Lung tissue behavior in the mouse during constriction induced by methacholine and endothelin-1

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Recent findings have suggested that lung tissue behavior is similar among various species, but little information is currently available. We questioned whether lung tissue behavior differs in mice. To address this question, we investigated whether tissue resistance (Rti) and lung elastic properties in the mouse would respond to varying lung volume, constriction induced by methacholine (MCh) and endothelin-1 (ET-1), and high-lung-volume challenge during induced constrictionable regardless of various conditions. Also, the relationship between tissue resistance and lung elastance (EL) was examined. From measured tracheal flow and tracheal and alveolar pressures in open-chest ICR mice during mechanical ventilation [tidal volume = 8 ml/kg, frequency (f) = 2.5 Hz], we calculated lung resistance (RL), Rti, alveolar resistance (Raw), lung elastance (EL), and hysteresivity ($\eta$). Under baseline conditions, increasing levels of end-expiratory transpulmonary pressure decreased Raw and increased Rti. The administration of aerosolized MCh and intravenous ET-1 increased Rti and EL in a dose-dependent manner. Rti increased from 0.207 ± 0.010 to 0.570 ± 0.058 cmH2O·ml−1·s after 10−7 mol/kg ET-1 (P < 0.01). After induced constriction, increasing end-expiratory transpulmonary pressure decreased Raw. However, $\eta$ was not affected by changing lung volume, constriction induced by MCh and ET-1, or high-lung-volume challenge during induced constriction. These observations suggest that 1) Rti is stable in mice regardless of various conditions, 2) Rti is an important fraction of Rti and increases after induced constriction, and 3) mechanical interdependence may affect airway smooth muscle shortening in this species. In mammalian species, including mice, analysis of $\eta$ may indicate that both Rti and EL essentially respond to a similar degree.

Materials and Methods

Animal Preparation

Male ICR mice (35–42 g) were studied. Animals were anesthetized with pentobarbital sodium (25 mg/kg ip) and ketamine hydrochloride (25 mg/kg ip) in combination and then paralyzed with pancuronium bromide (0.3 mg/kg ip). Anesthesia and paralysis were maintained by supplemental administration of 10% of the initial dose every hour. After tracheostomy, an endotracheal metal tube (1 mm ID, 8 mm long) was inserted in the trachea. Animals were mechanically ventilated (model 683, Harvard Apparatus, South Natick, MA) with tidal volumes of 8 ml/kg and frequencies of 2.5 Hz. The thorax was opened wide by means of midline sternotomy, and a positive end-expiratory pressure of 3 cmH2O was applied by placing the expiratory line under water. During the experiments, oxygen gas mixed with air was supplied to the ventilatory system. Under these ventilatory conditions, arterial pH, PO2, and PCO2 were 7.35–7.45, 100–180 Torr, and 30–45 Torr, respectively, at the end of experiments. A heating pad was used to maintain the body temperature of animals. The alveolar capsules were made out of 1-ml plastic syringes. One or two alveolar capsules were affixed to the pleural surface of the anterior portion of lungs with cyanoacrylate. The pleura underneath the capsule was punctured with an electrocautery needle through the central port of the capsule (depth <1 mm) to bring the underlying alveoli into communication with the capsule chamber. A piezoresistive microtransducer (model 8507C-2, Endevco, San Juan Capistrano, CA) was placed in the port of the capsule to measure alveolar pressure (Pa). Tracheal pressure (Ptr) was also measured by a piezoresistive microtransducer (model 8510B-2, Endevco) placed in a lateral port of the tracheal cannula. Tracheal flow was measured by means of a Fleisch pneumotachograph (no. 00000). Volume (V) was calculated by digital

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integration of the flow signal. All signals were amplified, filtered at a cutoff frequency of 100 Hz, and converted from analog to digital by a 12-bit analog-to-digital converter (model DT2801-A, Data Translation, Marlborough, MA). The signals were sampled at a rate of 200 Hz and stored on an IBM-AT-compatible computer.

Calculation of Resistance and $\eta$

$\text{Ptr}$ was corrected for both the tube resistance and the Bernoulli effect (16). From flow, $V$, and corrected $\text{Ptr}$, lung elastance ($\text{EL}$) and total $\text{RL}$ were calculated by finding the best fit for the equation of motion

$$\text{Ptr} = \text{RL} \left( \frac{dV}{dt} \right) + \text{EL} V + K$$

where $t$ is time.

