Tidal breathing affects airway responsiveness to methacholine

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ABSTRACT: *Tidal breathing affects airway responsiveness to methacholine. F. Salerno, P. Guido, A. Spanevello.*

Asthma is characterized by increased airway responsiveness and airway inflammation. Airway hyperresponsiveness may be caused by increased airway smooth muscle contractility or by a decrease in the mechanical load that opposes airway smooth muscle contraction. Under static conditions, the equilibrium between contractility and load will determine the final airway smooth muscle length and therefore airway caliber. Because of tidal breathing, however, lungs normally function under dynamic conditions where both airway contractility and opposing load are affected. The capability of tidal breathing to appropriately modulate airway function might be the mechanism that differentiates airways of asthmatics from those of normal subjects.
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Asthma is characterized by intermittent bronchial narrowing, airway inflammation and airway hyperresponsiveness. Airway hyperresponsiveness consists in the excess of bronchoconstriction to a variety of stimuli. The mechanisms involved in the airway hyperresponsiveness are not completely understood. There are many factors, in fact, potentially responsible for the excessive airway narrowing observed in asthmatic patients including factors that increase airway smooth muscle contractility and factors that reduce the mechanical load opposing airway smooth muscle contraction [1].

Potential mechanisms of airway hyperresponsiveness

There are many potential causes of increased airway contractility in asthma. Airways of asthmatic patients show an increase in airway smooth muscle mass compared with normal subjects or chronic obstructive pulmonary disease patients and, for a given degree of muscle activation, greater airway smooth muscle mass may allow the development of greater tension and thus more airway constriction [2, 3]. Alternately, increased airway contractility may be caused by an altered orientation of smooth muscle fibers in the airway wall. The smooth muscle, in fact, is not orientated circularly around the airway, and the relationship between airway narrowing and airway smooth muscle shortening is theoretically a function of the muscle fibers' orientation in the airway wall [4, 5]. Imbalances in the adrenergic/cholinergic or in the non adrenergic/non cholinergic (NANC) system are other investigated causes of increased airway smooth muscle contractility. Airway smooth muscle of asthmatics may also have an intrinsic increased shortening capacity. Airway smooth muscle is a heterogeneous population of cells expressing different contractile and pharmacological properties. It is possible that asthmatic airways contain cells that express different contractile properties in response to a variety of stimuli including inflammatory mediators [6, 7]. Indeed, a number of studies show, as a result of sensitization, modifications in the biochemical pathways of airway smooth muscle contraction and, from the mechanical point of view, an increase in maximal shortening velocity that under dynamic conditions may affect the final degree of airway narrowing [8–11].

Airway hyperresponsiveness may be caused by a decrease in the mechanical load that oppose airway smooth muscle shortening. Airways of asthmatic patients are thicker and structurally different from those of normal subjects [12]. The anatomical alteration of the airway wall is probably the consequence of chronic airway inflammation and may affect airway mechanics. In addition, during bronchoconstriction the airway wall undergoes a change in shape that represents an additional elastic load that in asthmatic airways may be reduced because of the airway remodeling [12, 13]. Abnormal mucosal thickness and abnormal mucosal folding caused by the airway remodeling may, during bronchoconstriction, be other reasons for decreased mechanical load. When airways narrow, in fact, the mucosa buckles forming folds that

affect airway lumen patency and create a mechanical load that further opposes airway smooth muscle shortening [14, 15]. The amount of the extra mechanical load will depend on the numbers and shape of the folds and on the thickness of the mucosa. Alterations in the airway-parenchymal interdependence and in the mechanical properties of the lung parenchyma are other potential causes of airway hyperresponsiveness. The effect of lung parenchyma on bronchial narrowing is mediated by the alveolar attachments that stretch the intraparenchymal airways. Moreover, during bronchoconstriction the parenchyma surrounding the airways undergoes stretch and distortion creating an extra mechanical load that will be a function of the amount of airway narrowing and the capacity of the surrounding parenchyma to resist stretch and deformation. A modification of the elastic and hysteretic properties of the lung parenchyma may, therefore, contribute to the excessive airway narrowing observed in asthmatic lungs as compared to normal ones [16–18]. Indeed, it has been shown in an animal model that when lungs are instilled with elastase, a protease that likely alters the mechanical properties of the lung parenchyma, airway narrowing following a smooth muscle agonist challenge is enhanced [19]. In addition, when lung volume is decreased under functional residual capacity, a condition where the tethering effect of lung parenchyma on airways is less, the degree of methacholine-induced bronchoconstriction is greatly increased, whereas ventilation at higher lung volume decreases the amount of induced bronchoconstriction [20, 21].

