-ray exposure. Phase difference between the Pb and VL cycles (φ) was measured from ΔPb values using both room- and body-temperature humidified box air. A similar analysis was used after aerosol exposures to bronchoconstrictor methacholine (Mch), except that φ depended also on increased Raw. In conscious mice, Vc (0.24 ml) doubled after Mch (50–125 mg/ml) aerosol exposure with constant Ve, frequency (f), ΔPb, and Raw. In anesthetized mice, in addition to an increased Vc, repeated 100 mg/ml Mch exposures increased both ΔPb and Raw and decreased f to apnea in 10 min. Thus conscious mice adapted to Mch by limiting phragm fatigue and failure.

bronchoconstrictor; methacholine aerosol; body plethysmography

THE EVALUATION of Raw in conscious unrestrained mice by means of barometric plethysmography requires the measurement of the box pressure excursion, Vt and Ve, under both room-temperature and body-temperature box air conditions (25). In the previous study (25), the values of Vt and Ve needed to compute Raw were measured in anesthetized mice by means of conventional methods. In conscious mice, Vt was measured only under control conditions from the box pressure excursions by means of the analysis of Drorbaugh and Fenn (13). This analysis is valid only for control conditions when the contribution of the effects of temperature and humidity to the box pressure excursions dominates the gas compression effects of airway resistance. Under bronchoconstricted conditions with increased Raw, a measure of Vt in conscious mice was not available. Accordingly, to compute Raw in conscious mice, we assumed values of Vc measured in anesthetized mice under both control and bronchoconstricted conditions, and further

assumed that Vt did not change with bronchoconstriction (25). In the previous study (25), the lack of a direct measure of Vc and Vt was a major deficiency and limitation of the barometric method for the evaluation of Raw in conscious mice.

The measurement of Vt in conscious mice is particularly difficult with available computed tomography (CT) and micro-CT imaging technology (15, 28) because of the relatively low temporal resolution with x-ray exposure times, which are too long to obtain lung images at end-inspiration and end-expiration at breathing frequencies approaching 5 breaths/s. Accordingly, in this study we used a single x-ray pulse of 10 ms to obtain single projection images of the thorax of conscious mice breathing spontaneously in a sealed box. The short exposure time minimized image blur due to ventilatory and cardiogenic motion. In anesthetized mice, we measured lung area from x-ray images obtained at Vc and after imposed increases in lung volume. With the assumption of uniform (isotropic) lung expansion, the mathematical relationship between lung area and lung volume was used to infer lung volume in conscious mice from x-ray images taken at different points during the box pressure cycle. The lung volume based on the lung area was corrected for tissue and blood mass measured in separate experiments. Under control conditions, the phase difference between the box pressure and lung volume cycle was measured from box pressure excursions with both room- and body-temperature humidified box air. A sinusoidal analysis was used to determine lung volume at associated points during the lung volume cycle and to determine Vt and Vc, both for control conditions and after exposure to the bronchoconstrictor methacholine (Mch) aerosol. The results showed that Vc increased in response to Mch aerosol in both conscious and spontaneously breathing anesthetized mice. However, Mch produced an increase in Raw in the anesthetized mice but not in the conscious mice.

Glossary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AL</td>
<td>lung area measured off x-ray images (cm²)</td>
</tr>
<tr>
<td>A0x</td>
<td>area under the box pressure-time curve for inspiration or expiration, mean of the two areas (cmH2O·s)</td>
</tr>
<tr>
<td>AFR/C</td>
<td>lung area measured off x-ray image taken at FRC (cm²)</td>
</tr>
<tr>
<td>b</td>
<td>subscript, box gas</td>
</tr>
<tr>
<td>°C</td>
<td>degree centigrade, unit of temperature</td>
</tr>
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LUNG VOLUME AND AIRWAY RESISTANCE IN MICE

METHODS

We studied BALB/c mice (20–24 g body wt, n = 35; Harlan, Indianapolis, IN). This study was approved by the University of Kentucky Animal Care and Use Committee.

Calibration of Lung Area From X-Ray Images vs. Lung Volume Measured in Anesthetized Mice

BALB/c mice were anesthetized with 50 mg/kg sodium pentobarbital delivered ip. After a tracheostomy, the supine animal was placed on a digital x-ray sensor (4.2 cm × 3.0 cm, Lightyear Technology) and ventilated (5 breaths/s and 0.15 ml tidal volume; Hugo Sachs Elektronik, Harvard Apparatus MiniVent, type 845). Airway pressure and ventilated (5 breaths/s and 0.15 ml tidal volume; Hugo Sachs x-ray source (10 ms exposure) at functional residual capacity (FRC) at images of the thorax (ventral-dorsal direction) were taken with an TA2000). With the trachea occluded at end-expiration, vertical x-ray images of the thorax were connected to a pressure transducer (Cobe) and a chart recorder (Gould VLmax VL at maximum box pressure

\[ \alpha \] phase angle from minimum \( P_b \) to x-ray exposure (°)

\[ \delta \] phase difference between \( P_b \) and \( V_L \), cycles (°)

\[ \phi \] phase difference between \( \Delta P_b \) and \( V_L \), cycles (°)

\[ \theta \] \( \tan^{-1} (\Delta P_b / P_b) \), phase angle difference between temperature-humidity and gas compression parts of the box pressure curve

\[ \Delta \] peak-to-peak excursion of peak-to-peak box pressure excursion, assumed equal to \( 2 \Delta P_b \)

\[ \Delta T \] body-to-box air temperature difference

\[ \Delta V_L \] increment of air volume from FRC

\( f \) respiratory frequency (cycles/s, Hz)

\( f_R \) functional residual capacity or end-expiratory lung air volume (Ve, ml)

\( g \) subscript, gas compression effects

\( h \) subscript, temperature-humidity effects

\( K \) coefficient relating \( V_L \) to \( A_L^{1.5} \)

\( Mch \) abbreviation for methacholine

\[ P_{adv} \] alveolar gas pressure (cmH\(_2\)O)

\[ P_b \] box gas pressure (cmH\(_2\)O)

\[ P_g \] gas compression part of box pressure (cmH\(_2\)O)

\[ P_h \] temperature-humidity part of box pressure (cmH\(_2\)O)

\( Q \) airway flow (ml/s)

\[ R_{aw} \] airway resistance if viscous pressure loss were entirely laminar measured by gas compression part of box pressure (cmH\(_2\)O·ml\(^{-1}\)·s)

\[ V_{adv} \] alveolar gas volume (ml)

\[ V_b \] box air volume (ml)

\[ V_e \] FRC, end-expiratory lung air volume (ml)

\[ V_{FRC} \] total lung volume at end-expiration (ml)

\[ V_L \] total lung volume consisting of volume of air, tissue, and blood (ml)

\[ V_{Lm} \] mean lung air volume, \( V_e + 0.5V_{t} \) (ml)

\( V_{t} \) tidal volume (ml)

1 subscript, control

2 subscript, intervention
measuring the resulting box pressure excursion. A small correction for
gas compression effects was made to the calculated tidal volume (Eq.
15 of Ref. 25), as discussed below.

