The myth of maximal airway responsiveness in vivo

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Brown, Robert H., and Wayne Mitzner. The myth of maximal airway responsiveness in vivo. J. Appl. Physiol. 85(6): 2012–2017, 1998.—A sine qua non of hyperresponsive airway disease in asthmatic subjects is the lack of a maximal response with increasing doses of aerosol agonist challenge. Normal subjects, however, often appear to exhibit an airway response plateau effect even when challenged with high concentrations of agonist. To investigate this question of maximal narrowing in individual airways in vivo, we used high-resolution computed tomography to visualize canine airways narrowed by two routes of agonist challenge. We compared airway narrowing induced by methacholine (MCh) via the conventional aerosol route to that caused by local atomization of MCh directly to individual airways. Our results showed that, with aerosol challenge, airway responses never reached a truly flat plateau even at the highest possible nebulizer concentrations. Airway closure was never observed. However, when MCh was delivered directly to the airway luminal surface, airways could be easily narrowed to complete closure at modest (10 mg/ml) agonist concentrations. Thus neither the elastic recoil of the lung nor limitations of smooth muscle shortening can be responsible for the apparent plateauing of dose-response curves. We suggest that the plateau results from limitations associated with the delivery of high concentration of agonists via the aerosol route.

airways; airway closure; asthma; dose-response curve; high-resolution computed tomography; hyperresponsiveness; methacholine

A SINE QUA NON of hyperresponsive airway disease in asthmatic subjects is the lack of a maximal response with increasing doses of aerosol agonist challenge (31). Increasing concentrations of agonist lead to further narrowing of the airways, which continues to the point where further challenge is deemed unsafe. Normal subjects, however, often appear to exhibit an airway response plateau effect even when challenged with high concentrations of agonist (31). Delivery of increasing concentrations of agonist to normal subjects causes no further narrowing in the airways and minimal respiratory symptoms. However, the literature support for such a maximal plateau in normal subjects is at best inconclusive. The percentage of normal subjects who demonstrate a maximal plateau in conventional pulmonary function test indexes ranges from 20 to 80% (17, 19).

When airways constrict in vitro, they do not exhibit a plateau effect to an agonist challenge, but they continue to constrict to increasing concentrations of agonist until complete airway closure occurs (2, 7). Thus any apparent limitation in airway responses in vivo must be due to mechanisms not directly related to limitations associated with airway smooth muscle contraction. The appearance of a plateau in vivo may result from elastic loads provided by the surrounding lung parenchyma or by limitations in the delivery of agonist to the airway smooth muscle.

To investigate this question of maximal narrowing in individual airways in vivo, we used high-resolution computed tomography (HRCT) to visualize canine airways narrowed by two routes of agonist challenge. We compared airway narrowing induced by methacholine (MCh) via the conventional aerosol route with that caused by local atomization of MCh directly to individual airways. Our results showed that, with aerosol challenge, airway responses never reached a plateau even at the highest possible nebulizer concentrations, and airway closure was never observed. However, when MCh was delivered directly to the airway luminal surface, airways could be easily narrowed to complete closure at normal agonist concentrations.

METHODS

Our study protocol was approved by The Johns Hopkins Animal Care and Use Committee. Six beagle dogs weighing ~10 kg were anesthetized with thiopental sodium (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 a 8.5-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 anesthesia was maintained by monitoring heart rate changes and eyelash reflex.

Imaging and analysis of airways. 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 (5). 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 (10, 30). 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 2012 8750-7587/98 $5.00 Copyright © 1998 the American Physiological Society http://www.jap.org
Challenges. We used two different methods to deliver the agonist agent. One method was a conventional aerosol challenge. Aerosol challenges were administered by a Hudson 3000 nebulizer (Hudson, Temecula, CA) driven by compressed oxygen at 10 l/min. Under test parameter conditions with an operating pressure of 50 lb/in.² and a flow rate of 10 l/min, the nebulizer produced particles of mass median diameter of 3.1 µm with a geometric SD of 3.2. Given such a relatively dispersed particle size distribution, good central and peripheral distribution should have been achieved (14). Approximately 1 ml of solution was administered per challenge.

The second method was a local atomization of agonist delivered directly to the epithelium of the same airway locations that were measured during the aerosol challenge. 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 (CT) 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 5- to 30-µl boluses caused the liquid to be sprayed on the adjacent airway wall. Neither placement of the catheter nor atomization of saline caused measurable changes in airway size.