Airway inflammation and airway hyperresponsiveness

Airways of asthmatic patients show a variable degree of inflammation. Studies carried out on biopsies specimens, bronchoalveolar lavage (BAL) or induced sputum show eosinophilic inflammation and in some cases of fatal asthma neutrophilic inflammation [22–24]. Other histological features of asthmatic airways are thickening of the airway wall, epithelial damage, thickening of the basal membrane, edema, goblet cell hyperplasia, airway smooth muscle hypertrophy, and intraluminal mucus [22]. Some investigators have found a relationship between airway hyperresponsiveness and airway inflammation, while others have failed to find any [25–27]. The relationship between airway responsiveness and airway inflammation remains a controversial issue, especially in view of the fact that variable degrees of eosinophilic airway inflammation are present in pathological conditions, i.e. allergic rhinitis, that are unassociated with airway hyperresponsiveness.

There are many possible mechanisms by which airway inflammation may cause airway hyperresponsiveness. Inflammation might have a direct effect on responsiveness, due to the release of mediators from the inflammatory cells causing increased airway smooth muscle contractility [26]. For instance, it has been shown that there is an increased airway smooth muscle velocity of shortening after passive sensitization. In this experiment, human bronchial rings incubated with human serum from atopic individuals increased their maximal contraction capacity and their maximal velocity of shortening [28]. An increased velocity of shortening may cause excessive airway narrowing under dynamic conditions. Alternatively, inflammatory mediators such as chemokines and cytokines may potentially alter structurally the airways and the parenchyma. The altered mechanical properties of airways and parenchyma, caused by the anatomical remodeling, may affect airway responsiveness to methacholine by modifying the mechanical load that opposes airway smooth muscle shortening [1, 12]. It is possible that the remodeling of the airways, probably triggered by the airway inflammation, may persist or evolve independently of the actual state of airway inflammation. If this is the case, a direct relationship between the degree of airway inflammation and the degree of airway responsiveness will be more difficult to find. Finally, airway inflammation might cause an alteration in the modulating effect of the lung volume dynamic maneuvers on airway smooth muscle contractility. The mechanism could be either an intrinsic alteration of the contractile properties of the smooth muscle or the mechanical dissociation between the lung parenchyma and the airway smooth muscle.

Airway hyperresponsiveness and tidal breathing

Under static conditions the final airway caliber following airway smooth muscle activation is determined by the equilibrium between airway smooth muscle contractility and load. Lungs, however, normally function under dynamic conditions. During tidal breathing, because of the forces of interdependence between the airways and the parenchyma, the changes in lung volume cause airway oscillation, and subsequently airway smooth muscle stretching and unloading.

When the airways are stimulated under dynamic conditions less constriction is obtained. In in vitro experiments, GUNST has shown that length oscillations decrease active force in isolated tracheal smooth muscle strips [29]. In this experiment, isolated strips of tracheal smooth muscle were oscillated at different frequencies and different amplitudes. Always, oscillated airway smooth muscle developed less active force as compared to isometric conditions. The effect of the dynamic oscillations on contractility was dependent on the rate of the airway smooth muscle stretching and unloading. The same group has shown, on isolated bronchi, that submaximal contractile stimuli produced airway closure under isobaric conditions, whereas cyclic volume oscillations decreased contractility [30]. In a following study it has been shown that in isolated canine lobes, a methacholine challenge causes, under static conditions, airway closure (at a transpulmonary pressure within the tidal range) [31]. Airway closure

did not occur, however, when the experiment was carried out during tidal oscillations. Moreover, in this experiment, the degree of induced bronchoconstriction in an isolated lobe, was higher than that observed in the whole lung, suggesting that factors other than the simple airway smooth muscle stretch may account for the limited maximal bronchoconstriction observed in the intact animal. The authors propose that the local release of humoral factors might limit maximal bronchoconstriction *in vivo*, or alternatively, that the airway smooth muscle stretch may cause bronchodilation (or protect against bronchoconstriction) *in vivo* by the release of bronchodilators or by the activation of neuronal pathways.

