Signal Transduction in Smooth Muscle
Selected Contribution: Airway caliber in healthy and asthmatic subjects: effects of bronchial challenge and deep inspirations

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Jensen, Andrew, Haytham Atileh, Bela Suki, Edward P. Ingenito, and Kenneth R. Lutchen. Selected Contribution: Airway caliber in healthy and asthmatic subjects: effects of bronchial challenge and deep inspirations. J Appl Physiol 91: 506–515, 2001.—In 9 healthy and 14 asthmatic subjects before and after a standard bronchial challenge and a modified [deep inspiration (DI), inhibited] bronchial challenge and after albuterol, we tracked airway caliber by synthesizing a method to measure airway resistance (Raw; i.e., lung resistance at 8 Hz) in real time. We determined the minimum Raw achievable during a DI to total lung capacity and the subsequent dynamics of Raw after exhalation and resumption of tidal breathing. Results showed that even after a bronchial challenge healthy subjects can dilate airways maximally, and the dilation caused by a single DI takes several breaths to return to baseline. In contrast, at baseline, asthmatic subjects cannot maximally dilate their airways, and this worsens considerably postconstriction. Moreover, after a DI, the dilation that does occur in airway calibration in asthmatic subjects constricts back to baseline much faster (often after a single breath). After albuterol, asthmatic subjects could dilate airways much closer to levels of those of healthy subjects. These data suggest that the asthmatic smooth muscle resides in a stiffer biological state compared with the stimulated healthy smooth muscle, and inhibiting a DI in healthy subjects cannot mimic this.

airway smooth muscle; airway resistance; methacholine

DURING INDUCED OBSTRUCTION, nonsymptomatic asthmatic subjects can transiently dilate their airways after a deep inspiration (DI) (6), whereas symptomatic asthmatic subjects at baseline cannot and may even constrict after a DI (17). Skloot et al. (21) have shown that, if healthy subjects refrain from taking any DIs during a methacholine challenge, then their airway responsiveness to methacholine (assessed spirometrically) becomes amplified. Recently, Brusasco et al. (5) found that inhibiting DIs in healthy subjects during a methacholine challenge increases airway sensitivity to methacholine but not to the level of asthmatic subjects. In contrast, inhibiting DIs in asthmatic subjects during a challenge did not alter their airway hyperreactivity. Changes in airway caliber depend on lung volume, smooth muscle tension, and net transmural pressure across the airway lumen (15, 22). Does the inability of a DI to relieve airway obstruction in asthmatic subjects stem from increased muscle stiffness or from a reduced tethering due to increased airway wall thickening and/or wall remodeling or both? Fredberg et al. (12) have suggested that airway smooth muscle in symptomatic asthmatic subjects may convert to a latch state in which force fluctuations from tidal breathing on the smooth muscle are not strong enough to break the actin-myosin cross bridges, leaving the smooth muscle in asthmatic airways in a stiffer state. Therefore, a DI cannot relieve constriction. The implication is that, in healthy subjects, force fluctuations from tidal breathing and/or periodic DIs on the smooth muscle are sufficient to keep the muscle from transitioning to this latch state, allowing a DI to be bronchodilatory (11).

The goal of this study was to track airway caliber during tidal breathing, during a DI, and after a DI in real time. Lung resistance at ~8 Hz reflects almost

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exclusively airway resistance (Raw) only [because tissue viscance becomes zero and there is no purely Newtonian component of tissue resistance (14, 19)]. Hence, we tuned a recursive least squares (RLS) method (20) to track Raw before and after a standard and modified (DI inhibited) methacholine challenge and after albuterol. Our results show that asthmatic subjects have a reduced ability to bronchodilate with a DI and reconstrict more rapidly than healthy subjects. We discuss these results in the context of potentially distinct biological states of airway smooth muscle in asthmatic subjects.

**METHODOLOGY**

**Subjects**

Measurements were made on 14 asthmatic subjects (5 men and 9 women) and 9 healthy subjects (7 men and 2 women), all nonsmokers, before and after a standard and modified (DI inhibited) methacholine challenge and after a bronchodilator (albuterol). Subjects ranged from 19 to 41 yr of age (22 \(\pm\) 6 yr). Table 1 lists the demographics for each subject. All asthmatic subjects were previously diagnosed with asthma by a physician and were currently taking inhaled bronchodilators. Of the 14 asthmatic subjects, 10 used short acting \(\beta_2\)-agonist (albuterol), 3 used albuterol in combination with inhaled corticosteroids, and 1 used albuterol and a leukotriene inhibitor (Accolate). Airway hyperreactivity was assessed by interpolating the methacholine concentration causing a 20% fall (PC20) in forced expiratory volume in 1 s (FEV1) from baseline. Inclusion criteria included no albuterol or caffeine 12 h before each day of the study. We also asked each subject to refrain from any other medications as directed by American Thoracic Society (ATS) guidelines for methacholine challenges (8). The study was approved by our institutional research committees, requiring informed consent from each subject.

