

Review article

Methacholine provocation test for diagnosis of allergic respiratory diseases

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Airway hyperresponsiveness is regarded as a characteristic feature of bronchial asthma, but it may also be present in other allergic or nonallergic respiratory disorders. The method most widely used to assess airway responsiveness is the methacholine inhalation challenge (1, 2), which mimics the effect of endogenous acetylcholine on airway smooth-muscle muscarinic receptors of the M_3 subtype. The major advantage in using methacholine instead of acetylcholine for bronchial challenge lies in its greater resistance to degradation by cholinesterase, which allows one to obtain cumulative dose-response curves.

The molecular mechanism by which methacholine causes airway narrowing is by activating the M_3 -Gq protein coupled receptors and the connected IP_3 pathway, an effect which ultimately results in Ca^{2+} release from intracellular stores and contraction. The mechanisms by which methacholine causes the airways of some individuals to constrict more than others are complex and still largely unknown. This complexity may in part explain the relatively low specificity of this test for the diagnosis of bronchial asthma. In this review, the practical relevance of the different determinants of airway response to methacholine will be discussed, with particular attention to those that may account for the presence of airway hyperresponsiveness in diseases other than asthma.

Mechanisms of airway narrowing

Upon application of a constrictor stimulus, the airway

smooth muscle generates a force, the magnitude of which depends on several factors. Among these are the interaction between receptor and agonist, the airway smooth-muscle contractile properties, and the amount of airway smooth muscle in the airway wall. These factors cannot be separated *in vivo* by analysis of the dose-response curve to methacholine.

In vitro, the descriptors of the dose-response curve to a constrictor agonist are the maximal response and the position of the curve, the latter being commonly reflected by the dose causing 50% of maximal response (ED_{50}). Position and maximal response are believed to reflect different mechanisms of response, which are briefly summarized in Table 1. *In vivo*, a reliable estimate of ED_{50} in the methacholine dose-response curve is difficult, as a true maximal response is seldom obtainable. The parameter used in clinical practice to report the response to methacholine is the threshold dose causing a predetermined change in a parameter reflecting airway caliber, most commonly a decrease of FEV_1 by 20% (PD_{20}). This parameter cannot be taken as a surrogate of ED_{50} , as it reflects both maximal response and the position of the dose-response curve. The difference between threshold dose and ED_{50} is better illustrated in Fig. 1. Therefore, whether airway hyperresponsiveness relies on abnormal transmembrane receptor-mediated response to agonist(s) cannot be determined from *in vivo* studies. In any case, the similarity of bronchoconstrictor response to different agonists does suggest that airway hyperresponsiveness is not sustained by the abnormality of a particular force-producing pathway. This observation

also suggests that the major causes of airway hyperresponsiveness are related to an abnormal response of airways rather than to an abnormal stimulation by inflammatory mediators.

There is experimental evidence that the maximum degree of airway narrowing in response to methacholine is greater in asthmatic than in normal subjects (3, 4). This feature can be modeled by assuming an increased airway smooth-muscle mass of the same magnitude as morphometrically determined in the airway walls of asthmatic subjects (5), suggesting that an increase in force-generation capacity by airway smooth muscle is an important determinant of airway hyperresponsiveness in asthma. Furthermore, the level of response to methacholine plateaus can be increased or decreased in normal individuals by simply decreasing or increasing lung volume, respectively (6). This suggests that the magnitude of airway narrowing depends on the external load against which the airway smooth muscle operates *in vivo*, thereby explaining the occurrence of airway hyperresponsiveness in diseases or conditions characterized by a decreased lung elastic recoil (pulmonary emphysema) or breathing at low lung volume (as in obesity and pregnancy).