$\text{Rti}$ was calculated by fitting the equation of motion to $\text{PA}$

$$\text{PA} = \text{Rti} \left( \frac{dV}{dt} \right) + \text{EL} V + K'$$

where $K$ and $K'$ are constants, the values of which were also estimated by multiple linear regression. The values of $\text{RL}$ and $\text{Rti}$ were accepted only when the values of $\text{EL}$ obtained from $\text{Ptr}$ and $\text{PA}$ were different by $\pm 10\%$ and both $K$ and $K'$ were $< 1$ cmH$_2$O different from the real value of end-expiratory transpulmonary pressure (Ptp).

Raw was calculated by subtraction

$$\text{Raw} = \text{RL} - \text{Rti}$$

where $\text{Rti}$ was the average of the values obtained from two capsules. Before and after induced constriction, the differences in the values of $\text{Rti}$ obtained from the two capsules were $4.8 \pm 1.7$ and $9.4 \pm 2.8\%$, respectively. Finally, $\eta$ (a structural damping coefficient) was calculated as follows

$$\eta = 2\pi f \frac{\text{Rti}}{\text{EL}}$$

Protocol

Effects of varying frequencies and tidal volumes. To examine frequency and tidal volume dependencies of resistance and $\eta$, we made measurements of 30-s duration at each frequency (0.313, 0.625, 1.25, and 2.5 Hz) and at each tidal volume (5, 10, and 20 ml/kg; $n = 5$). Frequencies and tidal volumes were changed in random order.

Effects of varying Ptp under baseline conditions. Baseline measurements were obtained 1 min after two deep inflations (peak $\text{Ptr}$ of 30 cmH$_2$O) were performed to standardize volume history. Measurements of 10-s duration were made at each level of $\text{Ptp}$ (3, 5, 7, 9, and 11 cmH$_2$O) and at each frequency (0.313, 0.625, 1.25, and 2.5 Hz; $n = 5, 5, 5, 5, 5, 5, 5, 5, 5, 5$). Ptp was altered in ascending order.

Effects of MCh administration. In seven mice, inhalations of saline and MCh were administered at Ptp of 3 cmH$_2$O. Aerosols were generated by an ultrasonic nebulizer (UltraNeb100, DeVilbiss, Somerset, PA), which produces particles with a mean aerodynamic diameter of 4.8 µm. Aerosols were delivered into the trachea for 30 s, and the aerosols of MCh were administered in a dose-response manner (0.01, 0.1, 1, 10, and 100 mg/ml).

Effects of ET-1 administration. Synthetic ET-1 (Peptide Institute, Osaka, Japan) was dissolved in phosphate-buffered saline (PBS) at concentrations of $10^{-9}$–$10^{-7}$ mol/kg. After two deep inflations (peak $\text{Ptr}$ of 30 cmH$_2$O) were performed twice to standardize volume history, baseline measurements of 10-s duration were sampled during tidal ventilation. After baseline measurements, 0.1 ml of PBS and ET-1 solutions was given intravenously in a half-log increasing manner in five mice. Each dose of ET-1 was administered 5 min after the previous dose to obtain a cumulative concentration-response curve.

Effects of varying Ptp during induced constriction. One minute after the administration of 100 mg/ml MCh aerosol or $10^{-7}$ mol/kg ET-1 (iv), measurements of 10-s duration were obtained at two different levels of Ptp (3 and 11 cmH$_2$O) in ascending order. These measurements were completed within 1 min, and preliminary experiments showed that the magnitude of bronchoconstriction remained unchanged during this period.

Statistical Analysis

Statistical significances were examined with analysis of variance (Fisher’s least significant difference test). $P$ values $< 0.05$ were taken as significant. Data are expressed as means $\pm$ SE.

RESULTS

Effects of Varying Frequencies and Tidal Volumes

The effects of different frequencies and tidal volumes are shown in Fig. 1. $\text{Rti}$ had a negative dependence on frequency and a slight positive dependence on tidal volume. In contrast, $\eta$ had a positive dependence on frequency and a slight negative dependence on tidal volume.

Fig. 1. Effect of different frequencies and tidal volumes (V$T$; ml/kg) on tissue resistance (Rti) and airway resistance (Raw) (A) and on hysteresivity ($\eta$; B) in mice. n, No. of measurements. $^*P < 0.05$ vs. frequency $= 2.5$ Hz. $^{**}P < 0.05$ vs. V$T = 5$ ml/kg.
Effects of Varying Ptp Under Baseline Conditions

Figure 2 summarizes the effects of changing Ptp on baseline RL, Rti, Raw, and EL. Increasing Ptp increased Rti and EL and decreased Raw significantly at Ptp $\geq 5$ cmH$_2$O. The $\eta$ was not affected by the changes in Ptp at each frequency.