**Effect of Body Temperature Box Air on Lung Volumes and Box Pressure in Conscious Mice**

The correction for the gas compression effect to the calculated tidal
volume required knowledge of the relative contributions of the gas
compression effect and the temperature-humidity effect to the box
pressure excursion. This correction was also needed to determine \( V_t \)
from x-ray data in mice with increased \( R_{aw} \). The correction was
determined by measuring the box pressure excursion first with room-
temperature box air, then with body-temperature humidified box air.
For the latter measurement, the box air temperature was maintained
at body temperature (37–39°C) with a thermostatic controller
(Physitemp, TCAT-2AC) that provided heat to the box from an
external infrared lamp when the temperature measured by the box
thermometer was below 38°C. Supplemental heat to the air within
the copper tube was provided by a voltage source to a silicon rubber
heater (100 W, c-03125–22, Cole-Parmer) bonded to the outside of
the copper tube. Humidity (100%) was provided by airflow from the
saline aerosolizer. The box air was stabilized at body-temperature
conditions for 1 h prior to placing the animal in the box. The box was
then sealed, and several x-rays were taken over a period of 6 min near
the midpoints of the pressure excursions that represented only gas
compression effects and airway flow. Accordingly, the midpoints
corresponded to maximum and minimum points on the volume cycle
that differed in phase by 90° from the flow cycle. By an independent
calibration with the CO2 probe, the 6-min period caused an increase
in CO2 partial pressure to 16 mmHg (2%) with no consistent change
in either the box pressure excursions or breathing frequencies.

**Effect of Mch Aerosol on Lung Volume in Conscious Mice**

The animal was placed in the box at 21–24°C and 100% relative
humidity with an airflow supplied by the saline aerosolizer. The box
was sealed and the box pressure excursions were measured over a
period of 6 min and used to calculate tidal volume (13, 25). Several
x-ray images of the thorax were taken and used to calculate tidal
volume (\( V_t \)) and mean lung volume (\( V_{m} \)), as described later. Box
pressure and x-ray images were remeasured after the box air was
increased to 37–39°C at 100% relative humidity. After a recovery
period of several hours, box pressure and x-ray images were measured
with room-temperature box air in response to a 1-min aerosolized
Mch (50 mg/ml) exposure. An increase in Mch concentration to 125
mg/ml together with repeated exposures for 1–3 min had no consistent
change on the box pressure excursions or the calculated \( R_{aw} \).

**Effect of Mch Aerosol on Lung Volume in Anesthetized Mice**

The foregoing procedures used in conscious mice to measure lung
volume at both room- and body-temperature box air conditions and
after Mch aerosol exposure at room temperature were repeated in
anesthetized spontaneously breathing mice in the prone position. The
mice were anesthetized with an ip injection of ketamine (100 mg/kg)
and xylazine (8.5 mg/kg) in 0.125 ml saline; this procedure provided
anesthesia for ~1 h. Under anesthesia, box pressure and x-ray images
were measured at room temperature and after box air conditions were increased to 37–39°C and 100% relative humidity. After allowing the animal to recover from the anesthesia overnight, box pressure and x-ray images were measured under anesthesia at room temperature with saline aerosol exposure and after Mch aerosol exposure (100 mg/ml) with a flow rate of 10 l/min. Preliminary studies showed no increase in the box pressure excursions after Mch aerosol exposure of 25–75 mg/ml. After 1 min of 100-mg/ml Mch exposure, the box was sealed, and box pressure was recorded for 1 min. The 1-min, 100-mg/ml Mch exposure was repeated until the box pressure excursions increased. Usually three Mch exposures were required to obtain an increased response in the box pressure. The increase in the box pressure excursions occurred simultaneously with a reduction in breathing frequency, terminating in apnea. During the increased box pressure response, x-ray images were recorded at several minimum and maximum points of sequential box pressure cycles. Post mortem, the lung and heart were removed, separated, and weighed. The lung was dried to a constant weight in an oven at 70°C, and the wet-to-dry weight ratio (W/D) calculated.

Blood and Tissue Mass in the Isolated Collapsed Lung

The equation for $R_{aw}$ (Ref. 25, see Eq. 3 below) requires air volumes at FRC, while the x-ray data provided the total lung volume, which included tissue and blood mass in addition to air volume. Thus, to correct for tissue and blood mass to obtain air volume, we measured tissue mass and blood mass at FRC via two separate experiments. In the first experiment, the blood mass was separated from the (blood-free) tissue mass in the isolated collapsed lung via the following procedure. In the anesthetized mouse, radioactive tracer $^{125}$I-albumin ($\sim 2 \times 10^4$ counts/s in 0.05 ml Ringer solution; bovine serum albumin, Perkin Elmer, Boston, MA) was injected into the jugular vein. Prior to use, any unbound $^{125}$I was removed by passing tracer through a desalting column (PD-10 desalting column; Amersham Biosciences, Piscataway, NJ; see Ref. 24). One minute after injection, a 0.4-ml sample of blood was withdrawn, and its specific radioactivity (counts/s per g) was measured in a gamma counter (WIZARD 1470, Perkin-Elmer, Billerica, MA). Post mortem, the lung was isolated and weighed, and its total radioactivity was measured. The trapped blood mass was calculated by dividing the total radioactivity of the blood by the specific radioactivity of the blood. Tissue mass was the difference between the mass of the isolated lung and the trapped blood mass. The density of both blood and tissue was assumed to be 1 g/ml.

Lung Blood Mass in Spontaneously Breathing Anesthetized Mice at FRC

In the second experiment, we measured the blood mass in anesthetized mice via a procedure similar to that used previously in unanesthetized mice (16). In the control anesthetized mouse, one minute after tracer $^{125}$I-albumin was injected into the jugular vein, the mouse was rapidly euthanized by immersion in liquid $N_2$ and placed overnight in a freezer ($\sim -20°C$). Then the frozen mouse was transected through a desalting column (PD-10 desalting column; Amersham Biosciences, Piscataway, NJ; see Ref. 24). One minute after injection, a 0.4-ml sample of blood was withdrawn, and its specific radioactivity (counts/s per g) was measured in a gamma counter (WIZARD 1470, Perkin-Elmer, Billerica, MA). Post mortem, the lung was isolated and weighed, and its total radioactivity was measured. The trapped blood mass was calculated by dividing the total radioactivity of the blood by the specific radioactivity of the blood. Tissue mass was the difference between the mass of the isolated lung and the trapped blood mass. The density of both blood and tissue was assumed to be 1 g/ml.

Statistics

Data are reported as mean values $\pm$ SD. We used paired $t$- and unmatched $t$-tests where appropriate to evaluate significant differences between two groups of data. We used $P < 0.05$ to be significant.

