Airway Hyperresponsiveness: From Molecules to Bedside
Invited Review: Complexity of factors modulating airway narrowing in vivo: relevance to assessment of airway hyperresponsiveness

Vito Brusasco1 and Riccardo Pellegrino2
1Dipartimento di Medicina Interna, Università di Genova, 16132 Genova; and 2Fisiopatologia Respiratoria, Azienda Ospedaliera S. Croce e Carle, 12100 Cuneo, Italy

Brusasco, Vito, and Riccardo Pellegrino. Invited Review: Complexity of factors modulating airway narrowing in vivo: relevance to assessment of airway hyperresponsiveness. J Appl Physiol 95: 1305–1313, 2003;10.1152/japplphysiol.00001.2003.—In vivo, the airway response to constrictor stimuli is the net result of a complex array of factors, some facilitating and some opposing airway narrowing, which makes the interpretation of bronchial challenges far from being straightforward. This review begins with a short description of the complex mechanisms of airway smooth muscle activation and force generation as the starting events for airway narrowing. It then focuses on gain factors modulating airway smooth muscle shortening and on the geometric factors determining the magnitude of reduction in airway caliber in vivo. Finally, in light of the evidence that mechanical modulation of airway smooth muscle tone and airway narrowing is at least as important as the inflammatory contractile mediators in the pathogenesis of airway hyperresponsiveness, the implications for the interpretation of bronchial challenges in clinical settings are discussed.
airway smooth muscle; contractility; airway remodeling; loads; deep inhalation

HISTORICAL BACKGROUND

Airway hyperresponsiveness (AHR) is defined as an excessive airway narrowing in response to a variety of chemical and physical stimuli that have little or no effects in healthy subjects and is documented in vivo by leftward and upward shifts of the dose-response curve to constrictor agents (112, 116). In the present review, we will refer to the leftward shift of dose-response curve as airway hypersensitivity and to increased maximal response as airway hyperreactivity.

In 1947, Curry (33) first reported that inhaled histamine and methacholine cause greater bronchoconstrictor responses in asthmatic than in nonasthmatic subjects, and the magnitude of response is related to the severity of disease. The concept of AHR was later introduced by Tiffeneau (103) by using a threshold dose to compare airway sensitivity to chemical and allergenic stimuli. Most of the studies on AHR conducted in the following three decades have dealt with autonomic factors (81) and mediators of immune reactions (35). However, no matter how important the release of inflammatory mediators in the pathogenesis of AHR may be, no studies have been able to fully explain how airway inflammation translates into airway narrowing, which is the basic event in bronchial asthma. Interesting in this respect is the hypothesis that AHR could result from an individual genetic predisposition to develop an abnormal response to inflammatory stimuli (9), possibly involving changes in airway smooth muscle (ASM) (108). The role of the interaction between airway inflammation and ASM has been further emphasized by studies focused on the phenotypic and mechanical heterogeneity of ASM (46).

In 1961, Nadel and Tiernay (82) first documented that a deep inhalation (DI) can effectively decrease airway resistance measured at the same volume of before DI in humans. Although this observation should have raised the clinically relevant question regarding whether a DI may affect the measurement of AHR, it was not until 20 years later that Fish and coworkers (37) reported that the specificity of methacholine challenge in separating asthmatic from hay-fever and normal subjects was dependent on whether or not measurements requiring full inflation were used to assess airway narrowing. Along this line, Woolcock and Permutt, reviewing the available literature for their chapter in the Handbook of Physiology (112), showed a better separation between normal and asthmatic subjects when airway responsiveness was assessed with 1-s forced expiratory volume (FEV1) than when specific airway conductance was used. Moreover, a series of studies conducted since the early 1960s documented that the well-known inverse dependence of airway re-
sistance on lung volume was strongly affected in disease by changes in either ASM tone or lung elastic recoil (21, 26, 106, 113–115). In particular, it was shown that increasing lung volume has less bronchodilating effects on asthmatic than on normal subjects, thus suggesting that airways and lung parenchyma are somehow uncoupled in the former.