Effects of allergic inflammation

Airway inflammation and airway hyperresponsiveness are both fundamental parts of the definition of bronchial asthma (7). From *in vitro* studies showing that several inflammatory mediators may enhance airway smooth-muscle contraction, it was postulated that the primary abnormality in the asthmatic airways is a particular kind of airway inflammation that gives rise to airway hyperresponsiveness. Accordingly, there was a great effort in the 1980s to demonstrate a strong relationship between the presence of inflammatory cells and airway hyperresponsiveness (8–11). If such a link could be demonstrated, the degree of airway hyperresponsiveness could be used as an index of airway inflammation. However, the relationships between baseline airway inflammation and hyperresponsiveness were either very weak (11) or absent (12). In particular, airway hyperresponsiveness was found in a number of asthmatic subjects even in the absence of airway inflammation, as assessed by bronchoalveolar lavage, bronchial biopsy, or induced sputum analysis (12). These observations suggest that the presence of inflammatory cells and mediators in the airways is not necessary for airway hyperresponsiveness to manifest itself. However, it has been found that subjects with allergic rhinitis without asthma may develop an airway inflammation that is indistinguishable from that observed in asthmatics, even though the former's airway responsiveness is not abnormal (13). This

observation suggests that the presence of inflammatory cells in the airways is not sufficient to cause airway hyperresponsiveness.

The lack of significant correlation between airway inflammation and airway hyperresponsiveness does not exclude a link between the two. In the first place, airway responsiveness increases in asthmatics when airway inflammation is primed by acute exposure to sensitizing allergen, either naturally during the pollen season or experimentally by allergen inhalation challenge (14). Second, subjects suffering from allergic rhinitis during the pollen season may develop an allergic inflammation that is associated with airway hyperresponsiveness (15). Third, *in vitro* studies (16, 17) indicate that allergic sensitization may alter airway smooth-muscle contractile properties to an extent that may theoretically influence airway responsiveness *in vivo*. Finally, sustained airway inflammation may result in airway wall remodeling, which has been regarded as a major cause of airway hyperresponsiveness (18).

Effects of airway wall remodeling

All layers of the airway wall (19) have been found to be thicker than normal in asthma, due to collagen deposition, glandular hypertrophy, persistent inflammatory cell infiltration, edema, airway smooth-muscle hypertrophy, and hyperplasia. Although it is well accepted that airway hyperresponsiveness is somehow related to airway remodeling, the relative role of each individual component is still uncertain.

Thickening of the airway smooth-muscle layer is characteristic of bronchial asthma, even though it may also occur to a lesser extent in chronic obstructive pulmonary disease (COPD). Even though there is no proof that an increase of smooth-muscle mass is directly associated with a proportional increase of force generation capacity, it is generally accepted that airway smooth muscle plays an important role in airway hyperresponsiveness (5). However, both animal models and postmortem studies suggest that abnormalities in smooth-muscle contractility may exist in asthma (20), although evidence for this in live human subjects is lacking.

For purely geometric reasons, thickening of the wall layer internal to the airway smooth muscle (mucosa) amplifies the effect of smooth-muscle linear shortening on airway caliber (21). Therefore, any condition characterized by mucosal inflammation may be associated with airway hyperresponsiveness, independent of the type of infiltrating inflammatory cells.

Another aspect of airway wall remodeling that has been regarded as a characteristic feature of bronchial asthma is subepithelial fibrosis (22), which results from the accumulation of fibronectin, tenascin, and collagen (types I, III, and V) in the lamina reticularis (23). Unlike

Table 1. Putative determinants of dose-response curve to methacholine (MCh)

	Position (ED_{50})	Maximal response
<i>In vitro</i>	MCh concentration and metabolism	Airway smooth-muscle contractility
<i>In vivo</i>	Access barriers to M_3 receptors	Airway smooth-muscle contractility
	Access barriers to M_3 receptors	Airway smooth-muscle mass
	Removal by blood	Magnitude of elastic loads
	Metabolizing cells	Airway wall thickening
	Binding to passive sites	

the thickening of mucosa and smooth muscle, the pathophysiologic significance of subepithelial fibrosis is not clear (24). In theory, subepithelial fibrosis may have opposite effects depending on its combination with other abnormalities of the airway wall. By itself, an increase in the amount of collagen should increase airway stiffness, thus providing an additional load on airway smooth muscle and limiting its linear shortening (25). In the presence of mucosal thickening, however, subepithelial fibrosis may decrease the number of mucosal folds that form upon airway smooth-muscle shortening, an effect which would enhance airway narrowing (26). Indeed, weakly positive correlations have been occasionally reported between subepithelial fibrosis and airway hyperresponsiveness in asthma (27, 28).