**Experimental Measurements**

We tracked Raw during tidal breathing, during a DI, and after a DI. The setup included a high-inertance tube that allowed the subjects to spontaneously breathe to atmosphere superimposing the 8-Hz flow oscillations delivered by a piston/cylinder. At 8 Hz, dynamic lung resistance (RL) is essentially Raw. The tube was 160 cm long with a 1.3-cm internal diameter. These dimensions caused the tube to act as a low-pass filter, enabling the 8-Hz oscillations to flow into the subject while presenting a tubing dead space (0.212 liter) small enough to ensure sufficient fresh gas for the subject each breath.

The experimental system delivered a sinusoidal flow at 8 Hz while collecting airway opening flow (\(V_{ao}\)) and transpulmonary pressure (Ptp). Specifically, a volume signal was sent to a servo-amplifier that drove a linear motor (Infomag, model 15) connected to a piston-cylinder arrangement. The Ptp was measured with a single Celesco differential 650-cmH2O pressure transducer (model LCVR) by measuring pleural pressure (estimated with an esophageal balloon) on one port and airway opening pressure with the other port. The V\(_{ao}\) was measured with a pneumotachograph (Hans Rudolph 4700A) connected to a single differential 62-cmH2O pressure transducer (model LCVR).

Table 1. Subject demographics

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FEV1, forced expiratory volume in 1 s; FEV1/FVC, ratio of FEV1 to forced vital capacity; PC20, index of AHR (i.e. concentration of methacholine causing a 20% drop in FEV1); M, male; F, female; N/A, not able to perform challenge.
Subject Classification

To provide some quantitative partitioning of the baseline conditions, we examined the PC_{20} in concert with measurements of the frequency dependence of dynamic Rt and lung elastance (El) taken between 0.15 and 8.0 Hz by using methods previously reported by us (13). We identified five subjects (i.e., subjects 11, 14, 15, 19, and 23) who presented with a PC_{20} < 0.01 (except for subject 11, who could not be challenged) combined with elevated baseline levels and frequency dependence of both Rt and El, all to the extent that their El was >0 throughout the entire 0.15–8 Hz range. Our previous study (13) had labeled subjects with strictly positive El data over this frequency range as Type B asthmatic subjects. By combining this criterion with PC_{20}, we chose to pool these five in a group that we labeled as asthmatic subjects with severe baseline constriction and reactivity, or “severe” for short. The other 10 asthmatic subjects were then classified as presenting with mild-to-moderate baseline constriction and reactivity. Note that our labeling of these groups as “severely” and “mildly to moderately” asthmatic was not intended to be consistent with a traditional clinical classification of their asthma, but with the degree of baseline asthmatic constriction (based on dynamic Rt and El data) and reactivity on the days of our study.

Data Analysis

At any single frequency, the lung may be modeled as a single resistance (Rl) and elastance (El) in series (16) represented by

$$P_{tp} = R_l \dot{V}_{ao} + El \int \dot{V}_{ao} + Po$$

where Po is Ptp when airway opening flow (Vao) and lung volume (Vao) are zero (i.e., at FRC). To track Raw in real time, we first high-pass filtered the data with a four-pole Butterworth digital filter (4-Hz corner frequency), allowing use to isolate the 8-Hz information that was superimposed on the lung behavior. This was accomplished by using a recursive least squares (RLS) algorithm similar to that introduced by previous studies (1–3, 13, 16). The APPENDIX displays the complete details of the RLS algorithm, but, briefly, the method provides a running estimate in which, after every new data point, the algorithm updates new parameter values. The algorithm employs a so-called “forgetting factor,” whereby only the most recent data contribute to the esti-

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Protocol

At baseline, before the standard methacholine challenge, subjects were trained on the 8-Hz-breathing system (5–15 min) until they were comfortable and able to perform the breathing maneuvers. Before placing the esophageal balloon in the subject, we measured baseline spirometry and estimated total lung capacity (TLC) by measuring thoracic gas volume by plethysmography and then integrating the subsequent flow through a pneumotach when subjects inhaled maximally. The balloon placement was then confirmed with an occlusion test (4). Once the balloon was set and the subject was relaxed, the pump was turned on to generate 8-Hz oscillations. Subjects were each asked to place their mouths tightly around a mouthpiece while firmly supporting their cheeks. Once set, they were asked to take five to six consistent tidal breaths followed by a slow steady inspiration to TLC, then by a passive expiration to functional residual capacity (FRC), and then by five to six consistent tidal breaths again.

These baseline measurements were made on 2 separate days, one leading to a standard methacholine challenge (day 1) and the other leading to a modified methacholine challenge (day 2; DIs inhibited during challenge). The methacholine was administered by use of a Rosenthal New Standard dosimeter (Pulmonary Data Service) via the five-breath dosimeter protocol as described by ATS, requiring, however, the subject to take roughly three-quarter DI breaths instead of inspiring to TLC (8). The methacholine dose sequence was 0.01, 0.1, 1, 10, and 25 mg/ml. Both days were no shorter than 3 and no longer than 5 days apart.