RESULTS: THEORY AND ANALYSIS OF DATA

Lung Tissue and Blood Mass in Anesthetized Mice

Table 1 (mean $\pm$ SD, $n = 5$) summarizes results of (blood-free) tissue and trapped blood mass measured in the isolated collapsed lung. Tissue mass averaged 0.10 g, and the trapped blood averaged 0.025 g. Table 2 summarizes the blood mass measured in the lungs of anesthetized spontaneously breathing mice. Lung blood mass averaged 0.21 g for the control mice and was reduced by 43% to 0.12 g after bronchoconstriction with three 1-min exposures to 100 mg/ml Mch aerosol. The reduced blood volume in anesthetized mice was attributed to an increased $V_e$ that dominated any Mch-induced vascular dilation (1). The values of tissue and blood mass were subtracted from the total lung volume measured via the x-ray measurements of lung area to obtain the lung air volume at FRC in conscious and anesthetized mice (see Calibration of X-ray Lung Area to Total Lung Volume With Anesthetized Mice).

Blood and Tissue Mass in Anesthetized Mice Formed a Uniform Lung Expansion

We assumed that the lung within the thorax was uniformly inflated so that total lung volume ($V_L$) was proportional to $A_L^{1.5}$, where $A_L$ is the area of the lung outline that includes the heart minus the area of the heart outline measured from each x-ray image (Fig. 3):

$$V_L = KA_L^{1.5} \quad (1)$$

Here K is a constant that converts the $A_L^{1.5}$ values to $V_L$, the sum of the air volume and the volume occupied by the tissue and blood.

Calibration of X-ray Lung Area to Total Lung Volume With Anesthetized Mice

An estimate of the total lung volume at FRC ($V_{FRC}$) was obtained by linear regression of the imposed air volume increments from FRC ($\Delta V_L$) vs. the measured $A_L^{1.5}$ values with the following equation:

$$\Delta V_L = KA_L^{1.5} - V_{FRC} \quad (2)$$

Here $-V_{FRC}$ is the intercept. Figure 4 shows a plot of $\Delta V_L$ vs. $A_L^{1.5}$ for five anesthetized mice: $\Delta V_L = 1.84A_L^{1.5} - 0.55$ ($R^2 = 0.75$, $n = 15$, $P < 10^{-5}$). The pooled data produced a

Table 1. Tissue and blood mass in isolated collapsed lung

<table>
<thead>
<tr>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collapsed lung mass, g</td>
</tr>
<tr>
<td>Blood specific activity, counts/s/g^-1</td>
</tr>
<tr>
<td>Lung total activity, counts/s</td>
</tr>
<tr>
<td>Blood mass in lung, g</td>
</tr>
<tr>
<td>Tissue mass of blood-free lung*, g</td>
</tr>
</tbody>
</table>

*Anesthetized mice were injected with $^{125}$I-albumin iv prior to euthanasia. Values are mean $\pm$ SD, $n = 5$. *Tissue mass of blood-free lung equals (collapsed mass − blood mass).
Table 2. Lung blood mass in spontaneously breathing anesthetized mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>After Mch Aerosol Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of lung samples, g</td>
<td>0.22±0.020</td>
<td>0.14±0.024</td>
</tr>
<tr>
<td>Blood specific activity, counts·s⁻¹·g⁻¹</td>
<td>6242±4667</td>
<td>3407±26879</td>
</tr>
<tr>
<td>Total activity of lung samples, counts/s</td>
<td>916±684</td>
<td>1701±855</td>
</tr>
<tr>
<td>Blood mass in lung samples, g</td>
<td>0.15±0.013</td>
<td>0.05±0.032</td>
</tr>
<tr>
<td>Tissue mass in lung samples, g</td>
<td>0.07±0.015</td>
<td>0.07±0.032</td>
</tr>
<tr>
<td>Blood mass/tissue mass</td>
<td>2.1±0.50</td>
<td>1.16±0.85</td>
</tr>
<tr>
<td>Whole lung tissue mass, g (see Table 1)</td>
<td>0.10±0.0051</td>
<td>0.10±0.0051</td>
</tr>
<tr>
<td>Whole lung blood mass, g</td>
<td>0.21</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Anesthetized mice were injected with ¹²⁵I-albumin iv prior to euthanasia and frozen in liquid N₂. Values are mean ± SD, n = 5.

K of 1.84 and V_{FRC} of 0.55 ml. Analysis of each mouse separately produced mean values of K and V_{FRC} of 1.90 ± 0.56 (SD, n = 5) and 0.58 ± 0.20 ml, respectively. In the control anesthetized mice, air volume at FRC was 0.24–0.27 ml, after correction for tissue and blood mass.

Figure 5 is a plot of V_L vs. A_L¹.5 after normalizing by dividing by V_{FRC} and A_{FRC}¹.5, respectively, the calculated V_L and measured A_L¹.5 values at FRC. The regression equation was: V_L/V_{FRC} = 0.97(A_L/A_{FRC})¹.5 (R² = 0.95, n = 14, P < 10⁻⁷). The constant of proportionality was 0.97, close to the value of 1 expected for uniform lung inflation. Values of V_L were predicted from measurements of A_L¹.5 with a 3% error.

**Theoretical Background: Relation of R_{aw} to Lung Volumes and Box Pressure**

The method used to evaluate R_{aw} by means of body plethysmography requires the box pressure excursion, lung tidal volume, and end-expiratory lung air volume under both room- and body-temperature box air conditions. A detailed description has been published (25). In brief, for an animal breathing spontaneously in a sealed box, R_{aw} is given by the following equation:

\[
R_{aw} = A_{ht}V_B/(V_TV_m)
\]

Here A_{ht} is the area under the gas compression part of the box pressure (P_B) vs. time (t) curve during inspiration or expiration. V_B is the box gas volume, V_T is the tidal volume, and V_m is the...
mean lung gas volume given by the end-expiratory gas volume ($V_e$) plus $V_t/2$. $P_b$ consists of two parts, one ($P_g$) due to gas compression and the other ($P_h$) due to the change in temperature and humidity of the inspired air from box air conditions to body-temperature conditions. The assumption of sinusoidal changes for $P_b$, $P_g$, and $P_h$ produces the following equation for amplitude $\delta P_b$ in terms of amplitudes $\delta P_g$ and $\delta P_h$:

$$\delta P_b = (\delta P_g^2 + \delta P_h^2)^{1/2}$$  (4)

The phase relationships among $P_b$, $P_g$, and $P_h$ are summarized by the vector diagram of Fig. 6:

$$\theta = \tan^{-1}(\delta P_b / \delta P_g)$$  (5)

$$\phi = 90 - \tan^{-1}(\delta P_b / \delta P_g) = \cos^{-1}(\delta P_b / \delta P_b)$$  (6)

In the experiments, $\delta P_b$ was calculated as half of $\Delta P_b$ (the peak-to-peak box pressure excursion), and $A_{st}$ was the average of the areas under the inspiratory and expiratory parts of the $P_b$ vs. $t$ curve, given by $\delta P_b/(\pi f)$ for a sine wave with frequency $f$.

