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Hypothesis: excessive bronchoconstriction in asthma

A.M. Bramley, R.J. Thomson, C.R. Roberts, R.R. Schellenberg

Hypothesis: excessive bronchoconstriction in asthma is due to decreased airway elastance. A.M. Bramley, R.J. Thomson, C.R. Roberts, R.R. Schellenberg. ©ERS Journals Ltd 1994.

ABSTRACT: Based on the strikingly different mechanical properties of airway smooth muscle preparations of different species, we hypothesized that a decrease in the elastance of nonmuscle elements within airway walls of asthmatics reduces the load limiting smooth muscle shortening, thereby allowing excessive smooth muscle shortening and bronchoconstriction.

Full thickness, circumferentially cut, lobar bronchial preparations were obtained from one asthmatic and six nonasthmatic lobectomy subjects.

Passive tension of the asthmatic preparation was less than that for any nonasthmatic preparation at all lengths below that for optimal force generation (Lmax). Maximal isometric force generation was greater in the asthmatic specimen (2.32 g) than in the nonasthmatic specimens (0.90±0.15 g), with stress threefold higher for the asthmatic tissue. Isotonic shortening of the asthmatic preparation was strikingly greater at starting lengths less than or equal to Lmax, with maximal fractional shortening being 31% *versus* 11±2% for nonasthmatic preparations. Morphometric analysis revealed no differences in cross-sectional areas of smooth muscle for asthmatic *versus* nonasthmatic preparations.

We conclude that the reduced tissue elastance may account for the greater muscle shortening by placing a lesser load upon the smooth muscle. Airway inflammation in asthma may alter connective tissue matrix elements within airway walls leading to this decreased elastance and excessive smooth muscle shortening.

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UBC Pulmonary Research Laboratory, St Paul's Hospital, Vancouver, BC, Canada.

Correspondence: R.R. Schellenberg
UBC Pulmonary Research Laboratory
St Paul's Hospital
1081 Burrard Street
Vancouver
BC V6Z 1Y6
Canada

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Hypothesis

Decreased elastance of asthmatic airway tissue allows exaggerated smooth muscle shortening.

Rationale

The ability of airway smooth muscle preparations to shorten appears to be inversely related to the amount of extracellular matrix elements present within the preparation utilized. We have previously demonstrated that human mainstem bronchi shorten minimally (maximal fractional shortening 25%) [1] when compared with comparable preparations from the dog or pig (maximal fractional shortening 70–80%) [2, 3]. Porcine trachealis tissue contains threefold greater amounts of muscle per cross-sectional area than does that of humans. It has correspondingly less extracellular matrix between muscle fibres as well as surrounding the muscle. These porcine preparations demonstrate low elastance when compared to the human counterparts. Thus, passive tension at the length for optimal force generation (Lmax) is 5% [2] of the maximal isometric force produced for porcine airway *versus* 60% for human [1]. These observations suggest that the limited amount of shortening of human airway preparations may be due to the greater tissue elastance, which places a load on the smooth muscle, limiting shortening. The fact that preparations with much greater muscle and less extracellular matrix have substantially less elastance suggests that elements within muscle cells themselves provide minimal resistance to shortening, when compared with elements extrinsic to the muscle cells. Compatible with this assumption is the very low stiffness of isolated smooth muscle cells [4], which demonstrate high fractional shortening [5].

These observations raise the possibility that smooth muscle within asthmatic airways could shorten excessively, simply due to a decrease in the load provided by extracellular matrix elements, without the requirement for altered properties of the muscle itself. This concept is depicted schematically in figure 1. Increased shortening would be magnified if there was a greater amount of smooth muscle within the airway, as this would have less elastance than nonmuscle tissue. Although the mechanisms whereby such changes in airway elastance might occur in asthma are speculative, the mechanical consequences can be evaluated.

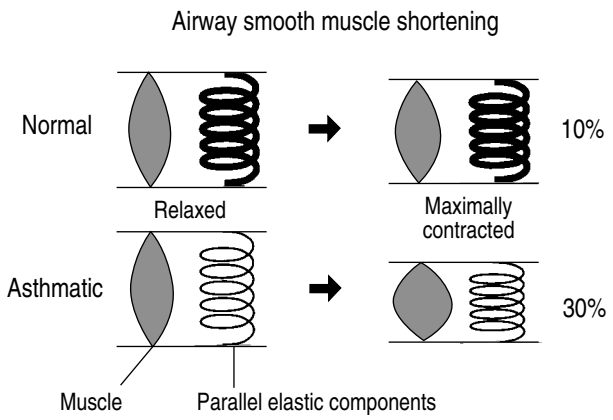

Fig. 1. – Schematic diagram illustrating how a decrease in stiffness of parallel elastic components (depicted by the springs) within airway tissue allows greater smooth muscle shortening. Percentage maximal fractional shortening values are those obtained for nonasthmatic (10%) *versus* asthmatic (30%) bronchial preparations.

