Lung volume history is known to have an important effect on airway tone and the airway response to bronchoconstrictors. In normal adults with induced bronchoconstriction, a deep inspiration results in bronchodilation. This effect has been measured as a decrease in airway resistance (Raw) or as an increase in forced expiratory flow during full compared with partial volume inspiratory maneuvers (1–5, 14–16, 18, 19). Recently, Skloot et al. (23) demonstrated that the inhibition of deep inspiration during methacholine (MCh) challenge results in a heightened airway response in normal subjects. In these normal subjects, the MCh challenge induced an airway response that was similar to that observed in asthmatic subjects. The mechanism for the effect of deep inspiration on airway responsiveness remains unclear, as does the mechanism for the often absent bronchodilating effect of a deep inspiration in asthmatic subjects (2, 17).

In isolated canine bronchi, the increase in transmural pressure induced by acetylcholine is greater under static conditions than when volume oscillations are imposed (9). As the size of the volume oscillation is increased, there is greater suppression of the contraction caused by the bronchoconstrictor. Similarly, in isolated canine tracheal smooth muscle strips, active force is lower when length oscillations are imposed than under static conditions (6, 22). As the amplitude of the length oscillations is increased, force generation decreases. In both isolated bronchi and tracheal smooth muscle strips, increasing the frequency of volume or length oscillation also decreases active force. Our recent studies of isolated canine tracheal smooth muscle strips suggest that non-cross-bridge mechanisms that function to adapt muscle contractility to changes in muscle length may play an important role in determining the effect of length oscillation on contractile force (7, 10, 13, 22).

We have previously shown that airway closure in response to MCh challenge occurs less frequently during tidal ventilation than under static conditions in rabbits and dogs (25, 27). These observations, in combination with our observations obtained in muscles in vitro, have led us to hypothesize that increasing the volume of tidal ventilation in vivo will decrease airway narrowing in response to bronchoconstrictors because it increases the magnitude of stretch on the airway smooth muscle. Our previous studies of airway smooth muscle in vitro also suggest that increasing the frequency of tidal volume oscillation will result in less airway response to bronchoconstrictors. In this study we evaluated the effects of the volume and frequency of tidal ventilation on the increase in airway resistance in response to MCh challenge in mechanically ventilated rabbits.

**METHODS**

**Experimental preparation.** New Zealand White rabbits (2.5–3.1 kg) were anesthetized with intravenous pentobarbital sodium (50 mg/kg). After tracheotomy, an appropriately sized tube was inserted and securely tied in place to prevent air leaks. Animals were mechanically ventilated (model 661, Harvard) with a tidal volume of 10 ml/kg at a frequency of 60 breaths/min. The expiration port of the ventilator was connected to a water column that maintained positive end-expiratory pressure (PEEP) at 2 cmH₂O. A jugular venous catheter was inserted to administer additional anesthetic, normal saline, and MCh. The abdominal and thoracic cavities were widely opened, and a warming pad was used to prevent cooling of the animal.

Tracheal pressure (Ptr) was measured with a piezoresistive pressure transducer (model 8507CQ-2, Endevco, San Juan Capistrano, CA), and tracheal flow (V) was measured with a screen pneumotachometer (model 8410A, Hans Rudolph, Kansas City, MO) and a differential pressure transducer (±2.25 cmH₂O; Validyne MP45, Northridge, CA) attached to the tracheotomy tube. Because the chest was open, changes in Ptr could be used to assess changes in transpulmonary pressure. Analog signals of flow and pressure were analog filtered above 50 Hz, amplified, and digitized at 100 samples/s (model DT2801-A, Data Translation, Marlborough, MA). Digital signals were stored in an IBM-compatible personal computer (Zeos 486, St. Paul, MN) by using data-acquisition software (RHT Infodat, Montreal, PQ, Canada).
Raw was assessed from changes in pressure and flow at the airway opening produced by forced oscillation with very small volumes (0.2–0.3 ml/kg) at 6 Hz. These oscillations were generated by using a small piston attached to a linear motor that was in parallel with the ventilator (21, 24). The digital signals of pressure and flow were digitally filtered to remove frequencies below 4 Hz, which were related to mechanical ventilation. Raw was then calculated by using a linear regression technique to fit pressure and flow signals to Eq. 1.

\[ \text{Ptr}(t) = \text{Raw}(t) \cdot V(t) + K(t) \]  

where K is a constant that absorbs any small offsets in the mean value of Ptr and t is time. By using commercial software (RHT Infodat), Raw was calculated by recursive least squares with a memory time constant of 4 s.