After baseline measurements on day 1, the standard challenge was started. Methacholine was administered starting at the lowest dose and readministered at increasing doses until at least a 20% drop in FEV1 (PC_{20}) was measured. During day 2, the same dose sequence was administered; however, the subject was asked to refrain from taking any DIs during the entire challenge (i.e., no spirometry between doses). To ensure this, the pleural pressure measured from the balloon catheter was continually monitored with an oscilloscope, allowing us to isolate the subject’s breathing between measurements. On each day, after we reached the maximum dose (25 mg/ml) or the dose causing at least a 20% drop in FEV1, the 8-Hz measurement was performed. Finally, 3–4 min after smooth muscle tone was relieved by albuterol, a final 8-Hz measurement was repeated.

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$$P_{tp} = R_l \dot{V}_{ao} + El \int \dot{V}_{ao} + Po$$

where Po is Ptp when airway opening flow (Vao) and lung volume (Vao) are zero (i.e., at FRC). To track Raw in real time, we first high-pass filtered the data with a four-pole Butterworth digital filter (4-Hz corner frequency), allowing use to isolate the 8-Hz information that was superimposed on the spontaneous breathing and DI maneuver (Fig. 1). Using the model described in Eq. 1, we implemented the filtered signals into a RLS algorithm similar to that introduced by previous studies (1–3, 13, 16). The APPENDIX displays the complete details of the RLS algorithm, but, briefly, the method provides a running estimate in which, after every new data point, the algorithm updates new parameter values. The algorithm employs a so-called “forgetting factor,” whereby only the most recent data contribute to the esti-

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Fig. 1. A flow chart of the signal processing done for each pressure and flow signal: Each signal was high-pass filtered, isolating the 8-Hz information and implemented into a recursive least squares (RLS) estimator using the single compartment lung model. The lung resistance we tracked at 8 Hz was presumed to approximate airway resistance (Raw). The phase of the output of the RLS estimator needed to be realigned from the filtering and the RLS estimator itself to correctly track Raw vs. time and against volume.
mates. We tuned the estimator so that $R_l$ reflected primarily the resistance from the most recent 8-Hz cycle. $R_l(8\,\text{Hz}) = \text{Raw}$ was then smoothed by using a low-pass digital filter with a 3-Hz cutoff, shifted to account for the filter phase response, and then displayed against time and against lung volume (calculated by digitally integrating prefiltered $V_{ao}$).

**Maximum dilation capacity and impact of tethering/remodeling.** We compared $R_{\text{min}}$ values within each subject group and across each group under each constriction condition. To assess the relative contributions of smooth muscle and parenchymal tethering on the subject’s ability to dilate airways during a DI, we compared $R_{\text{min}}$ before and after administering a bronchodilator (i.e., albuterol) for each subject group. Our presumption was that albuterol primarily relieved smooth muscle tone, without altering airway tethering (i.e., wall remodeling or thickening) conditions. We anticipate that $R_{\text{min}}$ will be elevated in asthma compared with healthy lungs, especially after bronchial challenge. Hence, if $R_{\text{min}}$ decreased post-albuterol, the degree of reduction would reflect how much of the originally elevated $R_{\text{min}}$ was due to airway smooth muscle stiffness. The remaining elevation in $R_{\text{min}}$ would reflect how much was due to tethering differences in asthmatic subjects.

**Impact of a single DI.** At baseline and after each condition, we calculated the increase in Raw during expiration of a DI normalized by the decrease of Raw that occurred during inspiration of the DI ($\Delta\text{Raw}_{\text{exp}}/\Delta\text{Raw}_{\text{insp}}$) as an index of reconstriction of airway caliber after a single DI.

**RESULTS**

**Assessment of Airway Caliber**

**Raw vs. time synchronized with volume.** Figure 2 compares typical Raw tracking results for the three example subject types before and after a methacholine challenge. Consistent with all subjects, Raw varied primarily 180° out of phase with changes in lung volume with some extraneous “notches” probably reflecting variations in glottal aperture during breathing. For example, as a subject inhales, the algorithm is able to track a decreasing Raw and vice versa during expiration. Each tracking result contained four key features: 1) intrabreath Raw fluctuations during tidal breathing, 2) the $R_{\text{min}}$ achieved during a DI at total lung capacity, 3) the dynamics of Raw after a single DI, and 4) the dynamics of Raw for several tidal breaths post-DI.

**Baseline**

Baseline intrabreath fluctuations in Raw for all healthy subject data were similar. During tidal breathing, the Raw averaged over all healthy subjects was $2.1 \pm 0.5$ cmH$_2$O·L$^{-1}$·s$^{-1}$. The specific healthy subject shown in Fig. 2A was tidally breathing $-2.5$ cmH$_2$O·L$^{-1}$·s$^{-1}$. At TLC during a DI, the Raw decreased

**Fig. 2.** Tracking Raw results at baseline (left) for healthy subject 9 (A), mild-to-moderate asthma subject 13 (B), and severe asthma subject 14 (C) shown in the solid lines and the corresponding data in these same subjects after methacholine challenge (D, E, and F, respectively). The volume of each subject’s breathing is displayed as a dotted line. Note that the Raw axis scales (in cmH$_2$O·L$^{-1}$·s$^{-1}$) are distinct for each subject type. Note subject classification based on reactivity and dynamic elastance data as described in the text.
to 0.9 cmH2O·L⁻¹·s and after a DI, Raw increased but only to a value ~20% below the pre-DI levels (i.e., 2.0 cmH2O·L⁻¹·s) and remained below the pre-DI levels for several breaths thereafter. Thus the DI produced a consistent residual dilatory impact that lasted for several tidal breaths.