In a more recent study, however, Milanese et al. (29) found a negative correlation between airway hyperresponsiveness and reticular basement membrane thickness in asthma. Furthermore, subjects with perennial rhinitis also had subepithelial fibrosis that was indistinguishable from that observed in asthmatics, although airway hyperresponsiveness was not present. Both these observations are in keeping with the theoretical prediction that increased collagen deposition tends to increase airway wall stiffness, thus providing additional internal load on airway smooth

muscle and limiting its linear shortening. Additional support for this interpretation comes from the observation that airway hyperresponsiveness in subjects with seasonal rhinitis was occasionally present during the pollen season, but the thickness of the reticular basement membrane was in the range of normality (29).

Finally, thickening of the airway wall layer external to the airway smooth muscle (adventitia) may be caused by inflammatory cell infiltration and edema. As the integrity of this region is essential for the transmission of the distending force from the lung to the airway wall, it can be postulated that abnormality of this layer may cause unloading of the airway smooth muscle, thus resulting in an increase of its shortening and airway narrowing (30).

External modulation of airway narrowing

The first report that the degree of airway narrowing can be modulated by external forces was published in 1961 by Nadel & Tiernay (31), who showed that a maneuver of deep inspiration attenuates airway narrowing in normal subjects. Only 15 years later, it was demonstrated that the effect of deep inspiration is different between normal and asthmatic subjects (32), and that this may profoundly affect the assessment of airway responsiveness (33). Indeed, Fish et al. (33) showed that with FEV_1 , a measurement that requires a deep inspiration, the dose-response curve to methacholine of hay fever subjects was flatter than that of asthmatic subjects but indistinguishable from that of normal subjects. In contrast, by specific airway conductance ($sGaw$), a measurement that does not require deep inhalation, the dose-response curve to methacholine of hay fever subjects was steeper than that of normal subjects but indistinguishable from that of asthmatic subjects. Thus, $sGaw$ appeared to be more sensitive than FEV_1 in the assessment of allergic diseases, but less specific for asthma. Although $sGaw$ and FEV_1 may reflect airway caliber at different levels of the bronchial tree, it is reasonable to assume that the above differences in dose-response curves are at least in part due to the different volume histories preceding the measurements of FEV_1 and $sGaw$.

Several studies published in the 1980s and 1990s have confirmed that a deep inspiration taken shortly after the inhalation of a bronchoconstrictor substance can dilate

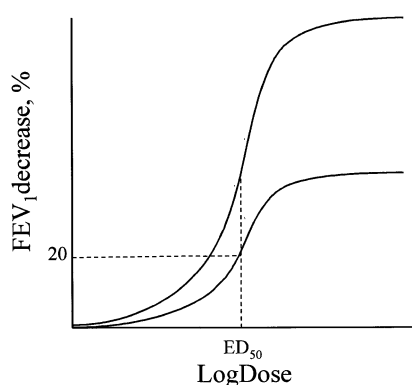


Figure 1. Schematic dose-response curves to bronchoconstrictor agonist. For each dose-response curve, dose causing half-maximal response (ED_{50}) is determined by crossing with vertical broken line, and dose causing 20% decrease of FEV_1 (PD_{20}) by crossing with horizontal broken line. Note that ED_{50} is same for both curves, whereas PD_{20} of curve with greater maximum response is less.