To assess just how local the drug delivery was with this new method, several pilot experiments were done. One such result from an 11-mm airway is shown in Fig. 1. Figure 1 shows the location of the catheter tip and the degree of constriction vs. distance for three sequential doses of histamine. Figure 1 clearly shows very little axial spreading of the constriction, being generally limited to <1 cm at all doses. Indeed, it was quite remarkable to observe airway closure over a range of only a few millimeters, with the airways being 50% of maximal size just a few millimeters either side of closure. MCh also produced a similarly limited local range of effectiveness, even at the point of closure. Here and throughout this paper, we use the term closure to indicate airways having a lumen that was not quantifiable on the CT scan. This occurs when the lumen decreases below 1 mm (30).

Protocol. Dogs were anesthetized and ventilated as described above. To standardize lung volume history, the dogs were given a deep inspiration to 30 cmH₂O before aerosol challenges and also before atomization catheter challenges (see below). Initially the dogs received cumulative inhalation challenges of aerosol MCh (Sigma Chemical, St. Louis, MO), in concentrations of 50, 100, and 500 mg/ml. Five breaths at each dose were administered to a peak airway pressure of 15 cmH₂O, held for 1 s, and then released to atmospheric pressure. We did not use lower doses to document the entire dose-response curve, because in this study we were only concerned with effects at maximal dose. More complete dose-response curves in dogs with histamine and MCh challenges have recently been published (3). After completion of the scans at the highest dose, the dogs were allowed to recover with normal ventilation for 50 min.

Subsequently, a bronchoscope (Olympus BF-P30, Olympus, Melville, NY) was passed into the lungs (airway generation 2–4), and the atomization catheter was placed as described in Challenges. A 30-µl bolus of 10 mg/ml of MCh solution was sprayed on the adjacent airway wall, and the catheter was then pulled back into the bronchoscope. A second dose of 50 mg/ml MCh was subsequently sprayed on the wall in those airways that remained open after the first dose.

Finally, the dogs received intravenously 0.2 mg/kg atropine, a dose previously shown to effectively block vagal tone in the dog (6) and completely relax the airways. HRCT scans were repeated after atropine to allow us to normalize the airway changes to the relaxed size (3).

Analysis. The completely relaxed airway after atropine was defined as 100% (relaxed state), and airway luminal areas were expressed as a percentage of the relaxed area. Each dog served as its own control. Mean airway areas as a percentage of relaxed areas were compared after each aerosol dose and each catheter dose by paired t-test. Significance was considered to be P < 0.05.

RESULTS

Twenty-one airways (4.2- to 11.7-mm relaxed diameter) were matched and measured under all challenge conditions. Aerosol doses of 50, 100, and 500 mg/ml of MCh caused the airways to narrow to an average of 23 ± 3, 12 ± 1, and 10 ± 1% of the relaxed area, respectively (Fig. 2). Airway narrowing did not plateau and continued to show further narrowing from the 100 to the 500 mg/ml dose (P = 0.023). Results from each of the individual airways of all dogs are shown in Fig. 3, left. Airway closure was never observed in any of these airways with any of the aerosol challenges. It is worth emphasizing that, although a narrowing to 10% of relaxed area may appear to be quite considerable, the actual change from baseline is much less. In dogs there is a substantial degree of baseline airway tone, averaging ~50% of maximally relaxed area (3). Thus, at the extreme dose used here, the average airway area is only reduced to 20% of baseline, which would increase airway resistance ~25-fold.

When the same airways were challenged with MCh via the atomization catheter, they were found to be much more responsive. Figure 4 shows an example of one 7-mm airway before and after constriction to closure with 10 mg/ml MCh. The airway lumen is not
detectable on the HRCT scan after the local MCh atomization challenge. This 10 mg/ml concentration of MCh delivered via the catheter caused complete closure in 13 of the airways and extreme narrowing in the remaining 8 airways to 11 ± 2% of the relaxed area (Figs. 2 and 3). In the airways not initially closed by the 10 mg/ml dose via the catheter, the narrowing was comparable to that seen in the same airways at the 500 mg/ml dose of aerosol MCh (P = 0.84). When the 50 mg/ml dose of MCh was delivered via the catheter to the eight airways remaining open, they also completely closed.