Protocol 1: To determine the effect of tidal volume amplitude on the airway response to MCh. Deflation pressure-volume curves for the lung were obtained in each animal before the MCh challenge as follows. The tracheotomy tube was disconnected from the ventilator, and the lung was inflated three times with a calibrated syringe from 0 to 25 cmH\textsubscript{2}O. After stress relaxation, Ptr decreased to between 20 and 25 cmH\textsubscript{2}O at this lung volume. After three inflations, volume was withdrawn from the lung in 10 equal volumes, and Ptr was recorded continuously. Two to 5 s were allowed for pressure equilibration after each step change in volume. The animal was then reconnected to the ventilator, and after several minutes of mechanical ventilation the pressure-volume measurements were repeated. The results of the two deflation pressure-volume curves obtained in each animal were averaged and expressed as a fraction of the lung volume measured during inflation from 0 cmH\textsubscript{2}O to the lung volume achieved after stress relaxation from 25 cmH\textsubscript{2}O (total lung volume).

Each of five animals was challenged with five doses of intravenous MCh (0.01 mg/kg). The first and the last (5th) challenges were performed under static conditions without ventilation. For these two challenges, the ventilator was turned off as the intravenous MCh dose was administered, and very small-volume (0.2 ml/kg) oscillations were initiated to measure Raw. After 40 s, the very small-volume oscillations were stopped and mechanical ventilation was resumed. Challenges 2, 3, and 4 were obtained when the animal was being ventilated by using tidal volumes of 5, 10, and 20 ml/kg at a rate of 15 breaths/min (0.25 Hz). The sequence in which the tidal volumes were studied in each animal was randomized. During MCh challenge, very small-volume oscillations were superimposed on the tidal volume oscillations from the mechanical ventilator for the measurement of Raw. At the end of each challenge, the animal was given three deep inspirations to total lung volume and then ventilated with a tidal volume of 10 ml/kg at a rate of 60 breaths/min (1 Hz) until Raw returned to baseline, ~20 min. The effect of each MCh challenge on Raw was expressed as the maximal Raw response. The maximal Raw response was quantitated as the mean of the Raw values during the time period from 2.5 s before the peak value of Raw to 2.5 s after the peak value of Raw. The results of the first and the last challenges obtained under static conditions were averaged to control for the effects of the timing and sequence of the MCh challenges.

Protocol 2: To determine the effect of ventilation frequency on the airway response to MCh. Four rabbits were challenged with intravenous MCh (0.01 mg/kg) while they were being ventilated at rates of 6 and 30 breaths/min (0.1 and 0.5 Hz) with a tidal volume of 5 ml/kg. Each animal received six MCh challenges. The first and the last (6th) challenges were performed under static conditions with no ventilation. The second to fifth challenges were performed while the animals were ventilated at 6 or 30 breaths/min (0.1 and 0.5 Hz). At each frequency, the effects of two challenges on the increase in Raw in response to MCh were determined, and the results obtained at each frequency were averaged.

Protocol 3: To determine whether differences in alveolar ventilation can account for the differences in airway response to MCh under static and dynamic conditions. In protocols 1 and 2, alveolar ventilation was very different under static and dynamic conditions of ventilation. Therefore, Raw was assessed under static and dynamic ventilatory conditions by using a closed-circuit constant-volume system with no fresh gas supply to the animal. Under these conditions, alveolar ventilation during tidal volume oscillations (dynamic conditions) was similar to that in the absence of tidal volume oscillations (static conditions). Five rabbits were challenged with intravenous MCh (0.01 mg/kg) under static conditions and under dynamic conditions with lung volume excursions of 10 ml/kg at 15 breaths/min (0.25 Hz). The sequence of the challenges under static and dynamic conditions was randomized.