The impact of the mechanical modulation of airway narrowing in the assessment of AHR and its relevance to the pathogenesis of bronchial asthma has been the object of a number of recent studies, most of which will be discussed in this review. The complex interaction between factors facilitating and factor opposing airway narrowing in response to constrictor stimuli is the object of the present review and is summarized in Fig. 1.

INTERACTIONS BETWEEN CONTRACTILE AGONISTS AND RECEPTORS

The ASM can be increasingly activated by a contractile agonist (79) up to the point when all its specific surface receptors are occupied. Thus the dose (log)-response curve has a sigmoidal shape characterized by position and maximal response. In vivo, the position of the dose (log)-response curve is determined by the amount of agonist binding to active receptors sites. This is limited by access barriers, agonist metabolism, and its removal by blood. An intact mucosa lined with surfactant layer is believed to represent an efficient physical and metabolic barrier between airway lumen and ASM (47). In diseased conditions, surfactant depletion (47), airway epithelial shedding (52), lack of elaboration of epithelium-derived relaxant factor on the ASM (38), and release of cationic proteins from inflammatory cells such as eosinophils (28, 104) may contribute to AHR. In addition, receptor pathways limiting ASM contraction may be dysfunctional, as suggested by airway inflammation being capable to uncouple β2- and M2 receptors (4, 41, 96, 99) and activate neural receptors, thus triggering constrictor responses (61). All these data support the well-accepted notion that alteration of epithelial structure and function may lead to enhanced AHR and impaired bronchodilatation.

FORCE GENERATION BY ASM

ASM contractile properties. For a given degree of activation, ASM generates a force that depends on the number and rate of interactions (cross-bridges) between its actin and myosin filaments (40, 101, 102) and their plastic adaptation within the internal cell scaffold (95). The capacity to generate force can be intuitively regarded as a major determinant of AHR in vivo. In addition to force-generating capacity, ASM contractility is defined by its velocity of shortening. This primarily reflects the quantity and total activity of myosin light chain kinase, which controls the actin-myosin cross-bridge cycling rate (5). Force and velocity are two distinct properties of the ASM, which may be inversely related to each other. For example, force is maximal when contractile filaments are arranged in parallel inside the smooth muscle cell, whereas velocity is maximal when they are arranged in series (92). Yet, both features are strongly dependent on the load ASM has to work against (see below). It has also been suggested that an increase in ASM shortening velocity may contribute to AHR in vivo (98), but experimental evidence is still lacking.

Despite theoretical predictions, whether an abnormal ASM contractility contributes to AHR in vivo has been a long-debated subject. At variance with early studies, which failed to show differences in contractile force between normal and asthmatic ASM, it has been recently found that isometric force as well as myosin light chain kinase and velocity of shortening are increased in passively sensitized ASM (2, 8, 54, 78) and in asthma (70a). Furthermore, sensitized ASM shows a contractile response to stretching that is absent in normal ASM (77, 100). It has been suggested that this so-called myogenic response may contribute to AHR in those asthmatic subjects who experience sustained airway narrowing after taking a deep breath (74, 88).

ASM mass. In healthy subjects, the proportion of ASM to total airway wall is minimal in the trachea and gradually increases along the bronchial tree. Pathological studies have documented an increased ASM mass in asthmatic airways, variably due to increase in myocyte number (hyperplasia) or size (hypertrophy) (36). Among the possible causes for ASM proliferation are polypeptide growth factors, inflammatory mediators, and cytokines derived from several cells, such as epithelial cells, macrophages, and mast cells (84). Two are the possible consequences of the increase in ASM in asthma. First, as predicted by theoretical models, an
increase in ASM mass could lead to an increase in force development and airway narrowing, thus possibly accounting for the increase in AHR (69). This hypothesis is, however, based on the unproven assumption that the proliferated ASM maintains the contractile properties of normal ASM, which makes the precise role of increased ASM mass in the development of AHR in vivo still uncertain. Second, the proliferated ASM mass may increase its capability to produce multiple cytokines, chemokines, and prostanooids (55, 56), which in turn would perpetuate and amplify the inflammatory process within the airway wall. In this sense, the increase in ASM mass in asthma may be regarded as a potential mechanism of AHR.