**Phase Difference Between $P_b$ and $V_L$**

Drorbaugh and Fenn (13) showed that the inspired gas volume $dV_L$ is proportional to $dP_b$, so that the phase relationships of $P_b$ and $P_e$ to $P_h$ (Eqs. 5 and 6) apply to $V_L$. In the experiments, $V_L$ was measured at different points along the $P_b$ vs. $t$ curve and corrected for the phase angle $\phi$ to determine the $V_L$ values at end-inspiration and end-expiration as well as $V_i$ (Fig. 7). For control (unconstricted) conditions with subscript 1, $\phi_1$ was calculated by means of Eq. 6 after determining $\delta P_{h1}/\delta P_{g1}$ from the following equation (Eq. 15 of Ref. 25):

$$\delta P_{g1}/\delta P_{b1} = [1 + (\delta P_{b1}/\delta P_{g1})^2]^{-1/2}$$  (7)

Here $\delta P_{b1}$ and $\delta P_{g1}$ were the box pressure amplitudes with room- and body-temperature humidified box air conditions, respectively. The calculation of $\phi_1$ for control conditions assumed that for constant $R_{aw}$, $\delta P_{g1}$ and $\delta P_{h1}$ (that is, $V_{t1}$) at room temperature did not change with body-temperature box air conditions. These assumptions were verified experimentally (see Effect of Body-Temperature Humidified Box Air Conditions on $\delta P_{g1}$, $V_t$, $V_e$, and $f$).

**Determination of $V_i$ and $V_e$ From X-Ray Images of Conscious Mice**

To evaluate the x-ray data, we assumed that the cyclic variations of box pressure $P_b$ and inspired volume were sinusoidal, and the total lung volume ($V_L$) analogous to alveolar air volume was represented by the following equation (Fig. 7, cf. Eq. A3 of Ref. 25):

$$V_{L1} = V_{Lmi} - 0.5V_t \cos(2\pi ft - \phi_1)$$  (8)

$V_{Lmi}$ is the total mean lung volume equal to $V_{FRC} + V_{t/2}$. Figure 7 illustrates box pressure $P_b$ and lung volume ($V_L$) excursions as two cosine waves with $\phi$ of 40°, $V_t$ of 0.2 ml, and $V_{Lmi}$ of 0.4 ml (Eq. 8).

In the experiments we collected x-ray images at different time points along several box pressure cycles. An example of an x-ray pulse recorded during a pressure cycle is shown in Fig. 2. The time ($t_0$) from the start (minimum point) of the cycle (phase angle $\alpha_1 = 0°$) to the time of x-ray exposure was measured for each x-ray image and converted to a phase angle ($\alpha_1 = 2\pi t_0$). $\alpha_1$ varied from 0° (cos 0° = 1), to 180° (cos 180° = -1) at the maximum box pressure, to 360° (cos 360° = 1) at the end of the cycle. Values of $A_{1.5}$ for the x-ray images were converted to $V_L$ values with K of 1.84 (Eq. 1), the value measured in anesthetized mice (Fig. 4). The linear regression of $V_{L1}$ vs. $\cos(\alpha_1 - \phi_1)$ values provided the tidal volume $V_t$ as twice the slope magnitude and $V_{Lmi}$ as the intercept (Eq. 8). In the event that $V_{L1}$ was measured at the maximum and minimum box.
With this procedure in general, the correction for \( \phi_1 \) affected only \( V_{t1} \) and not \( V_{Lm1} \).

Figure 8 is an example of \( V_{L1} \) vs. \( \cos (\alpha_1 - \phi_1) \) values measured in one animal. The regression equation (Eq. 8) was:

\[
V_{Lm1} = (V_{L1,max} + V_{L1,min})/2
\]

(9)

\[
V_{t1} = (V_{L1,max} - V_{L1,min})/\cos \phi_1
\]

(10)

With this procedure in general, the correction for \( \phi_1 \) affected only \( V_{t1} \) and not \( V_{Lm1} \).

Table 3. Values of tidal volume and total mean lung volume

<table>
<thead>
<tr>
<th>Value</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>( V_{t1} ) (box pressure excursion), ml</td>
<td>0.19±0.053</td>
</tr>
<tr>
<td>( V_{t1} ) (X-ray images), ml</td>
<td>0.21±0.051</td>
</tr>
<tr>
<td>Total mean lung volume, ( V_{Lm1} ) (x-ray images), ml</td>
<td>0.59±0.033</td>
</tr>
<tr>
<td>Total lung volume at FRC ( (V_{l,m1} - 0.5V_{e}) ), ml</td>
<td>0.49±0.045</td>
</tr>
<tr>
<td>Lung tissue volume (see Table 1), ml</td>
<td>0.10±0.0051</td>
</tr>
<tr>
<td>Lung blood volume (see Table 2), ml</td>
<td>0.21</td>
</tr>
<tr>
<td>( V_{e}, ) ml</td>
<td>0.18±0.045</td>
</tr>
</tbody>
</table>

Values are mean ± SD, \( n = 5 \). Values were measured via x-ray images in conscious mice under control conditions. Also shown are values of \( V_{t1} \) measured using the box pressure excursion, \( V_{t1} \), tidal volume; \( V_{Lm1} \), total mean lung volume; \( V_{e}, \) end-expiratory lung air volume.

Effect of Body-Temperature Humidified Box Air Conditions on \( \delta P_{g1}, V_{t}, V_{e}, \) and \( f \)

For control conditions, the foregoing corrections for \( V_{t1} \) based on \( \phi_1 \) and \( \delta P_{h1}/\delta P_{g1} \) used the assumption that both \( R_{aw} \) and \( \delta P_{g1} \) did not change with body-temperature humidified box air conditions (Eq. 7, Ref. 25). The constant \( R_{aw} \) with body-temperature box air conditions was substantiated previously in anesthetized mice (25). With body-temperature humidified box air, \( V_{Lm1} \) and \( V_{t1} \) were calculated from the measured x-ray areas taken at the midpoints of the box pressure excursions, representing \( \delta P_{g1} \) in phase with flow and thus end-inspiratory and end-expiratory volumes. The results are summarized in Table 4. Ratios of each parameter measured with body-temperature box air conditions to that measured with room-temperature box air were tested vs. 1 to evaluate any significant change. Note that body-temperature box air conditions had no significant effect on \( V_{t}, \) \( V_{e}, \) or \( f, \) indicating no change in the flow amplitude (\( \delta Q = \pi(V_{t}) \)); and with a constant \( R_{aw}, \) \( \delta P_{g1} \) equal to \( R_{aw}\delta Q \) did not change compared with the room-temperature box air conditions. By contrast, the evaluation of \( V_{t1} \) from the x-ray data with bronchoconstriction depended on any change in \( \delta P_{g1} \), which is treated in the following section.