Protocol 4: To determine whether the effect of tidal ventilation on the airway response to MCh is a consequence of the route of MCh delivery. Increasing lung volume can reduce pulmonary or bronchial blood flow (26). If this occurs, the increase in lung volume during tidal ventilation could reduce MCh delivery to the airways and thus decrease the airway response to MCh. In two rabbits, we therefore evaluated whether the effect of tidal volume oscillations on the airway response to MCh delivered by aerosol was similar to the effect observed when the MCh was delivered intravenously. MCh solution (40 mg/ml) was aerosolized with an ultrasonic nebulizer (Devilbiss), and the aerosol was delivered to the airways during 30 s of tidal ventilation with a peak airway pressure of 10 cmH\textsubscript{2}O (10 ml/kg) and a rate of 30 breaths/min. In the first animal, Raw was measured for 40 s in the absence of tidal ventilation (static conditions). After Raw returned to the baseline value, ~25 min, a second MCh challenge by aerosol was performed, and Raw was measured for 40 s during tidal ventilation at 20 ml/kg at a frequency of 15 breaths/min (dynamic conditions). In the second animal, the sequence of the two MCh challenges (static vs. dynamic) was reversed.

Protocol 5: To determine whether the differences in airway response to MCh under static and dynamic conditions can be attributed to vagal reflexes. In two rabbits, the vagus nerves were severed before intravenous MCh challenge under static (no ventilation) and dynamic conditions (tidal volume = 20 ml/kg; frequency = 15 breaths/min) as described in protocol 1. The sequence of the two challenges was reversed for the two animals.

Statistical analysis. All data are presented as means ± SE. For protocols 1 and 2, comparisons among the different conditions were made by using a nonparametric analysis (Kruskal-Wallis 1-way analysis of variance). For protocol 3, a paired t-test was used to compare the responses under static and dynamic conditions. For all statistical tests, P < 0.05 was considered statistically significant.

RESULTS

Pressure-volume curves. The mean of pressure-volume curves obtained in five animals is illustrated in Fig. 1. Volume is expressed as a percentage of the total lung volume. At a PEEP of 2 cmH\textsubscript{2}O, the mean lung volume was 32.6% of total lung volume. Tidal volumes of 5, 10, and 20 ml/kg at a PEEP of 2 cmH\textsubscript{2}O increased the end-inspiratory lung volume to 48.8 ± 5.7, 63.3 ±
7.3, and 93.5 ± 10.7% of total lung volume, respectively, and end-inspiratory transpulmonary pressures to 3.2 ± 0.1, 4.8 ± 0.1, and 16.2 ± 2.0 cmH₂O, respectively.

Effect of tidal volume on the airway response to intravenous MCh challenge (protocol 1). The mean effect of MCh challenge on Raw in five animals under static conditions (0 ml/kg tidal volume) and during tidal ventilation at volumes of 5, 10, and 20 ml/kg at a frequency of 15 breaths/min (0.25 Hz) is illustrated in Fig. 2. In response to each intravenous MCh challenge, Raw increased with time and reached a plateau within 30 s. The increase in Raw was greater under static conditions (tidal volume = 0 ml/kg) than during ventilation at any volume amplitude. Each increase in tidal volume amplitude decreased the response to MCh.

Figure 3 compares the mean maximal increases in Raw in response to MCh challenge when the lungs were ventilated by using tidal volumes of 5, 10, and 20 ml/kg or were held under static conditions. Tidal ventilation at each volume suppressed the maximal increase in Raw compared with static conditions (0 ml/kg). Each increase in tidal volume produced a statistically significant decrease in the response to MCh. At a tidal volume of 20 ml/kg, bronchoconstriction in response to MCh was almost completely abolished (Fig. 3).

Effect of ventilation frequency on the airway response to intravenous MCh challenge (protocol 2). Increasing the tidal ventilation frequency from 6 to 30 breaths/min (from 0.1 to 0.5 Hz) significantly decreased Raw in response to MCh challenge (Fig. 4). In addition, the response to MCh under static conditions was greater than the response during ventilation at either frequency.

Airway response to intravenous MCh under static and dynamic conditions under similar conditions of alveolar ventilation (protocol 3). The increase in Raw in response to MCh was significantly greater under static conditions.
conditions than during volume oscillations of 10 ml/kg at 15 breaths/min (Fig. 5) in a closed constant-volume circuit in which little or no gas exchange occurred. This indicates that differences in gas exchange under different ventilatory conditions cannot account for the reduction in the response to MCh during tidal ventilation.

Airway response to aerosolized MCh under static and dynamic conditions (protocol 4). The increase in Raw in response to MCh was four- to fivefold greater under static conditions than during volume oscillations of 20 ml/kg at 15 breaths/min in vagotomized animals (Fig. 6B). These results were similar to the results obtained from animals with intact vagus nerves (protocol 1, Fig. 3). Therefore, the smaller airway response to MCh under dynamic than under static conditions cannot be attributed to an effect of vagal reflexes.