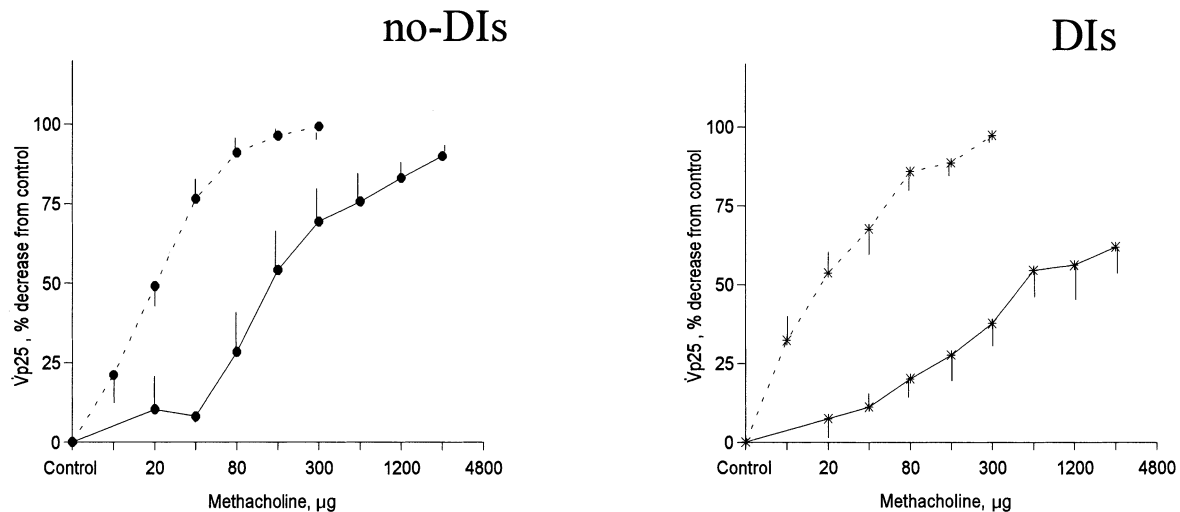


Figure 2. Dose-response curves to methacholine during prohibition of deep inhalations (no-DIs) and with five deep inhalations between dose steps (DIs) in 16 asthmatic (broken lines) and eight normal (continuous lines) subjects. $\dot{V}_{p25}$ is forced expiratory flow at 25% of control vital capacity determined plethysmographically. Data are mean $\pm$ SE. Modified from Brusasco et al. (39).

the airways in healthy subjects, but this effect is reduced or even reversed in asthma (11, 34–36). Experimental evidence has shown that the ability of deep inspirations to dilate constricted airways depends on the mechanical interdependence between airways and lung parenchyma (36, 37).

The relevance of the external modulation of airway narrowing to the assessment of airways hyperresponsiveness was again pointed out in 1995 by Skloot et al. (38), who found that the prohibition of deep inspiration during a methacholine challenge caused the concentration-response curve of healthy subjects to become indistinguishable from that of asthmatic subjects. This observation led them to conclude that bronchial hyperresponsiveness in asthma is a problem of limited smooth-muscle relaxation with deep inspiration rather than ease of constriction. In that study, however, changes in airway caliber were estimated by a parameter

that is not totally independent of changes in lung volume. Indeed, similar studies using changes in flow at the same absolute lung volume did not confirm the findings of Skloot et al. (39, 40). In particular, it was found that airway responsiveness to methacholine was greater in asthmatic than in normal subjects even when deep inhalations were prohibited (Fig. 2), suggesting that the determinants of airway hyperresponsiveness to methacholine in asthma are both the ease of constriction and the limited bronchodilation with deep inspiration (40). Nevertheless, this difference was much greater when repeated deep inspirations had been taken between dose increments. This was because deep inspirations shifted to the right the dose-response curve to methacholine of healthy, but not of asthmatic, subjects. This corroborates the opinion that the lack of sensitivity to increase in lung volume is an important characteristic of asthmatic airways.

In theory, repeated deep inspirations can attenuate airway narrowing by two mechanisms, namely, bronchodilation and bronchoprotection. These two effects are illustrated in Fig. 3. When exposed to a constrictor stimulus, airway smooth muscle generates a force that tends to decrease airway caliber. Every time lung volume is increased, a distending force is applied to the airway wall. The magnitude of this force depends on the degree of interdependence between airways and lung parenchyma and the magnitude of lung volume excursion. Therefore, the ability to dilate constricted airways with deep inspirations depends on the relative magnitude of the above opposing forces, i.e., the constrictor force generated by the airway smooth muscle and the dilator force provided by the lung elastic recoil. Furthermore, there is experimental evidence that repeated cyclic stretching maintains the airway smooth muscle in a relaxed status (41) and

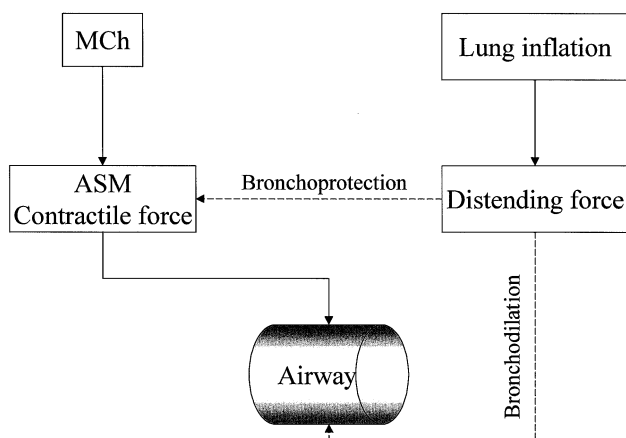


Figure 3. Mechanisms for bronchodilator and bronchoprotective effects of deep inhalation. Continuous arrows indicate enhancing effects, and broken arrows inhibiting effects. MCh: methacholine.

lowers its force-generation capacity (42). These effects are proportional to the amplitude of volume excursions and may account for a reduced bronchoconstrictor response when deep inhalations are performed before the administration of the agonist.