Determination of \( V_{t} \) and \( V_{e} \) After Bronchoconstriction Due to Mch Aerosol Exposure

The determination of tidal volume (\( V_{t2} \)) for the bronchoconstricted animals required the simultaneous solution of both \( V_{t2} \) and \( \delta P_{g2} \), the amplitude of the gas compression part of the box pressure curve that determines the increased \( R_{aw} \). Subscripts 1 and 2 refer to control and constricted conditions, respectively. We used the following procedure, analogous to that used for the control animals with the appropriate modifications.

Unique solutions for \( V_{t2}/V_{t1} \) (that is, \( \delta P_{h2}/\delta P_{h1} \) and \( \delta P_{g2}/\delta P_{g1} \)) were obtained from the experimental data as follows. First, starting with a trial value of \( V_{t2}/V_{t1} \) (e.g., 1) and \( \delta P_{h1}/\delta P_{g1} \) known from control conditions, \( \delta P_{g2}/\delta P_{g1} \) was calculated by means of the following equation (Eq. 19 of Ref. 25), obtained by applying Eq. 4 to control and constricted conditions:

\[
\delta P_{g2}/\delta P_{g1} = [((\delta P_{h2}/\delta P_{h1})^2[1 + (\delta P_{h1}/\delta P_{g1})^2]) - (\delta P_{h2}/\delta P_{h1})^2(\delta P_{g2}/\delta P_{g1})]^1/2
\]

\( \delta P_{h2}/\delta P_{h1} \) was the measured box pressure amplitude ratio. Second, \( \delta P_{h2}/\delta P_{g2} \) was given by the product of the three ratios:

\[
\delta P_{h2}/\delta P_{g2} = (\delta P_{h2}/\delta P_{h1})(\delta P_{h1}/\delta P_{g1})(\delta P_{g1}/\delta P_{g2})
\]

Third, the phase angle \( \phi_2 \) between the lung volume and the box
pressure curves for constricted conditions was computed by means of the equation analogous to Eq. 6:

\[ \phi_x = 90 - \tan^{-1}(\delta P_{g2}/\delta P_{g1}) \]  

(13)

Fourth, analogous to Eqs. 9 and 10, the predicted \( V_{Lm2} \) and \( V_{t2} \) values with two \( V_{L2} \) values at the maximum and minimum pressures \( (V_{Lm2 max} and V_{Lm2 min}) \) were given by:

\[ V_{Lm2} = (V_{L2 max} + V_{L2 min})/2 \]  

(14)

\[ V_{t2} = (V_{L2 max} - V_{L2 min})/\cos \phi_2 \]  

(15)

The trial \( V_{t2}/V_{t1} \) value was varied to match the predicted \( V_{t2}/V_{t1} \) value, thus producing unique solutions for \( \delta P_{g2}/\delta P_{g1} \) and \( \phi_2 \) ratio to match for tissue and blood mass. Note that no correction for \( \phi_2 \) was needed for \( V_{Lm2} \).

**Effect of Mch Aerosol Exposure in Conscious Mice**

The foregoing analysis was applied to measurements in conscious mice exposed to Mch aerosol. The \( R_{aw} \) ratio with bronchoconstriction was obtained by means of Eq. 3 with \( A_{bst} \) ratio equal to \( \delta P_{g2}/\delta P_{g1} \) divided by frequency ratio \( (f_2/f_1) \) together with the \( V_t \) and \( V_m \) ratios. Table 5 summarizes values for \( V_t \), \( V_m \), \( V_e \), \( \delta P_{g2}/\delta P_{g1} \), \( \phi_2 \), \( \delta P_{g2}/\delta P_{g1} \), and \( A_{bst} \) for control and Mch aerosol-induced conditions in conscious mice. These results were obtained after a 1-min exposure to 50 mg/ml Mch aerosol and were independent of dose (50–125 mg/ml), time of exposure (1–3 min), or number of exposures. \( V_{m2} \) was obtained from \( V_{Lm2} \) by correcting for tissue and blood mass measured in anesthetized control mice, since \( R_{aw} \) did not change with the Mch aerosol exposure. Values of \( \delta P_{g} \) and \( f \) were obtained from the box pressure curves associated with the x-ray images that were used to calculate \( V_t \) and \( V_m \). There was no consistent change in \( V_t \), \( \delta P_{g} \), or \( R_{aw} \) with the Mch aerosol exposure. The important effect of Mch was to increase \( V_m \) by 2-fold and \( V_t \) by 2.3-fold. Thus any bronchoconstriction due to Mch was eliminated by breathing at a higher lung volume.

**Effect of Mch-Induced Bronchoconstriction in Anesthetized Animals**

Table 6 summarizes values for \( V_t \), \( V_m \), \( V_e \), \( \delta P_{g2}/\delta P_{g1} \), \( \phi_2 \), \( \delta P_{g2}/\delta P_{g1} \), and \( R_{aw} \) for control and Mch aerosol-induced conditions in anesthetized mice. \( V_{m2} \) was obtained from \( V_{Lm2} \) by correcting for tissue and blood mass measured in anesthetized mice after 3 repeated 1-min expos.
LUNG VOLUME AND AIRWAY RESISTANCE IN MICE

Table 6. Lung volumes measured in anesthetized mice under control and constricted conditions with Mch aerosol

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>Mch (M)</th>
<th>Ratio (M/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_t$, ml (box pressure excursion)</td>
<td>$0.18 ± 0.046$</td>
<td>$0.27 ± 0.13$</td>
<td>$1.5 ± 0.37$</td>
</tr>
<tr>
<td>$V_t$, ml (X-ray images)</td>
<td>$0.19 ± 0.079$</td>
<td>$0.93 ± 0.21$</td>
<td>$1.4 ± 0.40^*$</td>
</tr>
<tr>
<td>$L_{tn}$ (x-ray images), ml</td>
<td>$0.55 ± 0.079$</td>
<td>$0.21 ± 0.0051$</td>
<td>$0.46^*$</td>
</tr>
<tr>
<td>Lung tissue volume (see Table 1), ml</td>
<td>$0.10 ± 0.0051$</td>
<td>$0.10 ± 0.0051$</td>
<td>$1.00^*$</td>
</tr>
<tr>
<td>Lung blood volume (see Table 2), ml</td>
<td>$0.21$</td>
<td>$0.12$</td>
<td>$1.52^*$</td>
</tr>
<tr>
<td>$V_m$, ml</td>
<td>$0.24 ± 0.079$</td>
<td>$0.71 ± 0.21$</td>
<td>$3.0 ± 0.69^*$</td>
</tr>
<tr>
<td>$V_e$, ml</td>
<td>$0.14 ± 0.095$</td>
<td>$0.58 ± 0.23$</td>
<td>$4.1 ± 0.90^*$</td>
</tr>
<tr>
<td>$\delta P_e$, cmH$_2$O</td>
<td>$0.042 ± 0.014$</td>
<td>$0.16 ± 0.022$</td>
<td>$4.0 ± 1.5^*$</td>
</tr>
<tr>
<td>$\delta P/\delta P_e$</td>
<td>$2.2 ± 0.21$</td>
<td>$69 ± 11$</td>
<td>$2.8 ± 0.40^*$</td>
</tr>
<tr>
<td>$\phi$, Hz</td>
<td>$24 ± 2.2$</td>
<td>$8.6 ± 0.46^*$</td>
<td>$1.5$</td>
</tr>
<tr>
<td>$f$, Hz</td>
<td>$4.0 ± 0.64$</td>
<td>$1.1 ± 0.23$</td>
<td>$0.28 ± 0.049^*$</td>
</tr>
<tr>
<td>$A_{tn}$, cmH$_2$O·s</td>
<td>$0.00091 ± 0.00032$</td>
<td>$34 ± 0.24^*$</td>
<td>$1.5$</td>
</tr>
<tr>
<td>$R_{sw}$, cmH$_2$O·ml$^{-1}$·s</td>
<td>$4.2 ± 1.3$</td>
<td>$8.0 ± 3.4^*$</td>
<td>$1.5$</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n = 5. *P < 0.05 compared with 1.

