Airway closure with high PEEP in vivo

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Brown, Robert H., and Wayne Mitzner. Airway closure with high PEEP in vivo. J Appl Physiol 89: 956–960, 2000.—When airway smooth muscle is contracted in vitro, the airway lumen continues to narrow with increasing concentrations of agonist until complete airway closure occurs. Although there remains some controversy regarding whether airways can close in vivo, recent work has clearly demonstrated that, if the airway is sufficiently stimulated with contractile agonists, complete closure of even large cartilaginous conducting airways can readily occur with the lung at functional residual capacity (Brown RH and Mitzner W. J Appl Physiol 85: 2012–2017, 1998). This result suggests that the tethering of airways in situ by parenchymal attachments is small at functional residual capacity. However, at lung volumes above functional residual capacity, the outward tethering of airways should increase, because both the parenchymal shear modulus and tethering forces increase in proportion to the transpulmonary pressure. In the present study, we tested whether we could prevent airway closure in vivo by increasing lung volume with positive end-expiratory pressure (PEEP). Airway smooth muscle was stimulated with increasing methacholine doses delivered directly to airway smooth muscle at three levels of PEEP (0, 6, and 10 cmH$_2$O). Our results show that increased lung volume shifted the airway methacholine dose-response curve to the right, but, in many airways in most animals, airway closure still occurred even at the highest levels of PEEP.

Airway closure

airway smooth muscle; maximal responsiveness; asthma; pulmonary interdependence; deep inspiration; positive end-expiratory pressure

When airway smooth muscle is contracted in vitro, the airway lumen continues to narrow with increasing concentrations of agonist until complete airway closure occurs (2, 11). Although some controversy remains as to whether airways can close in vivo, recent work has clearly demonstrated that, if the airway is sufficiently stimulated with contractile agonist, complete closure of even large cartilaginous conducting airways can readily occur with the lung at functional residual capacity (FRC) (8). This result suggests that the tethering of airways in situ by parenchymal attachments is small at FRC, a conclusion consistent with measurements of the effective pressure surrounding the airways and the blood vessels at this lung volume (16, 23). However, at lung volumes above FRC, the outward tethering of airways should increase, because both the parenchymal shear modulus and tethering forces increase in proportion to the transpulmonary pressure (15). It should therefore be possible to prevent airway closure if one increased the lung volume at which the airway is challenged, and such an effect was shown to exist in isolated canine lungs by Warner and Gunst (26).

Airway responsiveness is commonly assessed by administering bronchoconstricting agonist agent by the aerosol route to the entire lung. Thus, when the whole lung is challenged with aerosol, all smooth muscle is contracted, resulting not only in airway constriction but also in parenchymal stiffening (19), increased tissue resistance (17), and increased parenchymal shear modulus (15). However, when airway smooth muscle is stimulated with a direct local challenge, there are no global changes in the lung parenchyma. We previously used this latter approach, involving localized atomization of agonist delivered directly to a small section of epithelium (8), to demonstrate the possibility of complete airway closure at FRC.

In the present study, we used a similar methodology to test whether increased mechanical distending stress on the airways caused by increased positive end-expiratory pressure (PEEP) could limit or prevent airway closure in vivo. Our results show that increased PEEP shifted the airway constrictor dose-response curve to the right, but, in many airways, even the highest level of PEEP studied (10 cmH$_2$O) could not prevent airway closure. These results indicate that the ability of airway smooth muscle to shorten markedly in normal healthy lungs in vivo may be only minimally limited by parenchymal tethering forces.

METHODS

Our study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Five 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. After tracheal intubation with an 8.0-mm-ID endotracheal tube, the dogs were placed supine, and their lungs were ventilated with room air with a volume-cycled ventilator (Harvard Apparatus, Millus, MA) at a tidal volume of 15 ml/kg and a rate of 18 breaths/min. A stable depth of anes-
theinspiration to 30 cmH2O for 30 s.

**Agonist challenge.** A local atomization of MCh agonist was delivered directly to the epithelium of the same airway locations with repeated challenges. The atomization was accomplished with a specially designed catheter that could be placed with bronchoscopic visualization. A short (2-mm) plastic tube was inserted into a PE-190 catheter. This tube had six tiny (0.15-mm) side holes drilled circumferentially 1 mm from the end and was plugged at its distal end with a short (1-mm) stainless steel rod. This metal plug greatly aided visualization in the computed tomography scanner. In practice, the catheter was filled outside the lung with the desired agonist concentration and advanced 1.5 cm beyond the tip of the bronchoscope. Rapid injections of the 20-μl boluses caused the liquid to be sprayed on the adjacent airway wall. In previous studies, neither placement of the catheter nor atomization of saline caused measurable changes in airway size (8).