**ASM shortening.** Isolated ASM is able to produce enough force to shorten to 20% of its optimal length (101, 102). For geometric reasons, a linear shortening of such a magnitude would result in a complete airway closure (79). In vivo, however, a plateau of response to bronchoconstrictor stimuli is observed in normal subjects, suggesting that factors external to ASM limit its shortening. ASM could not be fully activated by aerosol delivering in vivo (13), but this issue remains unresolved in humans. In any case, for ASM shortening to happen, all noncontractile tissues arranged in parallel or in series with ASM must be stretched or compressed and deformed. This process absorbs part of the energy produced by ASM, thus limiting its shortening. Several pieces of evidence suggest that, in normal conditions, the load provided by the surrounding elastic structures is sufficient to strongly contrast and limit ASM shortening (71) at a much lower level than achievable in vitro (79). Thus the load on ASM can be regarded as the major determinant of the plateau response to bronchoconstrictor stimuli in normal individuals (71).

**Internal load.** The airway wall itself represents a load on ASM, owing to the presence of noncontractile tissues each having a different elastic modulus. In large airways, cartilage rings provide an important preload to ASM and their softening in inflammatory disorders may be associated with AHR (80). In small airways, the lack of cartilaginous support would render the airways more prone to obstruction and collapse. Noncontractile tissue may however behave like a load acting against ASM shortening. For example, a stiff airway reticular basement membrane could also represent a load on ASM (65, 67, 68) especially in asthma, where the basement membrane is thicker and presumably stiffer than normal due to subepithelial fibrosis (91). In vivo, however, changes in airway caliber are determined not only by ASM shortening but also by its effect on airway geometry, which may explain the conflicting results of studies attempting to relate basement membrane thickness and AHR (24, 76, 105). The available data on reticular basement membrane appear far from being conclusive in this regard.

**External load.** The major source of load against which the ASM has to work to short is provided by the lung elastic recoil, which decreases with lung deflation and vice versa. The response of normal subjects to bronchoconstrictor stimuli is increased during breathing at reduced lung volumes, thus making it similar to that of asthmatic subjects (34, 90). To be effective against ASM shortening, the force exerted by lung parenchyma must be properly transmitted to the external wall of the airways and from here across all tissues separating it from ASM. Any pressure dissipation at this level will necessarily result in loss of load, thus facilitating ASM shortening. Information is now available about the local interactions between lung parenchyma and airways in vivo. In theory, tissues softened by inflammatory changes may loose their ability to limit ASM shortening, especially at low lung volume (67). Also fluid accumulation around the airways would uncouple them from lung parenchyma, thus possibly impeding the transmission of lung elastic recoil force to airways. Changes in lung volume are also critical in this respect, as they reflect the ability of the tethering forces of lung parenchyma to keep the airways open (6, 34). Thus a decrease in volume, as in obesity (53), a decrease in elastic recoil (42), or a reduced number of deep sighs (39), as during sleep, may favor airway narrowing. Finally, altered autonomic neural regulation of the lung and ASM tone may control airway caliber, especially during sleep (49).

**Maximal force-velocity-load relationship.** ASM force and velocity of shortening are tightly linked to each other and inextricably to the load imposed by the functional and anatomic conditions. Although strongly influenced by the number and rate of interaction (5, 39, 40, 102), as well as by plastic adaptation of the actin and myosin filaments (95), ASM behaves like skeletal muscle in that its shortening velocity increases and force decreases when the load decreases and vice versa. Thus a decrease in load necessarily entails an increase in shortening velocity, and similarly an increase in velocity would imply a decrease in load.

A more detailed analysis of the mechanisms and consequences of airway wall remodeling in disease has been the object of another review of this series (75a).