Baseline tracking results were different in mild-to-moderate asthmatic than in healthy subjects. The mild-to-moderate subject in Fig. 2B exhibited an elevated Raw of 3.5 cmH2O·L⁻¹·s. During a DI, the subject was able to decrease Raw to only 1.4 cmH2O·L⁻¹·s (i.e., not to the level of the healthy subject). After a DI, Raw returned quickly to within ~10% of pre-DI levels so the DI had less residual bronchodilatory effect than that seen in healthy subjects.

The severely asthmatic subject in Fig. 2C exhibited a highly elevated Raw of 7 cmH2O·L⁻¹·s at baseline with intrabreath Raw swings of >2 cmH2O·L⁻¹·s compared with ±0.5 in the healthy subject during tidal breathing. During a DI, Raw decreased only to 2.6 cmH2O·L⁻¹·s but immediately after a DI, Raw quickly returned to pre-DI levels and in fact seemed to be higher. This is consistent with the findings of Lim and co-workers (17).

**Postchallenges**

Figure 2, D–F, shows the same three subjects after a methacholine challenge. During tidal breathing, the Raw in the challenged healthy subject was elevated from the baseline 2.5 (Fig. 3A) to 3.5 cmH2O·L⁻¹·s (Fig. 2D) and the tidal swings became ~1.0 cmH2O·L⁻¹·s. Nevertheless, during a DI, the healthy subject was still able to reduce Raw below 1.0 cmH2O·L⁻¹·s similarly to baseline. After the DI, Raw returned only to 2.0 cmH2O·L⁻¹·s, illustrating that the bronchodilatory impact from the DI was sustained. Moreover, Raw remained lower for at least 20 s post-DI.

The mild-to-moderate asthmatic subject in Fig. 2E exhibited an increase of Raw during tidal breathing to 6 with ± 1.5 cmH2O·L⁻¹·s intrabreath fluctuations compared with 3.5 ± 0.75 cmH2O·L⁻¹·s prechallenge. The Rmin during a DI was only 2.5 cmH2O·L⁻¹·s compared with 1.4 prechallenge. Thus, whereas the within-breath fluctuations in Raw were larger after the challenge, the net bronchodilatory capability was diminished. Then, after a DI, Raw rebounded almost completely back to pre-DI levels. The severely asthmatic subject in Fig. 2F exhibited an increase of Raw during tidal breathing to 10 ± 2 cmH2O·L⁻¹·s compared with 7 ± 1.5 cmH2O·L⁻¹·s prechallenge. The Rmin achievable during a DI was only 4.1 cmH2O·L⁻¹·s compared with 2.6 prechallenge. Again, although the within-breath fluctuations in Raw were larger after the challenge, the net bronchodilatory capability was diminished during a DI. Then, after a DI, Raw rebounded immediately back to pre-DI levels if not slightly above.

The modified challenge significantly amplified the impact on mean Raw in healthy subjects (P < 0.02) but not in asthmatic subjects. Mean Raw during tidal breathing in healthy subjects after a modified challenge was 3.0 ± 0.5 vs. 2.6 ± 0.6 cmH2O·L⁻¹·s after a standard challenge. An amplified Raw response was only seen in six of nine mildly to moderately asthmatic subjects (P < 0.03). The Raw increased to 4.2 ± 1.0 cmH2O·L⁻¹·s and 5.2 ± 1.7 cmH2O·L⁻¹·s after standard and modified challenges, respectively.

**Assessment of tethering via Rmin.** Rmin reflected the maximum ability to dilate airways at total lung capacity. For each subject group, we investigated Rmin at baseline, after each challenge, and after albuterol. Figure 3 summarizes the results for each subject group. At baseline and for both days, Rmin was 0.9 ± 0.1, 1.3 ± 0.3, and 2.0 ± 0.6 cmH2O·L⁻¹·s for the healthy, mildly to moderately asthmatic, and severely asthmatic subjects, respectively. Baseline Rmin were significantly higher in mildly to moderately (P < 7e⁻⁸) and severely asthmatic subjects (P < 7e⁻⁸) vs. healthy subjects. Thus, at baseline, asthmatic subjects cannot maximally dilate the airways with a DI to the same degree that healthy subjects can.

