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Smooth muscle in human bronchi is disposed to resist airway distension

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ABSTRACT

Studying airway smooth muscle (ASM) in conditions that emulate the *in vivo* environment within which the bronchi normally operate may provide important clues regarding its elusive physiological function. The present study examines the effect of lengthening and shortening of ASM on tension development in human bronchial segments. ASM from each bronchial segment was set at a length approximating *in situ* length (L_{insitu}). Bronchial tension was then measured during a slow cyclical strain (0.004 Hz, from 0.7 L_{insitu} to 1.3 L_{insitu}) in the relaxed state and at graded levels of activation by methacholine. In all cases, tension was greater at longer ASM lengths, and greater during lengthening than shortening. The threshold of methacholine concentration that was required for ASM to account for bronchial tension across the entire range of ASM lengths tested was on average smaller by 2.8 logs during lengthening than during shortening. The length-dependency of ASM tension, together with this lower threshold of methacholine concentration during lengthening *versus* shortening, suggest that ASM has a greater ability to resist airway dilation during lung inflation than to narrow the airways during lung deflation. More than serving to narrow the airway, as has long been thought, these data suggest that the main function of ASM contraction is to limit airway wall distension during lung inflation.

1. Introduction

Airway smooth muscle (ASM) is a structure found along the airway tree from the trachea down to the respiratory bronchioles. Because ASM cells and bundles are oriented nearly circumferentially around the airways (Smiley-Jewell et al., 2002), and because ASM shortens when activated, ASM seems well disposed to narrow the airway. Many putative roles have been assigned to bronchoconstriction induced by ASM (Fredberg, 2007; Mitzner, 2004; Seow and Fredberg, 2001 Mitzner, 2004; Seow and Fredberg, 2001). However, none have reached unanimity.

To better understand the potential function of ASM, one may need to consider that it normally operates in a dynamic environment. Indeed, lung volume excursions that are required for breathing impose oscillatory stress on the airway wall and the ASM embedded within it (Olsen et al., 1967; Sasaki and Hoppin, 1979). Studies emerging from work on the oscillatory mechanics of ASM have demonstrated that tidal straining of ASM decreases its contractile capacity markedly, thus acting like a bronchodilator (Fredberg et al., 1997; Gunst, 1983). These findings heralded a new era in the field of ASM mechanics wherein interest turned toward the understanding of the bronchodilator effect of breathing. In fact, the ability of oscillating strain to attenuate the contractile capacity of ASM has arguably become a mechanism by which deep inspiration relieve respiratory distress (Krishnan et al., 2008; Nadel and Tierney, 1961). Emanating from this concept were newly discovered contractile properties of ASM, such as the ability to tolerate or recover from oscillating strain (Chin et al., 2012), the velocity of shortening (Bullimore et al., 2011), the length-dependency (Lee-Gosselin et al., 2013) and plasticity (Pratusevich et al., 1995) of ASM force, the re-lengthening induced by imposed force fluctuations (Dowell et al., 2005; Oliver et al., 2007) and the maintenance of ASM shortening (Chapman et al., 2014). It was suggested, fur-

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ther, that any impairment in these newly identified contractile properties may ultimately contribute to airway hyperresponsiveness in asthma (Bossé and Paré, 2013). Because the physiological function of ASM is uncertain, it remains unclear whether targeting the molecular mechanisms underlying these newly discovered contractile properties would be helpful in respiratory diseases.

In human tissues, the effects of oscillating stress that simulates breathing maneuvers, such as tidal breathing and deep inspiration, have been studied in intact bronchi (Noble et al., 2013, 2011), isolated bronchial and tracheal ASM strips (Chin et al., 2010, 2012; Ijpma et al., 2015), as well as in lung slices (Lavoie et al., 2012). Another study conducted with human bronchi in isometric conditions have demonstrated that, within the physiological range of lengths that ASM undergoes *in vivo*, the length of ASM greatly influences its capacity to generate force (Lee-Gosselin et al., 2013). In isolation, each of these studies substantially improved our understanding of ASM on airway mechanics. However, a single study performed with human tissues in which all the elements that affect ASM's contractile capacity are considered together has never been conducted. Herein, human bronchial segments were studied at different levels of ASM activation, which spans across a fully relaxed to a maximally activated ASM, during imposed strain that spreads across the entire range of lengths that ASM susceptibly undergoes *in vivo*. Based on the 'length-dependency of ASM force' identified previously in isometric conditions (Lee-Gosselin et al., 2013), we hypothesized that in dynamic conditions ASM is better disposed to limit airway dilation during simulated lung inflation than to narrow the airway lumen during simulated lung deflation.

2. Methods

2.1. Human bronchial segments

Bronchial segments were isolated from lung specimens derived from patients undergoing lobectomy or segmentectomy for tumor removal. Consent was obtained and the use of human lung tissue was approved by the ethics committee of the IUCPQ (*Institut universitaire de cardiologie et de pneumologie de Québec*). In the days prior to surgery, spirometry was obtained (Table 1). The onsite tissue bank of the Respiratory Health Network of the FRQS (www.tissuebank.ca) was in charge of supplying resected lung specimens of gross healthy appearance (*i.e.*, away from tumors) the day of the surgery. From each lung specimen, bronchial segment with a mean length of 5.6 ± 0.2 mm were isolated.