**CHANGES IN AIRWAY GEOMETRY**

Modeling predicts that, for a given degree of ASM shortening, the thicker the airway wall the greater the reduction in airway caliber (14, 51, 79, 110). Increased airway wall thickness may be due to cellular infiltration, vascular engorgement, and interstitial fluid accumulation (51). Results of structure-function studies indicate thickening of the airway wall layer as the major contributor to AHR in chronic obstructive pulmonary disease, particularly if this occurs in the airway wall layer internal to ASM (110). Furthermore, a theoretical analysis suggested that thickening occurring in small airways may be the main determinant of airway closure in response to constrictor stimuli (72). Support to this prediction has been provided by the reduction in air trapping following steroid treatment in mild asthma (27). How small airway disease and closure determine AHR is a matter of speculation. Altered neural autonomic control, mucosa thickening, altered surfactant, inflammation of the interdependence zone,
involvement of ASM, airway remodeling, and selective closure of the collateral channels especially at night may be some of the many links with AHR (49, 57, 58, 64, 107).

It is believed that the airway mucosa folds when ASM shortens. For geometric reasons, it is expected that the lower the number of folds the greater the reduction in airway lumen (66). However, theoretical predictions on mucosal folding and its effects are heavily influenced by model assumptions (66, 93, 111), and no proof has been provided on how mucosal folding occurs in vivo and whether this is different between health and disease.

EXTERNAL MODULATION OF AIRWAY CALIBER IN VIVO

Effects of deep inspiration. In normal subjects with relaxed ASM, the airway caliber increases approximately with the cube root of lung volume. Therefore, under normal conditions, a DI is expected to be a potent bronchodilator mechanism, even though it leads to a transient decrease in distending pressure at a given lung volume on deflation (19). In various diseased conditions, the relationship between airway caliber and lung volume may be lost because the ASM tone is increased, due to the poor transmission of the force from the lung to the airways insufficient to distend them. For example, the ability to distend constricted airways by DI may be lost in any condition associated with limited lung volume excursion, such as obesity (117), supine position (90), chest wall disease (10), chronic heart failure (1), or when lung elastic recoil is decreased (25, 42).

During induced bronchoconstriction in normal subjects, a DI is followed by an increase in airway caliber that persists for several seconds. This bronchodilator effect of DI increases with the degree of induced bronchoconstriction (17) and is likely the result of an increase in airway hysteresivity (39). In asthma, the ability of DI to dilate constricted airways is partially or totally lost and the subsequent recovery of resistance occurs faster than in normal subjects (89). A blunted bronchodilator effect of DI during induced bronchoconstriction has been found to be associated with an increase in quasi-static lung hysteresis (17, 19, 86), thus suggesting inadequate stretching of ASM with the DI. Whether the increase in quasi-static lung hysteresis is due to true pneumoconstriction, inhomogeneity of bronchoconstriction, or parenchymal distortion near contracted airways cannot be determined in vivo. Alternatively, a DI could also be effective in asthma in determining bronchodilatation, but this may not be detected by usual measurements (maximum flow and/or flow resistance) because of a faster airway re-narrowing occurring after DI, possibly due to a transient reduction of lung elastic recoil or an increase in ASM shortening velocity or both (50).