In healthy subjects, Rmin slightly but significantly increased after a standard (P < 0.05) and a modified (P < 0.0009) challenge, and the increase in Rmin after a modified challenge was amplified over that occurring with a standard challenge (P < 0.04). Nevertheless, these increases in Rmin compared with baseline were very small so that the capacity to maximally dilate airways was not compromised much with either challenge. In contrast, in the mild-to-moderate asthma subjects, Rmin increased 30% after standard challenge and 50% after modified challenge. Also, Rmin postchallenge was far greater than healthy Rmin postchallenge. The severely asthmatic subjects showed elevated Rmin, even at baseline.Challenging them further reduced their capacity to bronchodilate with a DI (i.e., after the standard challenge, Rmin = 3.0 cmH2O·L⁻¹·s vs. 1.0 cmH2O·L⁻¹·s in healthy subjects).

Of particular interest are responses to a DI post-albuterol. After bronchodilator administration, the Rmin values in the mild-to-moderate and severe asthmatic groups dropped substantially and nearly back to that seen in healthy subjects at baseline. For example,
in the mildly to moderately asthmatic subjects, Rmin was 2.0 ± 0.4 after modified challenge vs. 1.2 ± 0.2 cmH₂O·l⁻¹·s after albuterol. Likewise, in severe asthma Rmin dropped from 3.0 ± 0.9 to 1.4 ± 0.3 cmH₂O·l⁻¹·s. Again assuming that albuterol did not alter tethering forces, the decrease in Rmin postalbuterol reflected the effect of alleviation of smooth muscle tone. Any differences in Rmin postalbuterol of healthy vs. asthmatic subjects would have reflected the degree that decreased tethering inhibits maximum dilation (i.e., caused an increase in Rmin). These data give evidence that asthmatic subjects are able to transmit enough force to the airway lumen during a DI after albuterol to decrease Rmin to levels close to those of healthy subjects. The implication is that, in asthmatic subjects, the main cause of this reduced dilation capacity, particularly when challenged, lies at the level of the smooth muscle (i.e., it is too tense to stretch with a DI) rather than an inability to transmit a given force to it.

Assessment of bronchodilator effect of a single DI. During a single DI, the ratio of ΔRawexp/ΔRawinsp was computed and used as an index of single DI reconstriction (Fig. 4A). If ΔRawexp/ΔRawinsp was less than 1.0, a single DI was immediately bronchodilatory whereas it was bronchoconstrictive if greater than 1.0. Figure 4B shows the results of ΔRawexp/ΔRawinsp for each subject group at baseline, after each challenge, and after albuterol.

![Fig. 4. The ratio of reconstriction (ΔRawexp/ΔRawinsp) after a single DI, defined (A) as the change (increase) in Raw during the expiration after a DI divided by the change (decrease) in Raw created from the DI averaged over each subject group under each condition (B). Note a ratio < 1.0 indicates a single DI was bronchodilatory and > 1.0, bronchoconstrictive. Also shown are P values (paired t-tests) indicating whether the ratio is significantly above or below 1.0. Note subject classification based on reactivity and dynamic elastance data as described in the text.](http://jap.physiology.org/)

At baseline, healthy subjects as a group have an immediate and significant bronchodilatory impact from a single DI. When we challenged the healthy subject, a single DI was significantly and substantially more bronchodilatory, and if we constrict the healthy subject even more by inhibiting DIs during the challenge, a DI is extremely bronchodilatory. Specifically, ΔRawexp/ΔRawinsp after standard challenge and after modified challenge were 71 and 52%, respectively. Postalbuterol results were very similar to baseline results in healthy subjects.

In mildly to moderately asthmatic subjects, baseline and postalbuterol results were similar. As a group, ΔRawexp/ΔRawinsp, was 90% at baseline and 90% after albuterol. However, unlike in healthy subjects, the ratio was independent of challenge type (modified vs. standard) and, although still <1.0, the ratio was not significantly distinct in the modified challenge and was much greater than in healthy subjects.

Remarkably, severely asthmatic subjects showed no evidence of a single DI being bronchodilatory but rather it tended to be slightly bronchoconstrictive. At baseline and after each challenge, Raw returned either to or above pre-DI levels. Even after albuterol, a DI was not bronchodilatory.

Relation between inspiratory capacity and Rmin. Figure 2 suggested that the inspiratory capacity (IC) decreased with increasing severity of asthma. Specifically, for the healthy, mildly to moderately asthmatic, and severely asthmatic subjects shown, the inspiratory capacities were 4.0, 2.6, and 2.1 liters, respectively. Over the whole group, the mean inspiratory capacities were 3.5 ± 0.5, 2.8 ± 0.9, and 1.9 ± 0.3 liters, respectively. IC dropped postchallenge in all three subject groups. Over the whole groups, the mean values after modified challenged ICs were 3.0 ± 0.5, 1.8 ± 0.5, and 1.3 ± 0.4 liters for the healthy, mildly to moderately asthmatic, and severely asthmatic subjects, respectively.

