

**THE purpose of this study was the effect of epithelium damage on mechanical responses of airway smooth muscles under different resting tension. We performed acetylcholine (ACh) ( $10^{-5}$  M)-induced contraction on tracheal strips from 30 rabbits in five groups (0.5, 1, 1.5, 2 and 2.5 g) before and after epithelium removal. At low resting tension (0.5–1.5 g), the epithelium removal decreased the ACh-induced contractions. At 2 g resting tension, the epithelium removal increased the ACh-induced contractions of airways with intact epithelium about 20%. At 2.5 g resting tension, the elevation of contraction is about 25% ( $P < 0.01$ ). Consequently, after epithelium loss, the resting tension determines the airway smooth muscles responsiveness. In asthma, mediators such as ACh act on already contracted inflammatory airways, which results in additional increase of contraction. In contrast, low resting tension, a condition that simulates normal tidal breathing, protects from bronchoconstriction even when the epithelium is damaged.**

**Key words:** Epithelium, Airways, Smooth muscle pathophysiology, Bronchial asthma

## Airway responsiveness: role of inflammation, epithelium damage and smooth muscle tension

K. I. Gourgoulianis<sup>CA</sup>, A. Domali and P. A. Molyvdas

Department of Physiology, Medical School, University of Thessaly, Greece

<sup>CA</sup>Corresponding Author

Present Address: 22 Papakiriazi, 412 22 Larissa, Greece

Tel: (+41) 532 501

Fax: (+41) 255 420

Email: kgourg@med.uth.gr

## Introduction

The effects of lung-volume changes on airway tones can be mimicked in isolated bronchial segments *in vitro*, in which inflation–deflation cycles decrease the transmural pressure of contracted bronchi.<sup>1</sup> Studies with preparations of bronchial smooth muscles have been presented in the literature. A large range of resting tensions (0.5–2.5 g) was used in these experiments.<sup>2</sup> Resting tension determines the responsiveness of airway smooth muscles to contractile and relaxant agents. In normal human subjects, deep inspiration decreases airway resistance.<sup>3</sup> Fish *et al.* suggested that the major problem in asthma might be an impairment in the ability of inspiration to stretch the airway smooth muscle.<sup>4</sup> Cyclic stretch of airway epithelium also plays a key role in regulating inflammatory airway diseases including bronchial asthma, in which airway mechanics are altered.<sup>5</sup>

In this study, we evaluated the properties of airway smooth muscle under different resting tensions. First, we recorded the mechanical responses of smooth muscles during contraction with acetylcholine (ACh) and, second, we removed epithelium and ACh-induced contractions in a range of resting tension were also recorded.

## Materials and methods

Portions of tracheas were obtained from 30 rabbits (1–2 kg body weight) that have been previously

anaesthetized with pentothal (dose, 20 mg/kg intravenously, slowly). After immediate excision, the tracheas were placed in Krebs buffer (pH 7.4, 37°C) with the following composition: Na<sup>+</sup>, 137 mM; Mg<sup>2+</sup>, 1.1 mM; K<sup>+</sup>, 5.9 mM; Ca<sup>2+</sup>, 2.0 mM; Cl<sup>-</sup>, 123.0 mM; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 1.2 mM; HCO<sub>3</sub><sup>-</sup>, 24.9 mM; glucose, 9.6 mM. The solution was gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Muscle strips (2–3 mm) taken from the tracheas were superfused under 1 g of tension, in a bathing chamber that was continuously perfused with Krebs solution. Changes in tension were recorded on a Grass FT03C force displacement transducer and displayed on a Universal oscillograph (Harvard) recorder.

A single bolus dose of 0.2–0.5 ml of  $10^{-1}$  MACH was given to each muscle strip, to induce contraction. The preparation was then washed repeatedly and left until tension had returned to baseline. Each muscle strip was equilibrated at a resting load (1 g) for at least 60 min before starting the experiment.

We used ACh ( $10^{-5}$  M) to induce contractions at different resting tensions (0.5, 1, 1.5, 2 and 2.5 g). Each muscle strip in the same resting tension was contracted by ACh before and after epithelium removal. Experiments from six rabbits were performed for each resting tension. Epithelium was removed mechanically. ACh was purchased from Sigma.