Breathing Frequency

Frequency of breathing averaged 5.3 ± 0.26 Hz (Tables 4 and 5) in the conscious mice breathing 100% humidified room air. Body-temperature 100% humidified box air had no significant effect on $f$. Anesthesia reduced $f$ to 4.0 ± 0.64 Hz (Table 6), somewhat smaller than the reduction observed previously (25).

Methods

The use of the lung area from x-ray images of the thorax to obtain the lung air volume entailed several assumptions. First, lung volume was assumed to scale as $A_{tn}^{1.5}$ for uniform lung inflation. This was found to be a good approximation for anesthetized mice. Second, to obtain the lung area, we subtracted the area of the heart, which was assumed to remain at a constant volume (21). Third, the lung tissue and blood volumes measured independently in anesthetized mice were assumed to remain constant and to be distributed uniformly throughout the lung with lung inflation, and to be similar in conscious mice. We assumed that blood volume was constant with lung volume increments imposed in the anesthetized mice and with tidal volume in the conscious animals. This issue requires verification, since there is evidence that cardiac output is reduced with lung inflation (2, 3, 39).

The determination of tidal volume and end-expiratory volume from the x-rays taken during quiet breathing entailed several assumptions. First, the box pressure excursions were assumed to be sinusoidal. This was a reasonable assumption, since the sum of the areas measured under the inspiratory and expiratory parts of the box pressure curve was approximated well by $2\delta P/\phi f$, the value for a sine wave (25). Second, in the conscious mice we used x-rays taken during different respiratory cycles and assumed that the box pressure and lung volume excursions, $V_e$, and $f$ remained constant. This was supported by the measurements that showed little variation in box pressure excursion and frequency during the 10- to 30-min period for the x-ray measurements. However, the lack of a variation in the box pressure excursion did not exclude changes in $V_e$ that would contribute to the variation in $V_L$ observed at constant $f$ (21) value during repeated respiratory cycles (see Fig. 8). By contrast, in anesthetized mice when $R_{sw}$ increased after Mch aerosol exposure, x-ray images were collected while $\delta P_b$ was increasing and $f$ was decreasing. $V_t$ and $V_m$ computed...
under these conditions were similar to those of conscious mice, while the changes in $6P_b$ and $f$ represented average values over the response time to apnea. Third, the 10-ms x-ray exposure consisted of a single pulse that was short enough compared with the respiratory period ($\sim 200$ ms) to minimize image blur due to ventilatory motion on the x-ray image. The error in phase was 18°, amounting to 5% at the end-expiratory and end-inspiratory points of the box pressure cycle. However, the 10-ms x-ray exposure produced a greater blurring of the heart outline caused by a smaller cardiac period ($\sim 100$ ms). This effect sometimes produced an ill-defined heart outline on the x-ray images, which were subsequently discarded. Our approach using a 10-ms exposure contrasts with the 170-ms exposures that are needed when using available micro-CT scanners (15), which would not provide definitive images at end-expiration and end-inspiration, and thus $V_t$, in conscious mice breathing at 5 Hz. A similar limitation applies to clinical high-resolution CT scanners (4, 28). Fourth, the configuration of the thorax relative to the dorsal-ventral axis was assumed to be constant during the x-ray measurements. X-ray images that showed a gross asymmetry of the thorax, as observed by changes in curvature of the spine, were not reported. Last, the end-expiratory air volume from the x-ray data was obtained by subtracting the volume occupied by the tissue and blood that were measured in separate anesthetized mice by means of $^{125}$I-albumin injected iv prior to euthanasia. Tissue mass averaged 0.10 g in the collapsed isolated lung, which averaged 0.12 g (Table 1). The trapped blood was 0.02 g or 17% of the lung mass, somewhat smaller than the 25% measured in sheep (11). The lung blood mass averaged 0.21 g in the anesthetized BALB/c mice under control conditions and was reduced by 43% to 0.12 g after Mch aerosol exposure (Table 2). The control lung blood mass was greater than the value of 0.09 g measured in conscious male albino mice of the CF-1 strain (16). We used the blood mass measured in control anesthetized mice to determine the end-expiratory air volume in conscious mice when the total lung volume increased $\sim 40\%$. This most likely underestimated the increase in the end-expiratory air volume, since blood mass was reduced with the increased lung volume-induced reduction in cardiac output (2, 3, 39).

*Error in $\delta P_b/\delta P_g$ for Differences Between 37–39°C Box Air and Actual Body Temperature*

