Effects of tidal volume stretch on airway constriction in vivo

ROBERT BROWN AND WAYNE MITZNER
The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

Received 19 April 2001; accepted in final form 27 June 2001

Brown, Robert, and Wayne Mitzner. Effects of tidal volume stretch on airway constriction in vivo. J Appl Physiol 91: 1995–1998, 2001.—Tidal stresses are thought to be involved in maintaining airway patency in vivo. The present study examined the effects of normal stresses exerted by the lung parenchyma during tidal ventilation on recovery from agonist-induced airway constriction. In seven anesthetized dogs, one lung was selectively ventilated with a Univent endotracheal tube (Vitaid, Lewiston, NY). Airway tone was increased either transiently (intravenous bolus) or continuously (intravenous infusion) with methacholine (MCh). During one-lung ventilation, changes in the airway size of both lungs were measured for up to 40 min during recovery from constriction by using high-resolution computed tomography. After recovery to baseline, the alternate lung was ventilated, and the protocol was repeated. The absence of tidal stresses led to an attenuated recovery from either transient or steady-state airway constriction. The effectiveness or lack thereof of normal tidal stress in stabilizing airway size may be one factor that contributes to the lack of reversal with tidal breathing and deep inspiration seen in asthmatic subjects.

METHODS

Our study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Seven dogs weighing ~20 kg were anesthetized with thiopental (15 mg/kg induction dose followed by 10 mg·kg⁻¹·h⁻¹ intravenous maintenance dose). After induction of anesthesia, the dogs were paralyzed with 0.5 mg/kg of succinylcholine with occasional supplemental doses as required to ensure no respiratory motion during imaging. Dogs were intubated with an 8.0-mm (ID) Univent endotracheal tube (Vitaid, Lewiston, NY). This tube has a balloon catheter that, when inflated, allows all air flow to and from one lung to be completely blocked. After this endotracheal intubation, dogs were placed in the supine position and their lungs were ventilated with room air with a volume-cycled ventilator (Harvard Apparatus, Millis, MA) at a tidal volume of 15 ml/kg and a rate of 18 breaths/min. A stable depth of anesthesia was maintained by monitoring heart rate changes and eyelash reflex.

Imaging and analysis of airways. High-resolution computed tomography (HRCT) scans were obtained with a Somatom volume zoom scanner (Siemens, Iselin, NJ) by using a spiral mode to acquire 50 computer tomography images in a 9-s breath hold (apnea) at 137 kVp and 165 mA. The images were obtained with 9-s breath hold (apnea) at 137 kVp and 165 mA. The images were obtained with a spiral mode to acquire 50 computer tomography images in a 9-s breath hold (apnea) at 137 kVp and 165 mA.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org 8750-7587/01 $5.00 Copyright © 2001 the American Physiological Society
were reconstructed as 1-mm slice thickness and a 512 × 512 matrix using a 12.5-cm field of view and a high spatial frequency (resolution) algorithm that enhanced edge detection at a window level of −450 Hounsfield units (HU) and a window width of 1,350 HU. These settings have been shown to provide accurate measurements of airway lumen size in airways as small as 2 mm (11, 21). For repeated airway measurements in a given dog within each experimental protocol, adjacent anatomic landmarks, such as airway or vascular branching points, were defined and used to measure the airway size at the same anatomic cross sections.

HRCT images were analyzed using the airway analysis module of the Volumetric Image and Display Analysis image analysis software package (Dept. of Radiology, Division of Physiologic Imaging, Univ. of Iowa, Iowa City, IA) as previously described and validated (1, 3). HRCT images were transferred to a UNIX-based Sun workstation. An initial isocontour was drawn within each airway lumen, and the software program then automatically located the perimeter of the airway lumen by sending out rays in a spoke-wheel fashion to a predesignated pixel intensity level that defined the luminal edge of the airway wall. Intra- and interobserver accuracy and variability of the software program with the use of this HRCT technique in phantoms, consisting of rigid tubes to measure known areas, have been previously shown by our laboratory (11) and others (1) to be highly resistant to operator bias.

Protocol 1 bolus challenge. To examine the effects of transient airway constriction, dogs were anesthetized and ventilated as described above. To standardize lung volume history, dogs were initially given a deep inspiration of both lungs to 30 cmH₂O. Under direct fiberoptic visualization, the deflated blocker cuff of the Univent endotracheal tube was advanced into either the right or the left mainstem bronchus and inflated. One-lung ventilation was thereby established. After 10 min of one-lung ventilation, airway tone was induced by a bolus intravenous injection of 1 mg of MCh (Sigma Chemical, St. Louis, MO) in 5 ml of saline. Scanning was performed immediately after injection (−5 s) and at 30, 60, and 90 s and 2, 5, 10, and 20 min after the injection. One lung thus remained at functional residual capacity, unventilated for the entire 30-min experimental period. After recovery to baseline, with a 20-min period of normal ventilation, the blocker cuff of the Univent endotracheal tube was advanced into the alternate mainstem bronchus, inflated, and the protocol repeated.