## Results

Figure 1 shows that epithelium removal increases the ACh-induced contraction at high resting tension. At

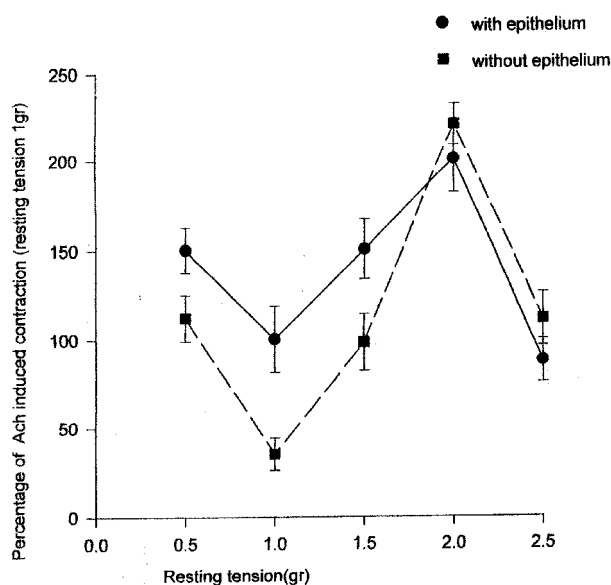


FIG. 1. Ach-induced contractions on different resting tensions.

2.5 g resting tension, the increase of contraction is 24.45% ( $P < 0.04$ ). At low resting tension, under 1.5 g, the epithelium removal decreases the ACh-induced contractions. The highest decrease was recorded at 1 g resting tension (72.30%) ( $P < 0.01$ ).

## Discussion

Our results showed that resting tension alters airway smooth muscle hyper-responsiveness. Epithelium removal increases airway smooth muscle contraction only in high resting tension. The influence of low resting tension on epithelial damage has a protective effect on airways muscle contraction.

In our experiments, ACh induces different contractions on different airway smooth muscle resting tension. Recent articles showed that tension applied to airway smooth muscles at the start of an isometric *in vitro* experiment is an important factor that determines responsiveness.<sup>6,7</sup> Airway epithelium also moderates the responsiveness of underlying smooth muscles. Hyper-reactivity of the airways is associated with damage of the epithelium.<sup>8</sup>

Intact epithelium has a protective effect because it releases relaxant substances such as prostanoids and nitric oxide.<sup>9</sup> Bronchial asthma is characterized by epithelium damage and airway smooth muscle contraction. Further contraction, induced by released mediators, depends on the presented findings. Endothelin-1, a 21-amino-acid peptide that has been identified in tracheal epithelial cells, has a dual action on guinea-pig isolated trachea. It evokes contractions at low resting tone, whereas it induces relaxations at higher resting.

Resting tension seems to influence the magnitude of non-adrenergic non-cholinergic (NANC) responses. Nitric oxide is produced in the airways, mainly from epithelium, and is the main mediator of the NANC system.<sup>10</sup> Airway epithelial cells metabolize arachidonic acid to biologically active eicosanoids, which contribute to regulation of airway smooth muscle tone.<sup>8</sup> Cyclic stretch of airways causes rapid inhibition of prostanoid synthesis.<sup>5</sup> All these data may have important implications for the pathogenesis of asthma, in which airway mechanics are altered. In our study, the resting tension affect the magnitude of contraction in the loss of epithelium. When the resting tension is low (normal condition), the damage of epithelium seems to induce additional relaxation. When the resting tension is high (bronchoconstriction, such as an asthma attack), the damage of epithelium increases the airways smooth muscle contraction.

A few years ago, Skloot *et al.* showed that airway hyper-responsiveness in asthma is a problem of 'limited smooth muscle relaxation' with inspiration.<sup>11</sup> This observation is based on the speculation of Green and Mead that in patients with asthma, maximal inspiration is unable to dilate the airways completely.<sup>12</sup> It is well known that maximal inspiration elevates the airway resting tension. The interaction between high resting tension and damage of epithelium which happen in asthma attack seem to be the real cause for 'limited smooth muscle relaxation'.

In normal humans subjected to bronchoconstrictions, deep inspiration decreases airway resistance and increases expiratory flow.<sup>13</sup> Normal tidal breathing also plays an important role in limiting airway responsiveness.

Lung-volume changes have an important effect on airway tone and the airway response to bronchoconstrictors.<sup>14</sup> Airway smooth muscle undergoes constant stretch and relaxation during the respiratory cycle. The responses of airways to a variety of stimuli are modulated by the epithelium.<sup>15,16</sup> In bronchial asthma, all the normal mechanisms are impaired. Additionally, an asthmatic airway has increased thickness of the smooth muscle layer, collagen deposition beneath the basement membrane and, especially, variable inflammatory products in the wall.<sup>17,18</sup>

In conclusion, mediators of asthma, such as acetylcholine, induce more contraction when acting on already contracted airways with epithelium damage, a condition mimicking asthma attack.<sup>19</sup> Despite this vicious circle of asthmatic airway, in physiologic conditions, normal tidal breathing protect from bronchoconstriction even when the epithelium is damaged.

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