We used a box air temperature of 37–39°C to eliminate the effect of temperature and humidity change on the box pressure excursion as the inspired box air changed to body temperature conditions. However, the body temperature of the anesthetized mouse decreased with time after anesthesia, and the difference between the box air temperature of 37–39°C and actual body temperature contributed to the gas compression effects measured by the box pressure excursion. The error in $\delta P_b/\delta P_g$ due to this effect was $\sim 10\%$ via a sensitivity analysis (25). This was verified by the following experiment. Fig. 9B shows the measured peak-to-peak box pressure excursion ($\Delta P_b$) of an anesthetized mouse breathing spontaneously in a sealed box vs. the body-to-box air temperature difference ($\Delta T$) as the box air was raised from 29 to 37°C over a 3-hr period. Humidity was maintained at 100% by saline aerosol, and body temperature was measured by a rectal probe. Fig. 9A shows the associated box air temperature and body temperature vs. $\Delta T$. Note that $\Delta T$ was 0°C when box air temperature equaled body temperature at 32°C, and body temperature increased from 32 to 34°C as box air temperature increased from 29 to 37°C. The linear regression of $\Delta P_b$ vs. $\Delta T$ was: $\Delta P_b = -0.00071\Delta T + 0.024$, $R^2 = 0.39$, $n = 23$, $P = 0.0013$. The correct gas compression contribution ($\Delta P_g$) to $\Delta P_b$ was the intercept 0.024 cmH$_2$O at $\Delta T$ of 0°C. The regression indicated that for negative values of $\Delta T$, $\Delta P_b$ was greater than $\Delta P_g$, and for positive values of $\Delta T$, $\Delta P_b$ was less than $\Delta P_g$. However, the values of $\Delta P_b$ below $\Delta P_g$ were not consistent with theory that showed $\Delta P_b$ increasing above $\Delta P_g$ at $\Delta T$ of 0°C for both negative and positive values of $\Delta T$ as $\Delta P_b$ contributed to $\Delta P_b$ (Eq. 4). The change in $\Delta T$ from negative to positive values is reflected in changes in phase between $P_b$ and $P_h$. For negative $\Delta T$ with box air below body temperature, $P_b$ lags (follows) $P_h$ by 90°, and $P_h$ lags $P_b$ by phase angle $\phi$ (Eq. 6 and Fig. 6). For positive $\Delta T$ as box air increases above body temperature, $P_b$ is opposite in direction to and leads $P_h$ by 90°, and results in $P_b$ leading $P_h$. Evaluation of the $\Delta P_b$ data for only positive values of $\Delta T$ showed the regression to be insignificant ($P > 0.14$). For a 3°C change in $\Delta T$ from $-3$ to 0°C, the change in $\Delta P_b$ was 0.0021 cmH$_2$O or 9% of the gas compression effect. Thus the gas compression pressure excursion was overestimated by 9%, and the measured $\delta P_b/\delta P_g$ (1.9) was overestimated by 10%. 

![Graph](http://jap.physiology.org/DownloadedFrom/10.22033/125.5.12731.png)
**Comparison With Previous Results**

Lung volumes during spontaneous breathing. $V_t$ measured in conscious mice via the box pressure excursion (0.26 ± 0.033 ml, Tables 3–5, n = 15) agreed with values measured via the lung area from the x-ray images (0.25 ± 0.050 ml). This agreement justified the procedures used for determining lung volume from single projection x-ray images. The measured $V_t$ values were comparable to those (0.21 ± 0.070 ml, n = 30) reported in a previous study (25). In anesthetized mice, $V_t$ was somewhat smaller than values measured previously (0.18 ± 0.047 ml, n = 5 vs. 0.29 ± 0.11 ml, n = 30; Ref. 25). $V_t$ averaged 0.28 ± 0.054 ml (Tables 3–5, n = 15) in conscious mice and 0.21 ± 0.15 ml (n = 10) in anesthetized mice. The latter values were comparable to those measured previously (25) by Ne dilution (0.25 ± 0.10 ml, n = 30).

In the present study, the response to Mch aerosol was to increase $V_t$ by 2-fold in conscious mice and by 4.1-fold in asthmatic attack (32, 40). In the conscious mice $V_t$ doubled by 40% in anesthetized mice exposed to Mch (25). Differences in response to Mch are most likely due to differences in the experimental protocols between the two studies (see below).

Our measurements of $V_t$ in conscious and anesthetized control mice span the range (0.14–0.40 ml) measured in mice with computerized tomography (CT) and micro-CT (28, 15). Our measurements of $V_t$ (0.18–0.33 ml) in conscious and anesthetized mice were generally greater than the value (0.09 ml) measured in anesthetized mice with micro-CT (15). In the latter study the x-ray exposure time of 170 ms during inspiration with a breathing period of 400 s produced the mean lung volume during inspiration, not the end-inspiratory lung volume, and resulted in an underestimate of tidal volume by a factor of two.

The measured increase in $V_t$ in response to aerosolized Mch exposure in conscious mice was consistent with the behavior often observed in humans with increased $R_{aw}$ caused by chronic obstructive pulmonary disease (COPD) or by an acute asthmatic attack (32, 40). In the conscious mice $V_t$ doubled with Mch exposure to partly offset the increase in the gas compression effect to the calculated $R_{aw}$. However, the increased $V_t$ cannot alone explain why conscious mice showed no increase in $R_{aw}$ with Mch exposure, because $R_{aw}$ increased in anesthetized mice with a similar increase in $V_t$. Lung hyperinflation secondary to Mch-induced airway constriction resulted in an increased expiratory force of the lung parenchyma on the airway that opposed the contractile force of the airway smooth muscle (5–10, 12, 20, 31, 32, 34–37). The response to Mch in conscious mice was largely independent of the Mch concentration and suggested a plateau in the bronchoconstriction that was always compensated by the lung expansion. A plateau in bronchoconstriction has been observed in some studies of normal humans (33, 38) and experimental animals (31, 34). However, a plateau was not observed in anesthetized dogs in one study that used CT (8) or in anesthetized mice in the present study. In humans the increase in $V_t$ with Mch was not attributed to the degree of bronchoconstriction or type of bronchconstrictor agent per se, but to flow limitation occurring during spontaneous breathing (32, 33). An important factor that would reduce $R_{aw}$ with Mch in the conscious mice is the bronchodilatory effect on the airway smooth muscle after a deep inspiration, as observed in humans (4, 23, 30) and anesthetized rabbits (20). These time-dependent effects might be related to the reduced contractility of airway smooth muscle observed with increases in cyclic changes in muscle length in vitro (18). The mechanisms responsible for these time-dependent effects have been reviewed (19).

By contrast to the results of the conscious mice in the present study, the Mch-induced increased lung volume in the anesthetized mice breathing room air showed no plateau in bronchoconstriction, but seemed to reach airway closure that resulted in gas trapping (5, 22, 32). This effect was verified in two mice whose post mortem lungs failed to collapse after the chest was opened and could not be deflated by suction with a syringe attached to the cannulated trachea. Further evidence for airway closure was provided in two anesthetized mice that were allowed to breathe 100% $O_2$. Here exposure to Mch aerosol similar to that of the mice breathing room air did not prevent the increased $R_{aw}$ or apnea. However, rather than the gas trapping that was observed in mice breathing room air, breathing $O_2$ caused the lung to collapse as $O_2$ was absorbed with closed airways. Thus, a collapsed atelectatic lung was observed on the x-ray images prior to apnea and after opening the chest post mortem, and airway closure was verified by the inability to expand the isolated atelectatic lung with high positive pressure (40 cmH$_2$O).

The observed response to aerosolized Mch in conscious mice was consistent with a plateau in the bronchoconstriction response to Mch concentration often observed in humans (4, 29, 38). The plateau in $R_{aw}$ (34) in anesthetized dogs has been demonstrated by means of CT with high doses of Mch aerosol and with relatively low doses of Mch directly applied to the airway smooth muscle (8); the difference was attributed to reduced Mch concentration with the aerosol. The latter effect was probably not the reason why airway collapse was not observed in our conscious mice, since the same Mch aerosol concentration produced airway closure in anesthetized mice.