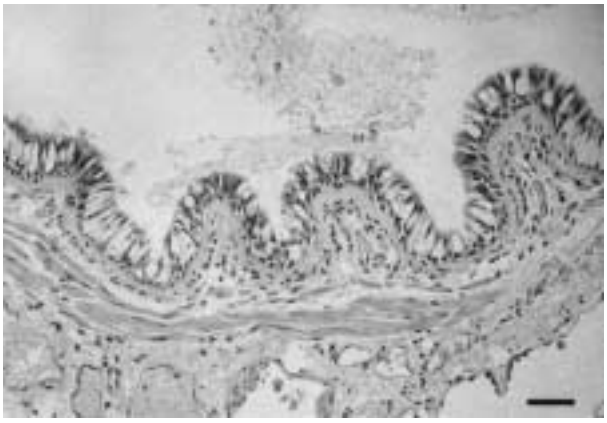

Fig. 2. – Histological section of airway from an asthmatic subject. Characteristic features of asthma include: epithelial desquamation and intraluminal mucus secretion (M); goblet cell metaplasia (G); collagen deposition below basement membrane (C); prominent smooth muscle (S); mucosal and submucosal inflammation. (magnification ×260. Scale bar = 50 μm).

Experimental evidence

Bronchial tissues from one asthmatic and six nonasthmatic patients undergoing lobectomy for lung carcinoma, were obtained within 30 min of surgery and placed in Krebs-Henseleit solution. A 1×2×5 mm full-thickness tissue strip, perpendicular to the longitudinal axis, was cut from the tumour-free bronchus and placed in an oxygenated tissue bath as described previously [1]. Measurements of muscle mechanics were performed using a special device for determining both isometric and isotonic responses of smooth muscle [2]. Length measurements were made using an optical micrometer (accurate to 0.001 cm) at increasing preloads between 0–2.5 g, and both active isometric and isotonic responses to electrical field stimulation (EFS) were obtained at each preload. The passive length-tension curve was continued until the length equalled approximately 110% of the length at which maximum force generation was obtained (Lmax). At the end of each experiment, contractile responses to acetylcholine (ACh) 10^{-4} M were obtained. For data analysis, muscle lengths were standardized by Lmax, the length at which maximum active tension (Pmax) was generated. Fractional shortening was calculated as percentage change in length/initial length at each preload. Morphometry was performed according to methods described previously [1].

The asthmatic subject, who also underwent lobectomy for carcinoma, was a 65 year old female nonsmoker with a long-standing history of mild asthma controlled with an inhaled β_2-agonist. She had never received inhaled corticosteroids and had not received oral corticosteroids within the six months prior to surgery. Forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) was 71%, with FEV_1 91% predicted and forced mid-expiratory flow (FEF_{25-75}) 58% predicted. Total lung capacity (TLC) and functional residual capacity (FRC) were normal. Allergy testing and bronchoprovocation were not performed. Histological evaluation of airways revealed characteristic features of asthma, including epithelial desquamation, intraluminal mucus, goblet cell hyperplasia, collagen deposition below the basement membrane, mucosal and submucosal inflammation, and prominent smooth muscle (fig. 2).

Passive/length tension relationships

The lobar bronchus from the asthmatic subject developed less passive tension with stretching than did the nonasthmatic lobar smooth muscle tissues. At Lmax, passive tension of the asthmatic smooth muscle was 75% of Pmax, whereas the nonasthmatic lobar tissues averaged 180% Pmax (n=6) (fig. 3). Values of absolute tension were comparable for the asthmatic and nonasthmatic lobar tissues at Lmax, but passive tension of the asthmatic tissue was significantly less at all lengths evaluated below Lmax, with none of the values falling within the range of those for the nonasthmatic tissues.