DISCUSSION

As early as 1976 (9) as well as in several studies in the 1980s (6, 17), it was known that the response of asthmatic subjects to DI was distinct from that of healthy subjects. Even within the asthmatic population, the sign of the response to a DI depended on whether the constriction was induced or spontaneous (6, 17). It has been suspected for some time that these distinctions are fundamentally related to the exaggerated airway narrowing in asthmatic subjects compared with healthy subjects. Most of the early literature focused on the ratio of maximum to partial peak expiratory flows with the presumption that if the inspiration part before a maximum expiratory flow maneuver had a bronchodilator effect, the peak flow would be greater than that achieved during a partial maneuver. More recently, the study of Skloot et al. (21) rekindled interest in the role of a DI in airway reactivity. Their study showed that airway reactivity in healthy subjects is amplified when a DI is prohibited during a
bronchial challenge. Subsequently, however, Brusasco et al. (5) revealed that, although prohibiting a DI amplifies reactivity in healthy subjects, it does not do so to the level of an asthmatic subject. One is left wondering: What are the distinctive characteristics of in vivo asthmatic smooth muscle behavior compared with those in healthy subjects? and Will prohibiting a DI cause the healthy airway smooth muscle to transition to behaving more like that of an asthmatic subject? To address these questions, we used Raw as an assay of airway caliber. With regard to a single DI, the capacity to maximally dilate airways appears to worsen as the severity of baseline asthma increases (Figs. 2–4). Airway provocation does not impact maximum dilation capacity in healthy subjects, even during a modified challenge, but does further inhibit it in asthmatic subjects (Figs. 2–4). After airways are provoked, the primary cause for asthmatic subjects being unable to increase airway caliber with a single DI appears to lie at the level of the smooth muscle in that the capacity to stretch is largely regained after albuterol (Fig. 3). Two mechanisms are likely in play here. First, the asthmatic muscle may simply have transitioned to a state in which it is too stiff to stretch. Second, asthmatic subjects may have more closure that cannot be alleviated by a DI but can be alleviated by albuterol followed by a DI.

Airway Dilation Capacity and Reconstriction Dynamics: Healthy vs. Asthma

Healthy subjects responded very differently to a single DI than asthmatic subjects. In healthy lungs, the residual dilation (i.e., reduction in Raw) due to a single inspiration is greater after provocation than at baseline and is still greater during a modified challenge compared with a standard challenge (Fig. 4). This is not the case in asthmatic subjects, and the residual dilation is identical with either challenge and can even produce further constriction, as was first indicated by Lim et al. (17) (Fig. 4).

With regard to the dynamics of airway caliber post-DI, the rate of reconstriction increases with the severity of asthma at baseline (Fig. 2). In a healthy subject after a modified challenge, the residual dilation due to a single DI is long lasting, more so than at baseline. In contrast, the asthmatic reconstriction rate is relatively the same before or after challenge and is largely ablated within a single tidal breath post-DI (Fig. 2).

With regard to interpreting changes in Raw in terms of airway caliber, we point out that, for fully developed laminar flow, resistance through airways can be approximated by Raw = k/d^4, where k is a constant and d is average airway diameter. Indeed, we can exploit this concept to derive an index of airway diameter. In Fig. 5A, we assign a relative diameter of 1.0 to represent the mean Raw (i.e., 2.0 cmH2O·l⁻¹·s⁻¹) averaged over all healthy subjects breathing around FRC. Then, in any subject at any time, we can derive the effective diameter change necessary to move this “healthy” FRC-based Raw to the measured Raw. Such a relative diameter reflects variations in mean airway caliber averaged over all the airways and does not directly reflect any one specific airway generation.

We applied this approach to the data of the three subjects shown in Fig. 2A to derive effective diameter variations shown in Fig. 5B at baseline and Fig. 5C postchallenge. During the DI, the healthy subject creates increases in the relative diameter to ~25% above its effective FRC levels. After the modified challenge (Fig. 5C), the mean diameter decreased by ~10%, but the maximum relative diameter achievable during the DI was the same as at baseline (i.e., constricting healthy subjects does not inhibit their ability to dilate...
airways maximally). In contrast, the $R_{\text{aw}}$ in both asthmatic subjects represent reductions in relative diameters during their tidal breathing and at maximal effort to TLC, both at baseline and even more so postchallenge. Moreover, we note that in principle the much larger intrabreath $R_{\text{aw}}$ fluctuations measured in the severely asthmatic subject (Fig. 2) do not require larger intrabreath effective diameter variations. In short, the $1/d^4$ relation amplifies the effect of small changes in diameter on the variations in an already high mean $R_{\text{aw}}$ in asthmatic subjects.

The reduced IC in asthma is consistent with the notion of increased severe narrowing and smooth muscle stiffness occurring simultaneously. To some degree, the reduced IC reflects dynamic hyperinflation. However, we did not find any large differences in the mean $P_{\text{tps}}$ or maximum recoil pressures of the asthmatic subjects. It is likely that those airways that contribute to reduced IC also contribute to the measured increase in $R_{\text{min}}$. In other words, the reduced IC likely represents increased amounts of highly narrowed and/or closed airways that are also subtending lung tissue that does not participate much in the DI. These same airways do not dilate during the DI. The net result is an elevation in the $R_{\text{min}}$.