The first report suggesting that deep inspiration may have bronchoprotective effects was by Malmberg et al. (43), who showed in normal subjects that shortening of the interval between methacholine dose-increments attenuated the decrease in FEV₁. Furthermore, they showed that this effect was greater when deep inspirations were taken before than after inhalation of methacholine. More recent work (44) has confirmed this finding and also shown that the bronchoprotective effect increases with the number of deep inspirations in normal subjects, but is totally absent in asthma (45). The limitation of these studies is that the changes in airway caliber were assessed by FEV₁, which needs a deep inhalation to be measured. Therefore, it cannot be distinguished whether deep inhalations before the administration of methacholine did reduce the force-generation capacity of the airway smooth muscle, or whether they made the airways more distensible with the deep inspiration that precedes FEV₁ measurement. Preliminary data from our laboratory suggest that the latter may be the case, as the protective effect of deep inspirations before challenge was apparent with FEV₁, but not *s*Gaw or forced expiratory flow from a partial flow-volume curve (46).

Practical implications

The knowledge that the bronchoconstrictor response to methacholine may be modulated by external factors, such as volume history, has important practical implications. Firstly, bronchial challenges to be compared should be performed by exactly the same standardized protocol. Indeed, whatever is the intimate mechanism of deep breaths (bronchoprotection or bronchodilation), the two protocols recommended by the recent American Thoracic Society guidelines (2) should be expected to give different results. Specifically, using inhalation of methacholine by five inspiratory capacity maneuvers (47) should be expected to produce larger PD₂₀ values in normal subjects than a protocol of tidal breathing inhalation (48). The available experimental data show that this is actually the case (49). Secondly, the choice of lung-function measurements to assess changes in airway caliber should be made on the basis of the clinically relevant question to be answered.

If a greater sensitivity of the test is sought, as, for example, to assess whether allergic nonasthmatic subjects have a greater degree of airway responsiveness than normal subjects, a measurement not requiring deep breaths (such as *s*Gaw) should be used. On the contrary, if a greater specificity is sought, as, for example, to separate asthmatic from allergic nonasthmatic subjects, a measurement requiring deep breaths (such as FEV₁) is preferable.

There is wide agreement that methacholine challenge is helpful in the diagnosis of asthma when baseline spirometry is normal in subjects with a suggestive history (50), i.e., with symptoms, such as wheezing, dyspnea, chest tightness, and cough, that occur upon exposure to allergen and cold air breathing, at the workplace, during respiratory infections. Indeed, the optimal diagnostic value of the methacholine test is when these symptoms are present and when the pretest probability of asthma is 30–70% (51). This requirement is dictated by the necessity to compensate for the low specificity of the test, as, owing to the complexity of the mechanisms of airway narrowing already described, methacholine challenge may be positive in diseases other than bronchial asthma, such as COPD (52), congestive heart failure, sarcoidosis, cystic fibrosis, bronchiectasis, and allergic rhinitis. Nevertheless, once the diagnosis of asthma has been established, the methacholine challenge may be valuable in optimizing pharmacologic treatment (53) and in monitoring changes of airway responsiveness, which may worsen during exacerbation due to exposure to allergen or upper airway viral infections, and which may improve after proper treatment (54–56). However, the strength of the methacholine challenge is its high negative predictive value, as a negative response is very unlikely to occur in asthmatic subjects, thus making it valuable in excluding the diagnosis of asthma (57).

Conclusion

The methacholine challenge is a useful tool in the diagnosis of allergic respiratory disorders, but the complexity of the physiology of airway narrowing does not allow us to make inferences about any single mechanism underlying airway hyperresponsiveness. In particular, the results of methacholine challenge cannot be taken as an index of airway inflammation, and vice versa, even though it is likely that a link does exist between airway hyperresponsiveness and airway inflammation.

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