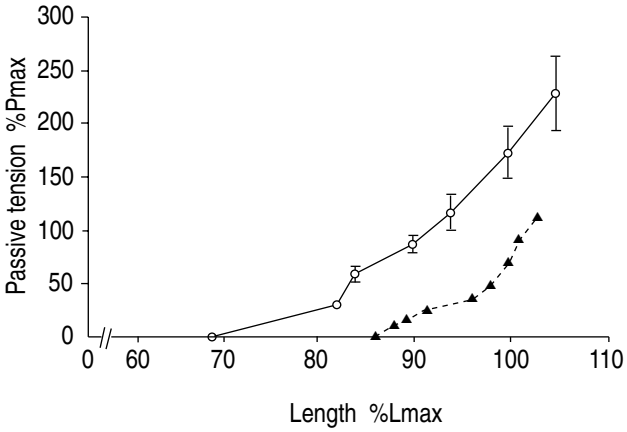

Fig. 3. – Passive length-tension characteristics of human nonasthmatic lobar bronchi (n=6) (—○—) compared with an asthmatic lobar bronchus (- -▲- -). Passive tension expressed as percentage maximum isometric force generated (%Pmax) and length as percentage length at which maximal isometric forced was obtained (%Lmax). Data for nonasthmatic tissues are presented as mean±SEM.

Table 1. – Parameters for asthmatic and nonasthmatic lobar bronchial smooth muscle

	Asthmatic	Nonasthmatic
Maximal fractional shortening %	31	11±2
Total tissue area mm^2	11.6	7.6±1.5
Muscle area mm^2	0.24	0.28±0.06
% smooth muscle	2.0	4.0±0.9
Force generation g	2.32	0.90±0.15
Stress $kg \cdot cm^{-2}$	0.98	0.30±0.05

Data are presented as mean±SEM (n=6) for nonasthmatic bronchi.

Isometric force

Maximal isometric force generation was significantly increased (2.32 g) in the asthmatic tissue compared with nonasthmatic tissues (0.9±0.15 g) (table 1). This enhanced response was observed at all starting lengths. Force per cross-sectional area (stress) was markedly greater for the asthmatic tissue (0.98 $kg \cdot cm^{-2}$) *versus* nonasthmatic tissues (0.3±0.05 $kg \cdot cm^{-2}$).

No changes in characteristics of the responses to EFS were noted. The asthmatic tissue demonstrated equivalent nonadrenergic, noncholinergic inhibition compared to the nonasthmatic preparations.

Isotonic shortening

Maximal fractional shortening occurred at similar starting lengths (~80% Lmax) in both the asthmatic and nonasthmatic lobar bronchial tissues (fig. 4). However, maximal fractional shortening was significantly greater in the asthmatic tissue (31%) compared with nonasthmatic tissues (11±2%). Shortening was greater for the asthmatic tissue at all starting lengths below Lmax, and was 16% compared with 6% for nonasthmatic tissues at Lmax. Maximum shortening to exogenously administered ACh was 33% for the asthmatic tissue *versus* 14±2% for the nonasthmatic.

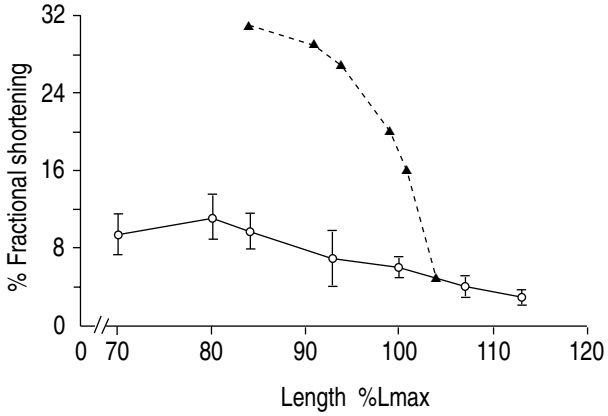

Fig. 4. – Fractional shortening of human asthmatic lobar bronchi (-▲-) and nonasthmatic lobar bronchi (n=6) (—○—) at various starting lengths during isotonic preloaded contractions. Data are presented as mean±SEM. %Lmax: percentage at which maximal isometric force was obtained.

Morphometrics

Results of the morphometric evaluation of the cross-sectional areas of muscle and total tissue areas showed no difference between the amount of smooth muscle of the asthmatic compared with the nonasthmatics (table 1).

When all extracellular matrix was subtracted, the smooth muscle represented only 2% of the total tissue area.