**Imaging and analysis of airways.** High-resolution computed tomography (HRCT) scans were obtained with a Somatom Plus Scanner (Siemens, Iselin, NJ) by using a spiral mode to acquire 33 contiguous images in a single 20-s breath hold (apnea) at 137 kVp and 165 mA. The images were reconstructed as 2-mm slice thickness and a 256 × 256 matrix by using a maximum zoom of 4.0 (12-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 measurement of airway lumen size in airways as small as 2 mm (14, 27). 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 the airways were matched by these adjacent landmarks and measured.

The HRCT images were analyzed by using the airway analysis module of the Volumetric Image and Display Analysis image-analysis software package (Div. of Physiologic Imaging, Dept. of Radiology, Univ. of Iowa, Iowa City, IA) as previously described and validated (1, 7). The HRCT images were transferred to a UNIX-based Sun workstation. An initial closed contour was hand 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 defines the luminal edge of the airway wall. Intra- and interobserver accuracy and variability of the software program using this HRCT technique in phantoms, consisting of rigid tubes to measure known areas, have been previously documented by our laboratory (14) and others (1) to be highly resistant to operator bias.

**Protocol.** Dogs were anesthetized and ventilated as described above. To standardized lung volume history, the dogs were initially given a deep inspiration to 30 cmH2O for 30 s. On separate days, in random order, a PEEP of 0, 6, or 10 cmH2O was applied. A bronchoscope (Olympus BF-P30, Olympus, Melville, NY) was then passed into the lungs (airway generation 3−6), and the atomization catheter was placed as described in Agonist challenge. Ventilation was stopped, a 20-μl bolus of MCh solution was sprayed on the adjacent airway wall, then the catheter was pulled back into the bronchoscope and the HRCT scans were acquired, and ventilation was resumed. This procedure was repeated with increasing doses of MCh of 0.3, 1.0, 3.0, 10, 30, and 100 mg/ml until either the airway appeared closed on HRCT scan (no visible lumen) or the highest dose was reached. After the final dose of MCh, 0.2 mg/kg atropine, a dose previously shown to effectively inhibit cholinergic tone in the dog and completely relax the airways (7), was administered intravenously to the dogs. HRCT scans were repeated 10 min after atropine during lung inflation to 10 cmH2O to allow us to normalize the airway changes to each relaxed airway’s size.

**Analysis.** The completely relaxed airway after atropine at 10 cmH2O was defined as 100% (relaxed state, maximum size), and airway luminal areas were expressed as a percentage of this maximally relaxed area. Each airway in each dog served as its own control. A Kruskal-Wallis nonparametric analysis was used to compare the overall amount of airway closure between the three PEEP levels, and Wilcoxon signed-rank tests were used for pairwise comparisons. In addition, the ED50 was calculated for each dog for each PEEP level. The mean values for each PEEP level were compared by paired t-test. Significance was considered to be P < 0.05.

**RESULTS**

Twenty-two airways (3−6 airways per dog, 1.5- to 6.7-mm relaxed diameter) in the five dogs were matched and measured. At 0 cmH2O PEEP, the airways measured in dogs 3 and 5 were closed after the administration of 10 mg/ml of MCh (Fig. 1), consistent with previous results (8). In dogs 2 and 4, all the airways were closed after administration of 30 mg/ml of MCh (Fig. 1). In dog 1, all the airways could not be completely closed at the highest dose (Fig. 1).

When the PEEP was set at 6 cmH2O, only dog 5 showed closure of the airway at the 30 mg/ml concentration, whereas dog 3 showed airway closure of all airways at the highest concentration of 100 mg/ml. Dogs 2 and 4 showed closure of some but not all the measured airways at the highest concentration, and dog 1 had no airway closure even at the highest concentration of MCh administered (Fig. 1).

When the PEEP was set at 10 cmH2O, only dog 3 showed complete closure of all airways at the highest concentration of MCh. Dogs 4 and 5 showed closure of some of the airways at the highest concentration of MCh, and dogs 1 and 2 showed no closure of the airways even when challenged with 100 mg/ml of MCh (Fig. 1).