DISCUSSION

In a recent report, we compared the response of individual airways to histamine and MCh challenge (3). These results demonstrated that the decrease in airway area with increasing doses of either agonist slows markedly at high doses. Although the dose-response curves give the appearance of a plateau, the airway area continues to fall slightly even up to doses of 500 mg/ml. This value of 500 mg/ml is an order of magnitude greater than the largest doses normally given to human subjects. We questioned whether the airways would actually close if the delivered doses could be made high enough. Unfortunately, it is not possible to challenge with aerosol concentrations much above 500 mg/ml, because solutions at higher concentrations are not stable at room temperature and begin to
precipitate agonist, and systemic drug absorption can lead to cardiovascular instability. For this reason we sought another method that would allow higher concentrations to be delivered to individual airways.

The approach we used was one that has been used to study the local clearance of aerosols (13, 28). We adapted this technique for agonist delivery, and results showed a very local effect of airway smooth muscle constriction as shown in Fig. 1. There is nothing in the literature that would have led to the prediction that airway closure could occur over so limited a region. We observed closure commonly occurring over a range of just a few millimeters, with the airway being as large as 50% of the relaxed size only a few millimeters distal on either side of closure. This result suggests that there are few axial constraints limiting bronchoconstriction and that severe airway obstruction in asthma might possibly result from relatively few intense local effects.

There are several studies of in vivo airway responsiveness in animal models that present variable results, particularly whether the dose-response curves show plateaus at the highest doses. Although lung resistance (RL) in the guinea pig showed no maximal plateau in response to histamine (11), results in the cat (25) and rat (20) showed a flat maximal plateau in airway resistance with MCh challenge. In dogs, the presence of a maximal plateau depends on the variable being measured and the experimental protocol. With an intravenous MCh challenge in dogs, Shioya et al. (24) measured the changes in RL and dynamic compliance in dogs to various agonists and were unable to demonstrate a maximal plateau. In another study that assessed the response to aerosolized MCh, the RL measured at normal respiratory rates showed a maximal plateau, but, when measured at a faster rate (that should make RL more closely reflect airway resistance), there was no plateau (12). Warner and Gunst (29) showed a plateau in RL in the first MCh dose-response curve in anesthetized dogs, but a second curve done 1 h later showed no maximal plateau. Even more puzzling are the results of Robatto et al. (22), who showed a maximal plateau in airway resistance in all dogs at doses as low as 0.5 mg/ml MCh. However, in a subsequent study 1 yr later in which an identical protocol was used, Robatto et al. (21) failed to find evidence of a response plateau in any of the dogs studied at doses up to 30 mg/ml MCh.

Studies of maximal responsiveness in humans are similarly inconsistent. In human subjects, airway contraction has been assessed either with spirometric indexes or RL. Several investigators have found only about one-third of normal subjects demonstrating an airway response plateau (17, 19). Others can only demonstrate a clear consistent plateau in normal and mild asthmatic subjects when measuring the flow at 40% of vital capacity after a partial expiratory flow-volume curve (27) and not with the more conventional forced expired volume in 1 s (FEV₁) (26). Although Woolcock et al. (31) fitted sigmoid curves to FEV₁ data and argued that normal and mild asthmatic subjects showed a plateau after histamine aerosol, the raw data presented show little evidence of a plateau. Ding et al. (8) also argued the presence of a maximal RL plateau in normal subjects in response to MCh. However, several of their subjects showed a maximal response at less than maximal dose; at higher doses, the response became smaller. Such a paradoxical response was also found more recently by Moore et al. (19), but the mechanism underlying it remains obscure. However, whatever is responsible for diminishing the response at the highest doses could certainly be responsible for the appearance of a flat plateau. On the basis of our present findings that demonstrate the ability of even large intraparenchymal airways to close, the appearance of a peak or plateau reflects either some agonist delivery problem or that the response variable being measured does not accurately reflect the degree of airway constriction.

We believe that the presence of an apparent response plateau may be determined by the dose of agonist that can be delivered. The results in Fig. 2 clearly demonstrate that the apparent plateauing seen on the dose-response curve during aerosol administration of MCh represents a limitation related to the aerosol administration. Even at concentrations 50 times lower than the maximal aerosol concentration, more than one-half of the airways studied were already closed when the MCh was administered directly to the luminal surface of the airway. Unfortunately we did not use lower doses, which would have allowed a more complete comparison of the relative dose-response curve.