Finally, those airways that do participate in the DI behave differently in asthmatic vs. healthy subjects. Consider the healthy subject postchallenge vs. the baseline mildly to moderately asthmatic subject in Fig. 2. They both start out with about the same mean $R_{\text{aw}}$ (3.5 cmH$_2$O·l$^{-1}$·s$^{-1}$) and both have the same IC. Yet, post-DI the dynamics of reconstriction are drastically different. In the asthmatic subject it is rapid and nearly complete at the end of the exhalation from the DI, whereas in the constricted healthy subject it is actually lengthened compared with control. If the constricted states of both subjects were due primarily to closures that first reopen during a DI and then reclose, both subjects would show similar dynamics of reconstriction post-DI. The drastic differences point to the smooth muscle as being a primary difference in the two subject types.

**Does the Primary Defect Lie at the Level of Smooth Muscle?**

We suggest the following scenario. First, the asthmatic smooth muscle behaves as if it is in a more frozen latchlike state (10) such that a DI can barely stretch the muscle and the stretch itself does not break many actin-myosin cross bridges. Hence, releasing the stretch causes a rapid reconstriction. The reconstriction would be a function of the viscoelastic properties of smooth muscle in concert with those of the airway walls. Hence, overshoot is a possibility as asthma severity worsens. In contrast, the healthy muscle, even after constriction, remains in a more “melted” and less stiff state (10) so that a single DI is more effective, cross bridges break, and the single DI has a long-lasting bronchodilatory impact; it takes several breaths for the muscle to reshorten back to its initial length and for cross bridges to reform. Indeed, after constriction it may be that a DI breaks even more cross bridges than before constriction and this causes the time for reconstriction to be even longer than during control conditions. An alternative explanation arises from the provocative study just published on the parallel vs. serial arrangement of cross bridges (7). Second, if we assume that albuterol primarily relaxes smooth muscle and does not change parenchymal tethering, we conclude that the inability to maximally dilate these airways lies at the level of the muscle being in a state too stiff to stretch (Fig. 3). After albuterol, the muscle relaxes and the asthmatic airway can stretch to levels much closer to healthy levels.

Stated succinctly, the smooth muscle of an asthmatic subject is capable of constricting to a much stiffer state than the smooth muscle of a healthy subject such that the asthmatic subject’s airways have a smaller internal lumen with many airways even to the point of closure. At that point, a DI to TLC is simply incapable of generating enough force to stretch and/or reopen the airway appreciably. What little stretch is achieved cannot last because of the rapid reshortening of the muscle’s series elastic component. The healthy subject’s smooth muscle during standard protocols that involve many DIs cannot constrict to the same degree as the asthmatic subject’s nor get itself in such a stiff state. Although prohibiting a DI during agonist stimulation causes more constriction than with DIs allowed, the constriction is still easily ablated by a single DI, and the dynamics postconstriction are quite slower and distinct from asthma. Thus it does not appear that prohibiting a DI fully mimics in healthy subjects the state of smooth muscle in asthma.

We ask then, Why can asthmatic subjects constrict so much compared with healthy subjects when exposed to the same stimuli? We offer two scenarios. First, perhaps with increased wall thickening there is less of a load on the muscle, and we combine this with the notion that some of this is actually smooth muscle thickening (remodeling) itself. The net effect is a stronger muscle and a lighter load, hence more constriction per agonist dose. Moreover, perhaps even before the constriction the muscle is in a state closer to latch than that of healthy subjects. Hence, even at baseline and before any dose, the asthmatic subjects display diminished dilatory capacity and distinct dynamics postdilation (Fig. 5). Thus all the effects described in the first part of this paragraph are amplified.

Airway thickening could serve to amplify constriction capacity without necessarily impacting dilation capacity. We point out that both phenomena are distinct force-transmission problems. During constriction, the muscle dynamically alters its tone while pulling on its local boundary. The capacity to shorten to a specific length is governed by the muscle’s force-generating capacity and the inner and outer wall loads. In contrast, when a DI is taken, a completely different force balance arises. Now a pressure of $\sim 30$ cmH$_2$O is pulling across the entire parenchyma and the outer airway wall before transmission of some force to the muscle...
occurs. Thus thickening that would amplify a muscle’s capacity to shorten does not necessarily have to also inhibit transmission of forces during a DI that act to stretch this muscle. But, if the muscle is now too stiff, these stretching forces are insufficient to dilate the airways.

These data leave several unanswered questions. First, it may be that albuterol, while simultaneously relaxing smooth muscle, also reduces adventitious thickness to a level sufficient to improve tethering. If so, then prealbuterol thicker walls could have caused loss of tethering and hence contributed to a reduced capacity of a DI to stretch airway smooth muscle. Second, it is likely that the airways constricthighly heterogeneously and that many small airways close. Indeed, we have recently shown this to be the case when examining dynamic lung resistance and elastance from 0.1–8 Hz before and after challenges (18). If this is the case, a DI is likely to reopen and/or stretch airways in a highly heterogeneous fashion. Thus some airway diameters may be increasing far more than that implied in Fig. 5 and some not at all. Regardless, our data clearly quantify that the capacity to stretch smooth muscle to dilate airways and/or reopen closed airways, when integrated over the whole lung, is severely diminished with increased asthma severity.