Overall, we observed airway closure in 19 of 22 airways at 0 cmH2O, 14 of 22 airways at 6 cmH2O, and 8 of 22 airways at 10 cmH2O (P = 0.006; Fig. 2). Furthermore, the number of airways that remained open even with maximal stimulation increased significantly when PEEP was increased from 0 to 6 cmH2O (P = 0.02; Fig. 2) and from 6 to 10 cmH2O (P = 0.001; Fig. 2). However, even at 10 cmH2O, 36% of the airways demonstrated complete closure.

We also calculated the ED50 of methacholine for the airways of each dog for each level of PEEP. The mean ED50 values were 0.15 ± 0.048, 0.24 ± 0.044, and 0.59 ± 0.063 mg/ml for 0, 6, and 10 cmH2O of PEEP, respectively. In each case, the increase in PEEP caused a significant increase in the ED50 (P < 0.05; Table 1).
DISCUSSION

Results in this study demonstrate that even relatively high levels of PEEP cannot always prevent airway closure when there is sufficient stimulation to the airway smooth muscle. At 10 cmH₂O of PEEP, more than one-third of the airways studied were closed by the MCh challenge. Increasing levels of PEEP shift the MCh dose-response curve to the right, indicating an attenuation of airway contraction by the increased lung inflation. The magnitude of this effect, however, was quite variable among dogs, with one animal showing minimal tendency for airway closure. Such variability among individuals is characteristic of measurements of airway responsiveness in both dogs and humans (5, 6, 10).

In this study, we delivered the MCh directly to the airway mucosal surface by using an atomization catheter previously employed (8). Rapid injection of the 20-μl boluses caused the MCh to be sprayed on the adjacent airway wall, which led to airway smooth muscle contraction localized to just a few millimeters along the airway length (8). In contrast, when the whole lung is challenged with aerosol, all airway smooth muscle is contracted, and this results in parenchymal stiffening (19) and increased tissue resistance (17). Such a stiffened parenchyma and increased shear modulus (24) should cause an increased elastic load on the airways that would reduce the degree of narrowing. Although this stiffened parenchyma would surely provide an increased load, it may not be sufficient to prevent closure in vivo. Indeed, using alveolar capsules in isolated canine lobes with MCh delivered by aerosol to the whole lung, Warner and Gunst (26) reported airway closure at a lung pressure from 7 to 10 cmH₂O. Furthermore, previous work from our laboratory showed that aerosol MCh challenge can by itself reduce airway luminal size to 10% of maximal size (6). The additional parenchymal distortion required to allow closure of the lumen would be expected to be quite small.

Although our protocol prevented the agonist-induced parenchymal stiffening, we systematically induced mechanical parenchymal stiffening with increasing levels of PEEP. However, even when the radial tethering forces on the airways were increased with PEEP values as high as 10 cmH₂O, a significant amount of airway closure still occurred in four of the animals when there was sufficient stimulation of the airway smooth muscle. We did not attempt to determine the pressure at which we could maintain patency in all the airways. Clearly, there would be a distribution of the balance between the required distending pressure to keep in-

Fig. 1. Methacholine (MCh) dose-response curves for each dog for each positive end-expiratory pressure (PEEP) level. A: dog 1. B: dog 2. C: dog 3. D: dog 4. E: dog 5. □: Solid lines, 0 cmH₂O PEEP; ○: dotted lines, 6 cmH₂O PEEP; ●: dashed lines, 10 cmH₂O PEEP. In all dogs at 0 cmH₂O of PEEP, and in 4 of the 5 dogs at 6 cmH₂O of PEEP, the airways measured were completely closed at less than the maximum dose of MCh. At a PEEP of 10 cmH₂O, only 36% of the airways were closed at the highest concentration of MCh administered (100 mg/ml).
AIRWAY CLOSURE WITH HIGH PEEP IN VIVO

Fig. 2. Number of airways closed at each level of PEEP. Overall, there was a significant difference in the number of closed airways ($P = 0.006$). Number of airways that remained open even with maximal stimulation increased significantly when PEEP was increased from 0 to 6 cmH$_2$O ($*P = 0.02$) and from 6 to 10 cmH$_2$O ($**P = 0.001$). Even at 10 cmH$_2$O, 36% of the airways were able to close with sufficient stimulation.