Effects of MCh Administration

MCh concentration-response curves for RL, Rti, Raw, EL, and $\eta$ are shown in Fig. 3. After 0.1 mg/ml of MCh, Rti and EL significantly increased, whereas Raw was increased at 10 mg/ml. Meanwhile, $\eta$ was not altered by increasing dose of MCh.

Effects of ET-1 Administration

Figure 4 summarizes the responses to ET-1 in mice. RL, Rti, Raw, and EL significantly increased after $10^{-7.5}$ mol/kg ET-1 administration. Rti increased from $0.207 \pm 0.010$ to $0.570 \pm 0.058$ cmH$_2$O·ml$^{-1}$·s after $10^{-7}$ mol/kg ET-1 ($P < 0.01$). The $\eta$ was not significantly affected by increasing dose of ET-1.

Effects of Varying Ptp During Induced Constriction

The effects of increased Ptp levels on Rti, Raw, and $\eta$ during MCh and ET-1 induced constriction are shown in Fig. 5. Increased Ptp levels significantly decreased Raw ($P < 0.01$) and increased Rti ($P < 0.01$), whereas changing Ptp did not affect $\eta$ during MCh- and ET-1-induced constriction.

DISCUSSION

The results of the present study show that $\eta$ is extremely stable in mice in the physiological range of breathing. The $\eta$ was not affected by changing lung volume, constriction induced by MCh and ET-1, and high-lung-volume challenge during induced constriction. Rti is an important fraction of RL and increases after induced constriction in mice. Increasing lung volume decreased Raw under the baseline conditions and during induced constriction, suggesting that mechanical interdependence may affect airway smooth muscle shortening in this species. These findings indicate that airway and lung tissue behavior, including $\eta$, in the mouse would not be different from that in other species.

Before the results are discussed, technical issues warrant consideration. It has been well described that lung mechanics, including resistance and elastance, depend on frequencies and tidal volumes (6, 8). In the present study, we investigated whether lung tissue behavior is affected by different frequencies and tidal volumes under unconstricted state. On the other hand, we were able to study the effects of agonist-induced constriction only at a single frequency (2.5 Hz).
and tidal volume (8 ml/kg), which were presumably in the physiological range of breathing of mice. As shown in Fig. 1, $\eta$ is most meaningful at low frequencies, where it is insensitive to frequency. However, we failed to obtain the data at low frequencies during induced constriction because severe bradycardia or cardiac arrest occurred during low-frequency ventilation. After agonist-induced constriction, we therefore analyzed the data obtained at 2.5 Hz, where $\eta$ is sensitive to changes in frequency.

The $\eta$ is the ratio of the energy dissipated per cycle to the stored potential energy at maximum volume (6). As shown in Fig. 1, $\eta$ had a positive dependence on frequency and a slight negative dependence on tidal volume. Meanwhile, in the physiological range of breathing, $\eta$ was not significantly altered by different levels of

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<th>Table 1. Estimates of hysteresivity calculated from mechanical data in living preparations</th>
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Values are means ± SE; n, no. of analyzed animals. $\eta$, hysteresivity.
inflation. Taken together, in mammalian species from mice to dogs, analysis of \( \eta \) may indicate that both \( R_{ti} \) and \( E_L \) essentially respond to a similar degree.

In mice, \( R_{ti} \) is a substantial fraction of \( R_L \) (40–60%) in the physiological range of breathing, suggesting that the relative contributions of \( R_{ti} \) and \( Raw \) to \( R_L \) are essentially similar in various mammalian species (9, 12, 14, 20–25, 27). One of the possible mechanisms to explain this phenomenon is as follows. One might assume that \( Raw \) follows Poiseuille’s law; i.e., \( Raw \) is related to \( L/r^4 \), where \( L \) is airway length and \( r \) is airway caliber. If all linear dimensions scale as \( V^{1/4} \), then it is expected that \( Raw \) would scale as \( 1/V \). \( R_{ti} \) is assumed to follow the structural damping law; i.e., \( R_{ti} \) is related to \( \eta E_L \). Because \( \eta \) is roughly constant and \( E_L \), the reciprocal of the compliance, scales \( 1/V \), \( R_{ti} \) is expected to scale as \( 1/V \). This theory suggests that both \( Raw \) and \( R_{ti} \) are dependent on animal size but that the ratio of \( Raw \) to \( R_{ti} \) would be constant. The results of the present study might support this mechanism.