DISCUSSION

Our findings demonstrate that tidal ventilation significantly decreases airway narrowing in response to MCh in rabbits in vivo. The suppression of the bronchoconstrictor response to MCh caused by tidal ventilation increased when either the amplitude or the frequency of the volume oscillations was increased. This effect of tidal ventilation on airway responsiveness cannot be attributed to effects on gas exchange, MCh delivery, or vagal reflexes because it was observed under conditions in which gas exchange was similar, when the MCh was administered by either the intravenous or aerosol route, and when the animals were vagotomized. These results are consistent with our previous observations that greater airway closure occurs in dogs and rabbits during bronchoconstriction under static conditions than in the presence of tidal ventilation (25, 27). The results of our present in vivo study are also consistent with our previous findings that isolated constricted bronchi and tracheal smooth muscle strips generate more pressure...
or force under static conditions than when volume or length oscillations are imposed (6, 9, 22). Our cumulative findings from both in vivo and in vitro studies suggest that increases in tidal volume during bronchoconstriction result in greater stretch of airway smooth muscle and that this decreases smooth muscle force generation and results in less airway narrowing.

In the present study, airway narrowing was assessed under both static and dynamic conditions of ventilation by using measurements of pulmonary impedance obtained by using very small-volume oscillations (0.2–0.3 ml/kg) at 6 Hz. Although we measured pulmonary impedance, we have previously shown that tissue resistance is negligible in rabbits at an oscillatory frequency of 6 Hz (24) and that changes in pulmonary impedance are correlated with changes in Raw at this frequency. Recent data suggest that increases in pulmonary resistance during bronchoconstriction are caused by airway narrowing and ventilation inhomogeneity and not by increases in lung parenchymal tissue resistance (12). In addition, our recent measurements in rabbits demonstrate that after MCh challenge, increases in pulmonary impedance at frequencies above 4 Hz are highly correlated with airway narrowing determined morphometrically (21). Therefore, we believe that the changes in pulmonary impedance that we measured are related to changes in Raw and reflect the effects of tidal ventilation on airway narrowing.

The changes in the frequency and volume of tidal ventilation in protocols 1 and 2 would be expected to result in differences in alveolar ventilation. In addition, under static conditions there is no alveolar ventilation. Therefore, we evaluated whether differences in alveolar ventilation could have accounted for the effects of ventilation on the airway response to MCh. The closed-circuit constant-volume system used in protocol 3 permitted no fresh gas to enter the system under either static or dynamic conditions. Greater airway narrowing was still observed under static than dynamic conditions even though little or no alveolar ventilation occurred under either condition. These results suggest that differences in alveolar ventilation during bronchoconstriction cannot account for the effects of tidal ventilation on the airway response to MCh in the present study.

The effects of large tidal volumes on airway responsiveness could potentially have been mediated by a decrease in the delivery of intravenous MCh to the bronchial circulation or by vagal reflexes in response to lung inflation (26). However, we observed a much greater airway response to MCh under static than dynamic conditions whether MCh was delivered by aerosol or intravenously. Thus it is not likely that decreased intravenous drug delivery during tidal ventilation can account for the effect of tidal ventilation on the airway response to MCh. In addition, because the much greater airway response to MCh under static than dynamic conditions was not abolished in vagotomized animals, it is unlikely that the results of protocols 1 and 2 can be attributed to the effects of vagal reflexes.

We found that increasing the magnitude of the tidal volume from 5–10 to 20 ml/kg resulted in a progressively greater suppression of the bronchoconstrictor response to MCh. In addition, at a constant tidal volume, an increase in the ventilation frequency from 6 to 30 breaths/min significantly decreased the response to MCh. The tidal volumes and frequencies employed in these studies were within the physiological range. Tidal volumes of 5–10 ml/kg are similar to those that occur in spontaneously breathing and mechanically ventilated animals and humans. The largest tidal volume (20 ml/kg) used in this study approached total lung volume at end inspiration and thus was similar to deep inspiration. Thus the observations suggest that changes in either the frequency or volume of tidal ventilation within the physiological range can modulate the airway response to bronchoconstrictors.