Finally, and perhaps most provocatively, we ask: Why would inhibiting a DI in healthy subjects amplify the constriction response while not moving the muscle to a state identical to that existing in asthmatic subjects? Compared with a standard challenge, the response to a DI is more dilatory after modified challenge in healthy subjects and less dilatory in asthmatic subjects (Fig. 3). Perhaps the baseline state of airway smooth muscle in asthmatic subjects is already latchlike because of the longstanding asthmatic environment (inflammation and remodeling). Thus, although not constricted much at baseline, it still behaves in a latchlike manner and fewer if any cross bridges are broken during a DI. A provocation on a latchlike muscle (especially a “thicker” one) then creates a much larger force and net diameter reduction. Healthy muscle simply never transitions to this level of “latch state,” and we are not sure why.

Prohibiting a DI during provocation might move it toward latch so that the muscle can generate more force and more constriction, but it never becomes fully stiff and in a condition such that it cannot be stretched with a DI. In Fig. 6, we show what happens in a single healthy subject after repetition of the protocol, with the first DI postchallenge now delayed for 45 min. The Raw dynamics (and hence smooth muscle) behave as before (i.e., show no evidence of transitioning to behave as in asthmatic subjects). The implication is that the altered state of airway smooth muscle in asthmatic subjects requires a much longer time and distinct environment. Simply prohibiting periodic fluctuations in a healthy airway wall environment for 45 min appears insufficient for a healthy airway to behave like an asthmatic airway.

In summary, we have shown that asthmatic subjects have a distinctly diminished capacity to stretch airways and that the reconstriction rate after a DI is much faster. Stretching capacity to levels seen in healthy subjects is largely regained after chemical relaxation of the smooth muscle. These results implicate the smooth muscle as the primary defect in asthma and that it exists in a distinct state and environment that (1) allows far more force during constriction, probably creating substantial airway closures, and 2) is far too stiff to stretch and allow reopening during a deep breath. How it transitions to this state remains a crucial question and is likely related to increased thickness and decreased periodic stretching.

APPENDIX

RLS Algorithm

Although the details of this appendix are also in the literature, we provide them here for completeness. Consider the single compartment model of the lung (Eq. 1), such that a single Raw (i.e., Rs at 8 Hz) and a single Ei can be estimated for a given data set. The parameter vector to be estimated is $\theta = [\text{Raw}, \text{Ei}]$. To track $\theta$ during breathing maneuvers, we apply a RLS approach (1–3, 13, 16), which updates the estimate of $\theta$ with each new data point, $k$.

$$
\theta_{k+1} = \theta_k + \left[ \frac{C_k X}{(\rho + X^T C_k X)} \right] (Y_{k+1} - X^T \theta_k) 
$$

(2)

$$
C_{k+1} = \frac{C_k}{\rho} + \left[ \frac{C_k X}{(\rho + X^T C_k X)} \right] \frac{X^T C_k}{\rho}
$$

(3)

where $C_k$ is related to the variances of the parameters and $\rho$, the “forgetting factor,” is an exponential weighting factor ($0 < \rho < 1$). The forgetting factor is related to the memory time constant, $\tau$ (i.e., amount of previous data used to estimate Raw and Ei for a given point in time) by

$$
\tau = \frac{-\Delta t}{\ln (\rho)}
$$

(4)

where $\Delta t$ is the time between data points. Therefore, the smaller the forgetting factor the less previous data used to estimate the parameters. If $\rho$ is too small, the algorithm then becomes sensitive to noise. If $\rho$ is too large (~1), the algorithm is less sensitive to transient changes in the parameters. We chose $\rho = 0.90$, making $\tau = 0.1$ s, which equates to
about a cycle of an 8-Hz oscillation. The covariance matrix for
the RLS algorithm is also calculated recursively by

$$
\text{COV} (\theta_{k+1}^\prime) = r_{k+1}^\prime C_{k+1}
$$

(5)

where

$$
r_{k+1}^\prime = pr_{k}^\prime + (1 - \rho)[1 - X^T C_k X] e_{k+1}^2
$$

(6)

and

$$
e_{k+1} = Y_{k+1} - \theta_X
$$

(7)

$r_{k+1}$ is the noise variance estimate and $e_{k+1}$ is the model
prediction error. If the error of the estimate was greater than
10%, then it was discarded. In all cases we initialized the
algorithm from separate measures of the frequency dependence
of resistance and elastance for each subject by using a
covariance matrix with 1,000 on the diagonal and zeros elsewhere.

To correctly align lung volume and the output of Raw from
the algorithm in real time, one must first compensate for
inherent phase shifts created from the filtering and the RLS
algorithm. The frequency response of the high-pass filter was
measured independently. The higher the forgetting factor,
the slower the algorithm is at being responsive to capturing
real-time changes in the parameters. This translates into an
apparent phase difference between the estimate and the true
parameter changes. This shift is specific to the choice of $\tau$.
Using a simulation study on a single compartment model
(Eq. 1) and applying 8-Hz oscillation to a sine wave character-
istic of normal breathing, we calculated a phase shift of
0.2 s.

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