The importance of DI in modulating airway narrowing was particularly stressed in a study in which airway responsiveness to methacholine was determined during a period of prohibition of DIs (97). The finding of a similar dose-response curve in normal and asthmatic subjects was interpreted as suggesting that the major reason for AHR was not a different response of ASM but the lack of ability to distend constricted airways by DI. Subsequent studies that used different measurements of airway caliber found that a difference in dose-response curve existed between asthmatic and normal subjects even when DIs were prohibited (15, 20), although such a difference was further increased with the DIs taken after each dose of methacholine (15). In conclusion, AHR in bronchial asthma appears to result not just from the inability of the DI to distend the airways but also from an intrinsic exaggerated reaction of the ASM to tracheal stimulation. An interesting question that has been the object of recent debate is whether DI causes bronchodilatation or bronchoprotection. Multiple DIs taken before a constrictor agent have been shown to blunt the response to a constrictor agent in healthy but not in asthmatic subjects (60, 73), thus suggesting the lack of a putative bronchoprotection mechanism in disease. This hypothesis has been, however, downsized in a recent study in rabbits (45) and not confirmed in a human study (31) in which functional parameters not preceded by a full inflation, such as specific airway conductance and partial forced expiratory flows, were used instead of FEV1. Specifically, in the latter study, a series of DIs taken before the airways were exposed to methacholine was unexpectedly associated with a significant decrease of the functional parameters not preceded by a full lung inflation similarly in asthmatic and healthy subjects compared with when DIs were avoided, thus suggesting paradoxical bronchoconstriction. It is tentatively speculated that a series of DIs before constriction is able to put the ASM in a condition to generate more force and achieve greater shortening, thus resulting in greater narrowing independent of the presence of disease. In healthy subjects, however, the series of DIs taken before inhalation of methacholine was capable of limiting the decrease in FEV1, although this is unlikely a sign of bronchoprotection.

Effect of tidal stretching. In isolated ASM, the force induced by a contractile agonist is significantly less in the presence than in the absence of cyclic stretching (39). Both in animals and in humans, increasing tidal volume and/or breathing frequency modulate the degree of airway narrowing induced by chemical agents (12, 89, 94, 95). This effect of cyclic stretching on the capacity of ASM to generate force and to shorten has been explained by an effect on cross-bridge cycling (40) or by a continuous adjustment of the contractile filaments within the cell scaffold (95). The molecular mechanisms and the experimental data supporting these theories, which are not mutually exclusive, are described in detail in another review of this series (44a).

PRACTICAL IMPLICATIONS

Choice of physiological measurements. The parameters most widely used in clinical practice to assess lung
function are the FEV\textsubscript{1} and the forced vital capacity (FVC). Common patterns observed during broncho-provocation tests vary between a decrease in FEV\textsubscript{1} with no decrease in FVC and a simultaneous decrease of both (83). A prevalent decrease in FEV\textsubscript{1} is commonly deemed to reflect airway narrowing at the choke point, which is generally located in rather large bronchi. The decrease in FVC, in contrast, is thought to reflect airway closure (43), without any consistent predilection for the small or large airways (62, 85). It has been suggested that an exaggerated fall of FVC during induced bronchoconstriction may help identify patients at risk of severe attacks (43), thus making this measurement recommendable on bronchial challenges.

Pulmonary resistance (R\textsubscript{L}) and dynamic elastance have been measured more frequently in animals than in humans because their measurements require the positioning of an esophageal balloon (89). R\textsubscript{L} includes both airway and tissue resistance. Correction for lung volume is problematic because airway and tissue resistance vary in opposite directions with it. Furthermore, tissue resistance and thus R\textsubscript{L} are greatly dependent on breathing frequency (18), which should be therefore carefully controlled.

Differences may be occasionally observed between dose-response curves to constrictor stimuli with the use of FEV\textsubscript{1} and other indexes of airway caliber. These differences may in part reflect different sensitivities to airway caliber occurring at different sites of the bronchial tree (48) but even more the effect of the full lung inflation required for spirometric measurements. For diagnostic purposes, spirometry is preferable in that it amplifies the differences between asthmatic patients (in whom DIs have no effect) and healthy subjects (in whom DIs have a bronchodilator effect).

Effect of lung inhomogeneity. From an anatomic point of view, the lung is a very inhomogeneous system; however, from a mechanical point of view, it behaves rather homogeneously. For the sake of simplicity, airway narrowing heterogeneities are classified as occurring along the airways (axial heterogeneities) or among anatomically similar airways (parallel heterogeneities). Although an exhaustive description of all lung and airway inhomogeneities would be almost infinite and is certainly beyond the scope of this review, it must be recognized that axial and parallel heterogeneities in reaction to a given constrictor stimulus are central with respect to the functional phenotype of the response (70).