This apparent increased responsiveness with direct luminal atomization raises a question regarding dose equivalency. In Fig. 2 we are comparing equivalent concentrations in the solutions, but it is clear that the amount of MCh delivered might be much different with the two methods. We have estimated the total amount of solution delivered to the whole lung during the aerosol challenge to be on the order of 1 ml. Thus, with the maximal aerosol dose, 500 mg of MCh were delivered to the entire airway surface. With the local catheter we delivered 0.3 and 1.5 mg of MCh, respectively, over the surface of the airway ~1 cm in length. It is difficult to know exactly how to quantify the relative amounts delivered with the two methods, but it is not unreasonable to suggest that the amount delivered with the direct delivery may be several orders of magnitude greater than that delivered by aerosol. If so, this would not only account for the significant left shift of the dose-response curve but also lead to the prediction that, if it were possible to deliver higher concentrations via aerosol, some airway closure would also be observed.

This prediction thus considers that the two curves in Fig. 2 are actually part of the same dose-response curve. The leftward shift of the catheter challenge curve only occurs because of our use of agonist concentration on the abscissa and not the actual dose delivered to the smooth muscle. Whether this is true depends on the relative importance of contracted parenchyma. That is, when the whole lung is challenged with aerosol, all airway smooth muscle is con-
tracted, and this results in parenchymal stiffening (18) and increased tissue resistance (15). When the airway is stimulated directly, there are no changes in the surrounding lung parenchyma. It is possible that an increased elastic load provided by stiffened parenchyma prevents airway closure. Although stiffened parenchyma would surely provide an increased load, we do not think that this would be capable of preventing closure. With aerosol challenge, the lumen can already be decreased to 10% of maximal size, so it seems that the additional parenchymal distortion required to reduce the lumen to closure would be small. Closure may also occur in airways too small to be visualized with HRCT, but the effect of such closure on parenchymal mechanics is uncertain. Another manifestation of parenchymal stiffening is its effect on lung volume. In dogs, increasing doses of MCh or histamine in similar concentrations used in the present study cause progressive decreases in lung volume (3). Such a loss of lung volume with the whole lung challenge would lead to a decreased load on the airways, thereby lessening the direct effect of parenchymal stiffening.

In comparing the two modes of challenge, there is one further difference that could possibly play a role. With the aerosol challenge, it is necessary to inflate the lung to deliver the aerosol, whereas the local challenge was delivered at functional residual capacity (FRC). It is known that both ventilation and deep inspiration can transiently decrease the airway responsiveness to MCh challenge (16, 23), so perhaps the decreased sensitivity and lack of closure with aerosol challenge resulting from the delivery procedure. We attempted to minimize this potential effect by delivering the aerosol in only five breaths at a modest peak pressure of 15 cmH2O. If this ventilation did lead to decreased responsivity transiently, we should have observed further airway constriction following the acquisition of CT scans. However, on return to the normal ventilation, the peak airway pressure did not increase; rather it slowly decreased to baseline. We therefore feel that this mechanism is unlikely to be playing a major role in our observations.

From the results presented in this study that clearly demonstrate the ability of normal conducting airways to close in vivo, we suggest that the appearance of a plateau in aerosol dose-response curves represents a limitation of the conventional aerosol delivery method. From this perspective, we can then rule out two suggested mechanical behaviors of airway smooth muscle in vivo that have been used to explain the observed response plateau and lack of airway closure. These are that the shortening of the muscle is itself limited and that there is a substantial and increasing elastic load on the smooth muscle as it shortens. Our results clearly show that, when locally stimulated, the airway smooth muscle is not limited in its ability to shorten to closure in vivo. Although this could have been predicted from in vitro experiments (9), as discussed above, it has not been easily demonstrated in vivo. Our results also show that the elastic recoil of the uncontracted lung parenchyma at FRC is insufficient to prevent the shortening of airway smooth muscle to closure. This may not be true at higher lung volumes and recall pressures or with exogenous contraction, but at the present time we only have data on maximal airway smooth muscle shortening at FRC. We also cannot speculate on why the shape of the dose-response curve in asthmatic subjects might be different from that in normal subjects. Clearly they respond at lower doses, but why the effects that serve to limit constriction in normal subjects do not seem to work in asthmatic subjects is not clear.

In summary, our results demonstrate that at FRC it is possible to cause closure of relatively large conducting airways. Thus neither the elastic recoil of the lung nor limitations of smooth muscle shortening can be responsible for the apparent plateauing of dose-response curves.

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