Table 1. ED$_{50}$ for methacholine concentration (mg/ml) for each dog and the mean (±SD) for all the dogs

<table>
<thead>
<tr>
<th>Dog No.</th>
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Mean ± SD $0.15±0.048$ $0.24±0.044^{*}$ $0.59±0.063^{†}$

Values are given in mg/ml. PEEP, positive end-expiratory pressure. $*P = 0.04$ compared with 0 cmH$_2$O PEEP. $†P = 0.007$ compared with 6 cmH$_2$O PEEP.

Balassy et al. (3), using alveolar capsule techniques in dogs to study the effects of PEEP on airway narrowing, similarly ascribe parenchymal tethering to the prevention of excessive airway narrowing and airway closure. Our results, showing a shift in the MCh dose-response curve with PEEP, are at least partially consistent with these previous studies. That the elastic load that the lung parenchyma imposes on the airway smooth muscle is a major factor in determining the degree of airway constriction was clearly demonstrated in studies by Bellofiore et al. (4). Their work supports the importance of the parenchymal elastic fibers on airway smooth muscle shortening in both the constrictor response to MCh and the dilator response to increased lung volume. Bellofiore et al. decreased the airway-parenchymal interdependence in the lungs of rats with elastase treatment to induce emphysema and quantified the decrease in parenchymal tethering by morphometric analysis. They found that disruption of the elastic fiber network increased the airway constrictor response to MCh at the highest doses tested. They also showed that, before the elastase treatment, the airway response to MCh was attenuated when end-expiratory volume was increased above FRC. However, after the elastase, they not only found greater constrictor response to MCh at FRC but also that increased end-expiratory volume no longer attenuated the constrictor response to MCh. Therefore, with decreased parenchymal interdependence, the dilation of the airways caused by increased lung volume was abolished. These results of Bellofiore et al., however, are not consistent with other findings in rats by Dolhnikoff et al. (13), who were unable to demonstrate significant morphological changes in parenchymal distortion after induced airway constriction with increased lung volume. They concluded that parenchymal tethering did not attenuate airway narrowing or prevent airway closure (13). Our results showing airway closure in dog airways in vivo are thus in agreement with this latter conclusion. Results from Okazawa et al. (21) in carbachol-challenged rabbit lungs also supported the notion that elastic loads exerted by the parenchyma are not sufficient to explain the attenuation of smooth muscle shortening in situ.

Several studies in human subjects, however, seem at odds with our conclusions. Ding et al. (12) studied the effects of changes in lung volume on airway responsiveness to aerosol MCh challenges in normal human subjects. They found an inverse correlation between increases in lung volume and the maximal response to MCh, but lung volume changes did not affect the sensitivity to MCh. We showed a clear decrease in the sensitivity to MCh (as defined from the ED$_{50}$) at higher lung volume. With respect to the maximal response, we do not believe there is a plateau in the dose-response relationship. Rather, the true maximal response is complete closure, and this cannot be exceeded. Moreover, as our laboratory previously demonstrated in an animal model, the response plateau to an agonist challenge in most human studies may only be an artifact of the limitation of the dose that can be delivered (6). This

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Many investigations have demonstrated that increasing lung volume leads to an attenuation of bronchoconstriction. Macklem (18) has suggested that the forces of interdependence of the parenchymal attachments on the airway wall may be the cause of attenuated bronchoconstriction in vivo. Robatto et al. (22) and

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effect could thus account for the results of Woolcock et al. (28) and Moore et al. (20), who both concluded that there was a maximal plateau with MCh challenge in normal human subjects. These substantial differences in results in humans showing a responsiveness plateau may be partially accounted for by the inability to provide sufficient contractile stimulation to the human airways and partially a result of measurement variability and reproducibility in indirectly assessing airway size in humans. In the work of Ding et al. (12), the evidence for a maximal plateau in airway resistance with increasing MCh challenge was not obvious in most of their subjects at FRC. The data presented by Woolcock et al. (28) and Moore et al. (20) similarly fail to present a convincing case for a truly maximal plateau. We believe that, if the airways were challenged with the method we used in dogs, the human airways would have constricted to a much greater extent.

In summary, our results clearly demonstrate the even high levels of PEEP cannot prevent normal conducting airway closure in vivo. These experiments extend our previous findings that showed that the elastic recoil of the uncontracted lung parenchyma at FRC did not prevent airway closure (8). Even with high levels of PEEP up to 10 cmH2O, the elastic tethering of the lung parenchyma is insufficient to create a maximal response plateau. We speculate that the ability of localized regions of airway smooth muscle to shorten markedly in normal healthy human lungs in vivo may be only minimally limited by parenchymal tethering forces.

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REFERENCES