Assessment of lung inhomogeneities with forced expiratory flow is not sensitive enough for axial and serial heterogeneous distribution of airway narrowing to cause a decrease in maximal flow only above a given threshold. The reason for this is that nonobstructed airways can carry extra flow, thus tending to maintain flow near normal values (flow interdependence) (75). Nevertheless, forced expiratory flows may help detect serial inhomogeneities if they are measured during maneuvers initiated from different lung volumes (17, 19, 86, 89). Similar evidence is reported with gases of different density (23). With the use of R\textsubscript{L} and dynamic elastance, studies have documented axial and serial inhomogeneous distribution of airway narrowing, including airway closure (7, 44, 59, 70, 85, 107).

Clinical interpretation of dose-response curves. In healthy subjects, the dose-response curve is characterized by a modest functional response that plateaus at high doses of agonist. In contrast, in bronchial asthma, the curve is typically shifted leftward and upward. The former feature embodies the concept of airway hyperreactivity, which is assessed from the dose that causes 50% of maximal response (ED\textsubscript{50}). Because ED\textsubscript{50} may not be measurable in disease due the absence of a plateau of response, the provocative dose or concentration of the agent (PD or PC, respectively) that determines a given response in clinical settings is taken as a surrogate of AHR. Yet, it is clear that PD or PC may be totally independent of maximal response and cannot be taken as pure indexes of airway sensitivity. Numerous diseases may lead to a leftward shift of the bronchial dose-response curve, such as bronchial asthma, viral upper respiratory tract infections, chronic obstructive pulmonary disease, bronchiectasis, rhinitis, and chronic heart failure (3, 22, 30, 63, 112, 116). Therefore, in clinical practice, a simple decrease of PD or PC cannot be interpreted as specific to bronchial asthma (29).

The upward shift of the dose-response curve depicts the degree of airway hyperreactivity and reflects the exaggerated response of the airways to a constrictor stimulus and ultimately airway closure. It is thought to be the result of increased ASM shortening, which in turn is due to either greater ASM force or higher velocity of shortening when the loads are reduced in disease (72, 79, 110). No studies have been able to identify the upward shift of the bronchial dose-response curve in diseases other than bronchial asthma. Therefore, airway hyperreactivity appears to be a specific marker of bronchial asthma (72, 116). It would be desirable to document the absence of plateau in clinical practice to assist the diagnosis, but direct evidence is hampered by the fact that induced excessive bronchoconstriction may be harmful and unacceptable to the patients. To partially overcome the problem, the decrease in FVC during a bronchial challenge has been proposed as an index capable of detecting exaggerated narrowing for it reflects the extreme form of it, i.e., airway closure (43). The fact that it somehow correlates with therapeutic aspects of the disease (43) would confer to this measurement a role worthy of greater attention than has been received until now. Another approach is based on the assessment of the bronchodilator effect of DI, which is expected to be impaired in asthma through the same mechanisms that determine exaggerated narrowing (17, 87).

Inflammation, remodeling, and other potential causes of AHR. The paradigm that airway inflammation and remodeling cause AHR in asthma stems from the reported relationship between inflammatory cells and AHR (11, 109). The concept that one or more cells cause AHR is a very attractive and simple one; however, the relationship between inflammation and AHR...
CONCLUSIONS AND FUTURE DIRECTIONS

Because inflammation is capable of affecting each of these factors, its potential role in determining and sustaining AHR is everything but minor. With this in mind, treating the airways as a simple tube that narrows when exposed to a constrictor agent is about as naive as assuming that a single or few inflammatory cells are capable of causing asthma. The emerging conclusion is that what we call AHR is nothing but the result of a complex mathematical equation made of a variable combination of multiple elements interacting with each other and possibly changing over time. Lumping all these variables together is a real challenge if we wish to understand the mechanisms of AHR, but perhaps it is the only realistic approach that offers directions for future effective treatment.

REFERENCES


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