Discussion

These results provide the first direct evidence that asthmatic airway smooth muscle shortens more than that from nonasthmatic subjects. Although these results are observations from a single asthmatic airway preparation, we feel the magnitude of the differences compared with all other lobar preparations studied is striking. Lobar preparations demonstrate significantly less shortening and higher passive tension than mainstem preparations. In our 27 lobar bronchial preparations so far studied using comparable experimental protocols, the highest value for the maximum fractional shortening was 22%, with the mean value=11±0.9%. Therefore, shortening in the asthmatic tissue (31%) was threefold greater than the mean for nonasthmatic tissues.

We found no difference in the value of smooth muscle area from asthmatic and nonasthmatic lobar bronchi, suggesting that the increase in force generation was not due to an increased amount of smooth muscle, which surprised us. Interestingly, our pathologist reported increased smooth muscle as a component of the histological evaluation in our asthmatic subject, whereas careful morphometric analysis showed this to be an inaccurate qualitative impression.

Previous studies have demonstrated increased amounts of smooth muscle in asthmatic airways using morphometric measurements [6–13]. However, these studies may have overestimated the amount of smooth muscle, due to techniques which limit the ability to exclude extracellular matrix within the measured muscle bundles. All studies utilized transverse airway sections which provide circumferential views of the smooth muscle. A qualitative comparison of circumferential *versus* cross-sectional views of muscle bundles reveals that differentiation of extracellular matrix within muscle bundles is much more difficult with circumferentially sectioned muscle bundles. Previous studies also utilized paraffin-embedding and, consequently, thick tissue sections, which further obscures the smooth muscle/extracellular matrix boundaries. Finally, these studies utilized low magnifications for measuring muscle bundle areas. This was probably done to keep entire circumferential muscle bundles in the field of view (*i.e.* the whole airway), which would result in poor resolving power with respect to the extracellular matrix elements within the bundles, which were probably included in the muscle area measurements. In our measurements, the majority of the extracellular matrix was subtracted from the total muscle bundle area when quantifying amounts of smooth muscle. It is clear that our technique provides

a more critical look at the amount of smooth muscle in airway tissue, and we would suggest that these methods be used in future studies.

It is probable that both parallel elastic elements within the airway wall and series elastic elements extrinsic to the muscle limit muscle shortening and, thus, bronchoconstriction in normal subjects, which is evidenced by the plateau of the bronchoconstrictor agonist dose-response curve [14]. The excessive bronchoconstriction noted in asthma has been suggested to reflect a loss of normal elements limiting airway narrowing [15]. Other investigators have suggested that excessive bronchoconstriction observed in asthma could be explained on the basis of the mechanical effects of thickened walls, without a need for increased muscle shortening [16, 17]. Due to the limited shortening of nonasthmatic human airway smooth muscle [1], this factor alone cannot account for the excessive bronchoconstriction noted in asthma. However, if asthmatic airway muscle shortening is increased threefold or more, large changes in airway resistance would occur, which would be further accentuated by thickened airway walls. The increased isometric force noted with the asthmatic preparation was inexplicable on the basis of increased muscle. This could be due to enhanced contractile properties of smooth muscle intracellular events, but might also be explained by a decrease in constraining matrix elements allowing a more parallel alignment of tangential muscle fibres to the vector of the force transducer.

The mechanisms causing decreased airway tissue elastance and increased muscle shortening are unknown. Many different inflammatory mediators have been implicated in asthma, but how they might bring about changes in smooth muscle or decrease the load on the muscle remains to be determined. We would suggest that the release of protease enzymes from infiltrating inflammatory cells or resident cells could degrade extracellular matrix elements. Elastases or collagenases have been shown to be secreted by neutrophils and macrophages [18–20]. The cytokines interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α) have been shown to release collagenase from neutrophils [21, 22], and TNF-α has been measured from alveolar macrophages following bronchial allergic challenge of subjects exhibiting a late asthmatic response [23, 24]. This hypothesis is supported by our recent preliminary findings that incubation of human bronchial preparations with collagenase *in vitro* decreases the tissue elastance and increases smooth muscle shortening [25]. Alterations in subepithelial elastin, as demonstrated by others [26], might also decrease the load limiting airway smooth muscle shortening.

To summarize, we raise the hypothesis that one of the most important determinants of increased smooth muscle shortening is a decreased load on the smooth muscle. Although the specific mechanism(s) by which such changes occur in asthma remain to be defined, our results showing increased shortening and decreased airway elastance of bronchial tissue suggest that a decrease in the load provided by extracellular matrix components may be a consequence of airway inflammation in asthma. Such altered smooth muscle mechanical properties could account for airway hyperresponsiveness.

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