Protocol 2 continuous challenge. To examine the effects of a stable level of airway constriction, on a separate day, dogs were anesthetized and ventilated as described above. First, a stable state of airway tone was induced with a continuous intravenous infusion of 67 μg/min MCh. After 10 min of stable constriction, one-lung ventilation was established. After 10 min of one-lung ventilation (20 min of stable MCh-induced airway constriction), scanning was performed after discontinuing the infusion (at 5 and 60 s and 2, 5, 10, 15, and 40 min). One lung thus remained unventilated for the entire 50-min experimental period. After recovery to baseline with a 20-min period of normal ventilation, the blocker cuff of the Univent endotracheal tube was advanced into the alternate mainstem bronchus, inflated, and the protocol repeated.

Analysis. All measurements of airway area were normalized to the respective size when completely relaxed, as assessed at the end of the experimental protocols. Previous work has demonstrated that 0.2 mg/kg of atropine is sufficient to abolish cholinergic and baseline smooth muscle tone in dogs (3). The completely relaxed airway after atropine was defined as 100% (relaxed state), and airway lumenal areas were expressed as a percentage of this relaxed area. Each airway in each dog thus served as its own control. Two-way analyses of variance were used to compare the airway size during ventilation to nonventilation over time during MCh constriction separately for the bolus and the continuous infusion challenges. Scheffe’s correction for multiple pairwise comparisons was used, and \( P \leq 0.05 \) was considered significant.

RESULTS

In each dog, 13–18 airways, ranging in size from 1.5 to 5.5 mm in diameter, were matched and measured at functional residual capacity.

Protocol 1. During one-lung ventilation before MCh bolus, the mean size of the two airway populations was not different whether they were ventilated [50 ± 4% (SE) of relaxed state] or nonventilated [47 ± 3% of relaxed state, \( P = 0.44 \)]. Constriction of the airway with the bolus of MCh was similar in the ventilated and nonventilated airways. Immediately after the intravenous bolus injection of MCh, the ventilated and nonventilated airways constricted to 22 ± 1.6 and 21 ± 1.5%, respectively \( (P = 0.65) \). However, at 30 s after constriction and for the duration of the measurements (20 min), the ventilated airways relaxed (i.e., recovered from the constriction) to a greater extent than the nonventilated airways \( (P < 0.0001) \).

Protocol 2. During continuous MCh infusion and one-lung ventilation, the mean size of the airways at maximal constriction was not different whether they were ventilated (33 ± 2% of relaxed state) or nonventilated (31 ± 2% of relaxed state, \( P = 0.39 \)). After discontinuation of MCh infusion and over the next 40 min, the ventilated airways again relaxed to a greater extent than the nonventilated airways \( (P < 0.0001) \).

DISCUSSION

Our results show that the loss of rhythmic tidal stretching, which normally occurs with ventilation,
The potential importance of smooth muscle relaxation in asthma has been emphasized in several recent studies that have even suggested that the inability of airways to relax might be a primary defect in this pathology (14, 17). Skloot et al. (17) also showed that, in the absence of a deep inspiration during MCh challenge, normal subjects had a greatly exaggerated and sustained response to this agonist. It was suggested that asthmatic airways could be modeled by this condition in normal subjects. More recently, our laboratory has shown that, in normal subjects who are prevented from taking a deep breath, the spirometric changes associated with aerosol MCh challenge are in fact reflected in the narrowing of the conducting airways as measured by HRCT (2).

Although it seems clear that rhythmic tidal stresses do indeed have the ability to reduce airway smooth muscle shortening, both the energetics model of Fredberg et al. (6) and the plasticity model of Shen et al. (15, 16) invoke mechanisms only related to the contractile apparatus within the smooth muscle. Neither local removal nor degradation of agonist was considered in these models that implicitly assumed a steady-state level of stimulation and contraction. Stable levels of contraction in vivo, however, are more the exception than the rule, regardless of the cause of contraction. Aerosol challenges might be longer lasting than an intravenous bolus, but they are still quite transient phenomena (13), and allergic challenges that result in contraction of airway smooth muscle are similarly not steady-state phenomena. These considerations highlight the importance of understanding the nature of the recovery process of the smooth muscle from exogenously induced contraction.

The recovery phase of agonist-induced smooth muscle challenge normally occurs over a much longer time course compared with the onset of contraction. Indeed, following an intravenous bolus of agonist, the contraction normally peaks within a few minutes, but recovery is normally prolonged over tens of minutes (13). Although this is partly due to different concentration gradients in the vascular wash-in and washout of agonist, recovery also depends on several additional factors less directly involved in the acute onset of contraction. These factors include local bronchial blood flow, intrinsic smooth muscle contractility, and biochemical degradation of the agonist. Although bronchial blood flow is clearly important (19), the biochemical or muscle contractility components must play a significant role because a major portion of the recovery from bronchoconstriction occurs even in the absence of perfusion (12).