The reason for the apnea in anesthetized mice after the Mch exposures are speculative and might involve many factors. First, the 8-fold increase in $R_{aw}$ and 4-fold increase in $V_t$ with Mch might result in diaphragmatic fatigue due to the extremely high expansive force required to expand the lung with closed airways (17). This was evident from the Mch-induced 4-fold increase in box pressure excursion ($\Delta P_{bx}$) relative to control (Table 6). The increase in box pressure was consistent with a 3-fold increase in the force generated by the diaphragm, as reflected in the alveolar pressure amplitude ($\Delta P_{alv}$) that equaled the viscous pressure loss ($R_{aw}\Delta Q$, Ref. 25) due to airflow ($\Delta Q$ is flow amplitude). In the control anesthetized mice with an $R_{aw}$ of 4.2 cmH$_2$O·ml$^{-1}$·s (Table 6) and $\Delta Q$ of 2.4 ml/s ($\pi V_t$, with $V_t$ of 0.19 ml and f of 4 Hz), $\Delta P_{alv}$ was 10 cmH$_2$O. With Mch aerosol exposure, $R_{aw}$ increased 8-fold to 34 cmH$_2$O·ml$^{-1}$·s, and with a flow amplitude of 0.93 ml/s ($V_t$ of 0.27 ml and f of 1.1 Hz), $\Delta P_{alv}$ increased to 32 cmH$_2$O. Such a sustained effort from the diaphragm might result in diaphragm fatigue and in turn a reduced f. Second, diaphragm fatigue was exacerbated by the lung inflation-induced low cardiac output (2, 3, 39) and $O_2$ delivered to the overworked and overcontracted muscle, and resulted in diaphragm muscle failure (17). Similar effects on cardiac output most likely occurred in the mice breathing 100% $O_2$ after airway closure.
and lung atelectasis. A low cardiac output was consistent with a reduced blood volume measured in the constricted lung (Table 2). Reduced cardiac output, increased Ve and Raw, and gas trapping produced by iv Mch has been reported in anesthetized dogs (2). Diaphragm muscle failure was secondary to the reduced blood flow and was not due to lack of O2 to the blood. The blood flowing through the lung was well oxygenated with no observable evidence of cyanosis or O2 desaturation caused by pulmonary edema, since the lung W/D (4.87 ± 0.23, n = 5) measured post mortem was normal. Airway secretions due to the Mch aerosol, as observed in rats (22), might result in airway closure and gas trapping. However, this effect should also occur in the conscious mice and thus cannot by itself explain the apnea in the anesthetized mice.

One factor that might have contributed to the reduced Mch-induced Raw in conscious mice compared with anesthetized mice was the reduced f with anesthesia (25). The reduced f with anesthesia was not attributed solely to the lung inflation-induced inhibition of active respiration (Hering-Breuer inflation reflex) because f did not decrease in conscious mice with a Mch-induced increased Ve. Airway and parenchymal tissue elastance and resistance, demonstrated in vivo (31) and in vitro studies (18), might change with frequency under control conditions and in response to Mch.

Airway resistance (Raw). Raw measured in control conscious mice in the present study (3.1 cmH2O·ml−1·s, Tables 4 and 5) was 60% greater than values (1.9 cmH2O·ml−1·s) estimated previously for conscious mice based on Ve values measured in anesthetized mice (25). In the conscious mice of the present study, Raw showed no increase in response to aerosolized Mch, in contrast to the 8-fold increase estimated previously for both anesthetized and conscious mice (25). These differences in the control Raw and R Raw ratio with Mch between the two studies might be caused by the following differences in the experimental protocols. First, the need to take x-rays of the thorax in the present study required placing the conscious mouse within a tube to position its thorax over the imaging sensor, and the mouse might have reacted to the physical confinement by an increase in Raw. This confinement might produce an emotional stress that contributed to the response to Mch, similar to the effects observed in humans with asthma (26). Second, the measurements of Vent and Vc required x-rays to be taken after several exposures to aerosolized Mch. The steady-state box pressure excursions obtained over the 10- to 30-min experimental period were smaller than the transient values produced over the 30-s exposure to Mch in the previous study. Third, the present study showed an increased Ve (2.3-fold) in response to Mch, which acted to offset the contribution of the A Raw ratio (1.6-fold) to the calculated Raw, ratio, while in the previous study Ve was assumed to be unchanged with Mch. Thus any airway constriction induced by Mch in conscious mice was effectively reduced by breathing at a higher lung volume. Raw in the anesthetized mice averaged 4.2 cmH2O·ml−1·s, comparable to the value measured previously with a similar technique (25), 2.5-fold greater than measurements (1.7 cmH2O·ml−1·s) obtained via end-inflation airway occlusion (14), but 8-fold greater than values (0.5 cmH2O·ml−1·s) measured via the forced oscillation technique (27). The much smaller Raw measured by forced oscillation was most likely due to the relatively small viscous pressure loss under the imposed laminar flow conditions. However, the present technique measured both laminar and turbulent flow contributions of spontaneous breathing to the total viscous pressure loss that determines Raw (25). The relatively high Raw values measured in the present study were most likely not due to a significant contribution of the upper airway, since similar high Raw values were measured previously in tracheostomized animals (25). By contrast to conscious mice in the present study that showed no change in Raw with Mch, Raw in the anesthetized mice with Mch increased 8-fold, comparable to the increase of the previous study (25). However, the Raw response in the present study occurred only after three repeated 1-min exposures to 100 mg/ml Mch aerosol, which resulted in apnea after several minutes. This is in contrast to the previous study, which used a lower dose of Mch (25 mg/ml) with a shorter exposure time of 30 s followed by box pressure measurements of 20–30 s in a sealed box. Here changes in pressure excursion with Mch increased on average by 50% relative to control with no change in f, Vc, or Vt.

Concluding Remarks

We developed a method using single projection x-ray images of the thorax to estimate Ve and Vt in spontaneously breathing mice. We used the technique to show that conscious mice responded to repeated exposures to aerosolized Mch by doubling Ve with little change in Raw. This behavior was largely independent of dose up to 125 mg/ml and the number of exposures, and was consistent with the establishment of a plateau in Raw. By contrast in anesthetized mice, three repeated 1-min exposures to 100 mg/ml Mch aerosol increased Ve by 4-fold and Raw by 8-fold, with breathing frequency decreasing to apnea within 10–20 min. This behavior was consistent with an increasing bronchoconstriction to airway closure and gas trapping, which resulted in diaphragm fatigue and failure exacerbated by the lung inflation-induced reduced blood flow and O2 supplied to the diaphragm muscle.

GRANTS

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