Our findings support our hypothesis that the bronchodilating effect of changes in lung volume is caused by the direct effect of stretch on force generation by the airway smooth muscle (6, 8, 9, 22, 25, 27). In the present study, we found a direct relationship between the magnitude of the increase in lung volume during tidal ventilation and the magnitude of the suppression of airway narrowing during bronchoconstriction. Increases in the magnitude of the tidal volume were associated with increases in end-inspiratory transpulmonary pressure (Fig. 1), which should result in an increase in the transmural pressure across the airway wall. The increased airway transmural pressure during volume oscillation should result in greater stretch of the smooth muscle in the airway wall.

The effects of the amplitude of volume oscillations on the airway responsiveness may result from a plasticity of smooth muscle cell structure (7, 10, 11, 20). We have postulated that the suppression of the contractile response of tracheal smooth muscle caused by stretch or length oscillation is related to an effect of stretch on the organization of the contractile filaments in smooth muscle cells, which might involve adjustments in the sites of attachment of actin filaments to the membrane as well as changes in actin or myosin filament length and orientation (7, 10, 20, 22). When the amplitude of the length oscillations is increased, the organization of the contractile filaments within the cells adapts to the longest length to which the muscle is being stretched, effectively lengthening the contractile element in relation to cell length. As the muscle length is decreased during each individual oscillation cycle, active shortening of the contractile element begins from a longer starting point, resulting in lower overall active force development during the imposed shortening. If the magnitude of the stretch of the muscle is reduced by decreasing the amplitude of the oscillation, the contractile element length "set point" adjusts to a shorter length; however, adjustments of the contractile element length set point occur slowly relative to the active shortening velocity of the muscle. Because the rate of adjustment of the contractile element length set point is slow relative to the rate of active shortening, changes in the contractile element length set point do not occur...
during a single oscillation cycle unless the oscillation frequency is extremely slow, probably much slower than the frequencies associated with normal ventilation.

The prolonged depression of airway responsiveness that has been observed when tidal breathing is resumed after deep inspiration may also be explained on the basis of the plastic properties of the smooth muscle. A sudden decrease in the amplitude of volume oscillation would abruptly reduce the magnitude of the stretch on the muscle. Under these conditions, the contractile element length set point would adjust slowly to accommodate the reduced amplitude of smooth muscle stretch. This would result in a prolonged depression of airway responsiveness until the contractile element length set point readjusted to the reduction in stretch on the muscle. In the absence of large stretches of the airway smooth muscle, force generation is greater and approaches the static response (6, 22). This mechanism can also account for the heightened airway responsiveness observed in normal subjects when deep inspiration is inhibited during the bronchial challenge (23).

In the present study, we also observed an effect of ventilation frequency on airway responsiveness. This is also consistent with our previous observations of airway tissues in vitro in which force generation decreased with increases in the frequency of oscillation (6, 22). The effects of frequency on the contractile response are likely to be related to the rate of imposed shortening relative to the rate of active shortening. An increase in the frequency of oscillation at constant oscillation amplitude would not alter the contractile element length set point but would decrease the time allowed for active shortening of the contractile element and thereby lower force development during the shortening phase of the oscillation cycle. If the rate of oscillation were increased sufficiently above the shortening velocity, active shortening of the contractile element would not occur at all and the oscillation would result only in stretch and retraction of series elastic elements. However, our previous calculations suggest that at oscillation frequencies comparable to normal rates of tidal ventilation, some active shortening of the contractile element probably occurs (22).

Asthmatic subjects often exhibit an absence of bronchodilation with a deep inspiration (1–4, 14–16, 18). This phenomenon may be related to inherent differences in the contractility of the smooth muscle of asthmatic subjects or to lower forces of interdependence between the airways and the lung parenchyma in asthmatic subjects. In the latter case, deep inspiration may not produce the same stretch of the airway smooth muscle of asthmatic individuals as occurs in nonasthmatic individuals, thus resulting in greater airway responsiveness.

In summary, the results of this study demonstrate that airway narrowing during bronchoconstriction is greater under static conditions than during tidal ventilation in mechanically ventilated rabbits. The suppression of airway narrowing in response to MCh increases as the magnitude of the volume oscillations is increased from normal tidal breathing to deep inspiration and as the frequency of tidal volume oscillations is increased. These findings are consistent with previous in vitro data obtained in isolated bronchi and tracheal smooth muscle strips. Our findings suggest that the effect of a change of lung volume on airway responsiveness in vivo is related to the effects of stretch on the airway smooth muscle.

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