Despite the clear finding in the present work that the ventilated airways recover to a greater extent, our results leave open several important questions on the effect of ventilation on the recovery from constriction. Significant differences were found in the recovery from steady-state vs. transient smooth muscle contraction. With the steady-state constriction by the continuous intravenous infusion of MCh, ventilated and unventilated airways constrict to comparable levels. After the agonist was stopped, however, the ventilated airways eventually

*Fig. 2. Percent change in mean airway area during ventilation (•) and nonventilation (○) during a continuous infusion of MCh. After discontinuation of the MCh infusion and during the next 40 min, the ventilated airways again relaxed to a greater extent than the non-ventilated airways (P < 0.0001).*

attenuates the recovery of airway smooth muscle constriction to an agonist agent in vivo. Furthermore, the attenuation appears more pronounced on the magnitude than on the time course of the recovery. In addition, this attenuation was observed after both a short and a prolonged induction of airway contraction.

The effect of rhythmic cycling in decreasing smooth muscle tone has been shown in vitro (8, 10). Tidal stresses have also been shown to effect the degree of airway closure in vivo (18). Warner and Gunst (20) originally demonstrated that the rhythmical stretching associated with tidal breathing decreased not only the baseline lung resistance but also the response to MCh. This effect of tidal breathing limiting the degree of airway smooth muscle constriction was supported by the more recent studies of Tepper et al. (18) and Shen et al. (16). The latter group proposed a mechanistic explanation that involves changes in the plasticity of the smooth muscle cellular cytostructure (15, 16). Another hypothesis to account for the mechanism underlying similar observations was published in recent papers by Fredberg and colleagues (5–7). In this work, it was proposed that steady-state muscle forces would be determined by a balance between high- and low-energy cross-bridge dynamics. If the contractile stimulus is increased, then the number of cross bridges increases, and, given enough time at a constant load, the rapidly cycling cross bridges progressively convert to slowly cycling latch bridges and muscle stiffness increases. The model of Fredberg et al. (6) argues that the stresses associated with normal tidal breathing are sufficient to keep the airway smooth muscle from attaining a low-energy latch state. Neither of these models, however, focuses on recovery from constriction. If minimization of tidal stresses does have the potential to augment airway narrowing either because of plastic changes in the cytoskeleton or increased latch bridge formation, then, at least qualitatively, the dynamics of recovery from the constriction might be expected to be measurably decreased. Our results would suggest that this is the case.
reach a larger size. Why the ventilated airway got significantly bigger despite starting from the same level of constriction is not clear. Nor is it clear why the ventilated and nonventilated airways constricted to the same degree. However, if the level of contractile status is indeed similar in the two conditions, then there does not seem to be any obvious reason why the ventilated lung should now relax to a greater degree. One potentially important possibility is that the effect of tidal stretching on airway smooth muscle is dependent on the magnitude of contractile stimulus. Perhaps the effect of tidal stresses is more effective at small or moderate levels of constriction, and, with the strong stimulus that continuous infusion imposed, this effect was obscured. Although visual inspection of the mean results in Fig. 2 does not suggest that there is any substantive difference in the dynamic recovery, we did attempt to quantify the dynamics of recovery with an exponential fit. Unfortunately, there was too much variability in the data to reliably determine the final plateau and recovery time constant.

With a bolus intravenous injection of agonist, it is well accepted that the airway muscle will constrict over a time course measured in seconds and then relax over a time course measured in minutes. Our results in Fig. 1 show this dynamic response for both the ventilated and nonventilated airways. In the ventilated airway, the airway size was greater both at the peak of constriction and during recovery. Thus, in contrast to results with continuous MCh stimulation, the airways constricted to a significantly greater degree when they were not subject to the rhythmic tidal stretching. This behavior is what one would expect based on previous theoretical and experimental work. It is also worth noting that this is consistent with the above speculation that the effect of tidal stretching is dependent on the initial baseline level of constriction. In the case of the transient bolus, the peak level of constriction was more than with continuous infusion, and an augmented contraction was observed in the unventilated airways.

One final note is that our present experiments did not attempt to determine the specific level of reduction of tidal stresses needed to change the response. Our experimental design of totally eliminating the tidal stresses in one lung was chosen both for experimental simplicity and to maximize any potential effects. There would, of course, be an expected dose-time effect on the magnitude of the response, but we do not know how linear that effect would be. This information may be very relevant to the difference between the chronic airway constriction seen in some asthmatic patients.

In summary, absence of tidal stresses leads to an attenuated recovery from either transient or steady-state airway constriction. The magnitude of this effect may depend on the baseline level of tone in the airways. Changes in tidal stresses may be one factor in the pathology of airway constriction and lack of reversibility with tidal breathing and deep inspiration seen in asthmatic subjects.

This study was supported by National Heart, Lung, and Blood Institute Grant PO1 HL-49545.

REFERENCES