2.2. Experimental setting

The airways studied are too small (average internal luminal diameter of 2.0 ± 0.4 mm) to use a setting where the internal pressure is controlled (Harvey et al., 2015), which would be the ideal setting to simulate physiological loading. The bronchial segment was thus mounted in an organ bath attached to a servo-controlled force-length transducer (300B model of dual-mode lever arm systems from Aurora Scientific Inc.) in between platinum electrodes. A schematic of the setup is shown in Fig. 1. More precisely, one side of each of two stainless steel triangles was inserted into the bronchial lumen. The bronchial segment was held in place by applying a distending force (the resting tension) that simulated a transpulmonary pressure at functional residual capacity (FRC) (*i.e.*, 5 cmH_2O) as previously described (Lee-Gosselin et al., 2013). The length at which ASM freely adjusted to in response to this distending force is called L_{insitu} because it is assumed to be the length at which ASM operates *in vivo* when the lung is at FRC. The bronchial segment was then subjected to a period of equilibration, which consisted of repetitive 20-s electrical field stimulations at 5-min intervals until the bronchial segment generated a stable isometric force (Lee-Gosselin et al., 2013).

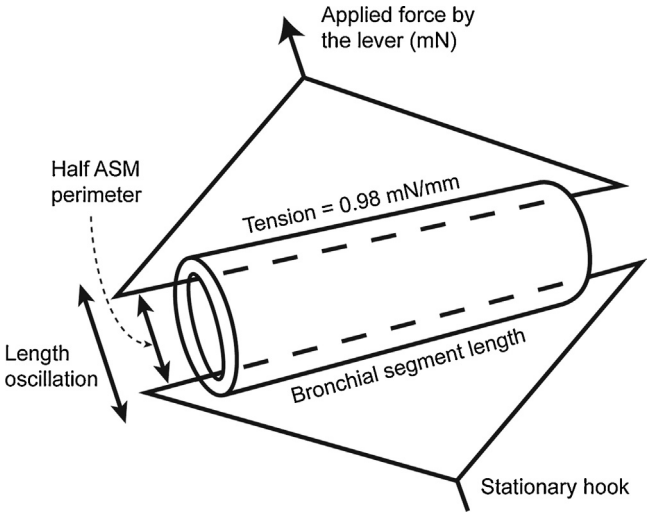

Fig. 1. Schematic of the experimental setup used to assess ASM contraction in segments of small bronchi. The distending force applied to the bronchial segment was calculated to simulate a transpulmonary pressure at functional residual capacity (FRC) (*i.e.*, 5 cmH_2O); merely by converting a transmural pressure of 5 cmH_2O into tension based on the radius of the bronchus (tension = pressure × radius). The calculated tension was then multiplied by two. This is because the bronchial segment flattened when exposed to distending stress and the bronchial wall on either side of the stainless-steel triangles' rod has to be exposed to the calculated tension. The tension was then converted to force based on the length of the bronchial segment. In a representative bronchial segment with an internal diameter of 2 mm and bronchial segment length of 5 mm, tension in one side of the bronchial segment = 0.49 KPa × 1 mm. To account for the 2 sides, the total tension was therefore 0.49 mN/mm × 2 sides = 0.98 mN/mm. The force applied by the lever to simulate the calculated tension = 0.98 mN/mm × 5 mm = 4.9 mN. Flattening of the bronchial segment also straightened the ASM. This was convenient to calculate the magnitude of the length oscillation, which was 30% below and above the half-length of ASM perimeter. In the same representative bronchial segment, the half perimeter of ASM is π * diameter/2 = 3.14 mm. The distance excursion between the two stainless-steel triangles that was used to oscillate ASM from 0.7 to 1.3 L_{insitu} was thus 1.88 mm; *i.e.*, from 2.2 to 4.08 mm.

2.3. Experimental protocol

The ASM was then submitted to length oscillations. During one cycle, the length of ASM fluctuated from 0.7 to 1.3 L_{insitu}, which represent the extreme limits of the physiological range of lengths that ASM can experience *in vivo* due to both breathing maneuvers (Fredberg et al., 1997) and changes in body posture (Tawhai et al., 2009). The rate of length change was set at 30% L_{insitu}/min (the cycling frequency was thus 0.004 Hz).

Length oscillations were initially performed until a steady-state loop was obtained (Fig. 2). The intrinsic state of ASM activation was then assessed. This is because intrinsic tone is often observed in excised human bronchi (Ellis and Undem, 1994; Taylor et al., 1984; Watson et al., 1998). To fully control the magnitude of ASM activation, it was necessary to inhibit (or to confirm the absence of) intrinsic tone. A set of experiments was thus undertaken to assess the origin of intrinsic tone. Different pharmacological agents were added cumulatively into the organ bath during the length oscillations in the following sequence: atropine, indomethacin, montelukast and fexofenadine in order to block the muscarinic receptors, the generation of endogenous prostaglandins, the CysLT1 receptor of cysteinyl-leukotrienes and the H_1 receptor of histamine, respectively. Cycling proceeded after the addition of each agent until a new steady-state loop was achieved. Atropine and indomethacin had no effect (not shown), suggesting that acetylcholine and endogenous cyclooxygenase products are not involved