Of note, also the mechanical load that opposes airway smooth muscle contraction is affected by lung volume oscillations. During lung volume oscillations, in fact, the elastic recoil of both airways and lung parenchyma, because of their hysteretic properties, is altered [32]. Moreover, the effect of lung volume oscillations on elastic recoil is more pronounced after induced bronchoconstriction as the hysteretic properties of the lung parenchyma and airways are increased during airway smooth muscle activation [33, 34].

It has been hypothesized that the lung volume oscillation that occurs during tidal breathing may protect the airways from reaching excessive narrowing during contractile stimulation *in vivo* [35]. Indeed, modifications in tidal volume amplitude and frequency have been shown to modulate airway narrowing during agonist challenge in animal models *in vivo*. SHEN *et al.* have demonstrated in mechanically ventilated rabbits that the degree of airway narrowing, after induced bronchoconstriction, is highly dependent on whether the experiment is carried out under static or dynamic conditions [36]. The intravenous infusion of methacholine, under static conditions or with a low tidal volume, in fact, caused a degree of airway narrowing that was virtually eliminated at a tidal volume of 20 ml/kg. In another experiment, the effect of tidal volume amplitude on induced airway narrowing has been shown to be independent of modifications in mean bronchial pressure [37]. In fact, when the end expiratory pressure is kept constant and tidal volume is modified mean bronchial pressure changes, and a change in mean bronchial pressure may alter the tethering effect of lung parenchyma on the intraparenchymal airways and therefore airway caliber. In this study, carried out on open chest dogs, the two lungs from the same animal were independently ventilated at two different tidal volume amplitudes but the same end expiratory pressure. The lungs ventilated with a lower tidal volume always showed a greater increase in lung elastance and lung resistance in response to the intravenous infusion of methacholine. When the experiment was repeated adjusting throughout the protocol the end expiratory pressure so that, on average, the mean bronchial pressure was maintained constant, the effect of tidal volume amplitude on airway resistance and lung elastance was similar [37].

There has been great effort made in recent years to try and understand the mechanisms underlying airway behavior under dynamic conditions. Although other mechanisms cannot be excluded, airway smooth muscle is likely to be the structure responsible for the airway behavior under dynamic conditions. FREDBERG *et al.* have proposed that the cyclical length change of the airway smooth muscle would interfere with the actin-myosin bridge formation and, therefore, force generation [35]. The continuous variation in muscle length would impede airway smooth muscle from reaching the latch state, a condition characterized by low energy consumption and low frequency of actin myosin formation, and, from the mechanical point of view, by high stiffness, high force generation and low velocity of shortening. In this model, myosin interaction with actin tends, normally, toward that present under isometric conditions. The tidal changes of muscle length, however, cause an increase in the rate of attachment/detachment between actin and myosin which if fast enough does not allow the muscle to reach static equilibrium.

PRATUSEVICH *et al.* have proposed that the efficiency of the smooth muscle contraction over a wide range of lengths is facilitated by a variation of the number of contractile units in series [38]. In this model, after a length variation of the smooth muscle, temporary force depression would occur because the contractile filaments will be moved from their optimum overlap. GUNST *et al.* hypothesized that the decreased airway smooth muscle contractility observed under dynamic conditions is a function of the plasticity of the cellular organization of contractile filaments [39]. The airway smooth muscle has the property to function over a wide range of muscle lengths, and length adaptation is proposed to result from the dynamic modulation of contractile and cytoskeletal filaments within the smooth muscle fiber [40]. This mechanism would optimize force development to the shape of the smooth muscle cell. During tidal ventilation, because of the relatively fast change in muscle length, the contractile elements will not have the time to adapt to the continuous change in length.

In conclusion, airway hyperresponsiveness, a defining feature of asthma, may be caused by excessive airway smooth muscle contractility or by the reduced mechanical load that opposes airway smooth muscle contraction. Under static conditions the airway caliber will be determined by the equilibrium between contractility and load. Airway smooth muscle, however, normally functions under dynamic conditions as the lungs and airways are normally stretched and unloaded during normal breathing. Airway behavior under dynamic conditions is profoundly different compared to under static conditions, and airway smooth muscle properties under dynamic conditions are likely to be important in the regulation of the degree of airway narrowing during airway smooth muscle activation. A failure of the protective effect of lung volume oscillations on excessive airway constriction may be the cause of airway hyperresponsiveness and asthma in humans.

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