Under baseline conditions, increasing lung volume decreased \( Raw \) and increased \( R_{ti} \) in mice, as has been previously described in other species. In dogs, it has been reported by Ludwig et al. (12), who partitioned pulmonary resistance into \( Raw \) and \( R_{ti} \) with use of alveolar capsules, that lung inflation decreased \( Raw \). In rats and guinea pigs, the effects of changing lung volume on \( Raw \) and \( R_{ti} \) have also been reported, whereas the bronchodilating effect of lung volume is less in the guinea pig (24). In mice, \( Raw \) was reduced by 83% at Ptp of 11 cmH\(_2\)O compared with Ptp of 3 cmH\(_2\)O, which was a similar degree to that observed in rats (24).

After constriction induced by MCh and ET-1 in mice, increases in \( R_{ti} \) were observed, as has been shown in several other animal species (9, 12–14, 20–25, 27). However, the mechanisms that give rise to increases in \( R_{ti} \) are not clearly explained. Potential mechanisms include constriction of parenchymal contractile elements (10), changes in alveolar geometry and the behavior of the air-liquid interface (1, 3), and microatelectasis (6, 30, 31). In addition, changes in the conducting airways causing alterations in parenchymal stress and elasticity might be related to the observed increase in \( R_{ti} \) (18). Meanwhile, Kimmel et al. (11) have made theoretical analysis and concluded that changes in \( R_{ti} \) associated with parenchymal distortions are slight compared with those associated with volumetric expansion.

As expected, \( Raw \) increased after MCh- and ET-1-induced constriction in mice. In mice, \( Raw \) significantly increased after the administration of 10 mg/ml MCh or 10\(^{-7.5}\) mol/kg ET-1. In guinea pigs, it has been shown that \( Raw \) significantly increased after 1 mg/ml MCh (23) or 10\(^{-9.5}\) mol/kg ET-1 (20), suggesting that mice are more resistant to exogenous administration of MCh and ET-1 than are guinea pigs. The mechanisms that may explain this airway narrowing include smooth muscle contraction, airway wall thickening, and intraluminal secretions (19, 26). Potential alterations in airway compliance after induced constriction might also be involved in the observed increases in \( Raw \) (28).

During constriction, increasing lung volume decreased \( Raw \), suggesting that mechanical-interdependence forces might affect bronchoconstriction in this species. It has been reported in several species that airway-parenchymal interdependence affects the magnitude of bronchoconstriction. In humans, it has been demonstrated by Ding et al. (4) that the maximal \( R_L \) after inhaled MCh decreases at higher lung volumes. In dogs, Ludwig et al. (13) have reported that \( Raw \) decreased at the higher lung volume during agonist-induction constriction, whereas it has been shown by Gunst and colleagues (7, 32) that airway closure during induced constriction in both in vitro and in vivo canine lungs could be reversed at higher Ptp values. In cats, it has been reported that increasing lung volume reduces the level of bronchoconstriction (29). In rats, Bellafiore et al. (2) have described that the response to MCh was enhanced in elastase-treated animal model of emphysema and have suggested that the increased response in \( R_L \) was caused by loss of lung elastic recoil. In guinea pigs, it has been demonstrated that airway-parenchymal interdependence is important in determining the level of bronchoconstriction, whereas the lung volume dependence of \( Raw \) is less compared with that in rats (24). The present study suggests that the mechanical interdependence between airways and parenchymal lung tissues could modify the airway smooth muscle shortening as an impedance and affect the magnitude of bronchoconstriction in mice, one of the smallest mammalian species.

In summary, we demonstrated that \( \eta \) was stable in mice in the physiological range of breathing and was not affected by changing lung volume, constriction induced by MCh and ET-1, and high-lung-volume challenge during induced constriction. \( R_{ti} \) was an important fraction of \( R_L \) at baseline and increased after exogenous constriction in mice. Increasing lung volume reduced \( Raw \) during induced constriction, suggesting that mechanical interdependence between airways and parenchymal tissues may modify airway smooth muscle shortening in this species. These observations might be useful to understand the pulmonary mechanics in the mouse and provide an important step for the dynamic study of lung behavior in the mouse, one of the most extensively used animal models.

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REFERENCES


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