Bronchodilatory effect of deep inspiration on the dynamics of bronchoconstriction in mice

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Bates JH, Cojocaru A, Lundblad LK. Bronchodilatory effect of deep inspiration on the dynamics of bronchoconstriction in mice. J Appl Physiol 103: 1696–1705, 2007. First published September 20, 2007; doi:10.1152/japplphysiol.00698.2007.—We recently developed a computational model of an airway embedded in elastic parenchyma (Bates JH, Lauzon AM. J Appl Physiol 102: 1912–1920, 2007) that accurately mimics the time dependence of airway resistance on tidal volume and positive end-expiratory pressure (PEEP) following methacholine injection in normal animals. In the present study, we compared the model predictions of bronchodilation induced by a deep inflation (DI) of the lung following administration of the bronchial agonist methacholine to corresponding experimental measurements made in mice. We found that a DI in mice caused an immediate reduction in airway resistance when it was administered soon after intravenous injection of methacholine, while the airway smooth muscle was in the process of contracting. However, the magnitude of the reduction in resistance was greater and its subsequent rate of increase less than that predicted by the model. We found that this effect was most pronounced when the DI was given within ∼3 s following methacholine injection, again in contrast to the predictions of the model. The reduction of airway resistance was virtually independent of the rate of lung inflation during the DI, however, which agrees with model predictions. We conclude that while the model accounts for a substantial fraction of the post-DI reduction in airway resistance seen experimentally, there remain important differences between prediction and experiment that suggest that the effects of a DI are not simply due to eccentric contraction of the airway smooth muscle.

airway smooth muscle; airways hyperresponsiveness; airway-parenchymal interdependence; computational model

INTEREST IN THE ABILITY OF A DEEP INHALATION (DI) TO REVERSE BRONCHOCONSTRICTION HAS A LONG HISTORY because of its differential effects in subjects with asthma versus normal subjects (10). The physiological effects of a DI have been studied extensively in recent years in both humans (e.g., 1, 20, 21, 33, 34) and in animal models (e.g., 1, 17, 36). There are several hypotheses that have been proposed to explain how DIs reverse bronchoconstriction normally and why they may fail to do so in asthma (1). One is that some of the effect of a DI is due to stretch-activated release of a bronchodilatory mediator (8), which may be deficient in asthatics. Another possibility is that stretch affects airway smooth muscle (ASM) tone via neural pathways (23, 32). Alternatively, it has been suggested that a DI somehow directly affects the contractility of the ASM (11, 35) in a way that is different in asthmatics compared with normal subjects (27). Yet another hypothesis is that the effects of a DI are purely mechanical and that their lack of efficacy in asthmatics is either because the airway ASM is too stiff (21), the airway has become mechanically decoupled from the parenchyma (1), or differences exist in the relative hysteresis of airways and parenchyma that allow the airways to overcontract when the DI is released (45). These hypotheses are all biologically plausible and indeed may all be operative to varying degrees in different individuals. Their relative contributions in asthma, however, are still poorly understood (1).

The mechanisms by which a DI affects bronchoconstriction in healthy versus asthmatic lungs would become clearer if we knew how to predict the precise consequences of each of the candidate possibilities using accurate theoretical models. Although such models have yet to be developed for a number of the proposed mechanisms, current knowledge makes it possible to take this approach with regard to the mechanical hypotheses outlined above. Indeed, some sophisticated computational models of ASM contractility have been proposed (2, 12, 29). In the present study, however, we take the view that it is appropriate to start simple and add complexity as needed. A minimalist attempt to account for the dynamics of bronchoconstriction is represented by our recently developed computational model of an airway embedded in elastic parenchyma (5). The airway narrows due to the contraction of a circum-scribing ring of ASM, which itself is governed by a hyperbolic force-velocity relationship. This in turn determines the time course over which airway resistance increases once the ASM is activated. There are no humeral or neural effects on ASM tone caused by lung volume changes in this model, nor are there any alterations in the intrinsic ASM contractility. The way in which activated ASM becomes stretched in this model is simply through eccentric contraction caused by an increase in lung volume acting via the forces of airway-parenchymal interdependence. Nevertheless, this model accurately mimics the time dependence of airway resistance on tidal volume and positive end-expiratory pressure (PEEP) following methacholine injection in normal rats and rabbits (5).

The model described above also predicts that active bronchoconstriction will be transiently reversed by a DI through eccentric contraction of the ASM and that bronchoconstriction will become reestablished at its original level after lung volume is returned to baseline on completion of the DI. This model represents the simplest plausible hypothesis for explaining the dynamic effects of a DI on airway caliber in vivo so we felt that, at the very least, it should be applicable to normal experimental animals. Accordingly, in the present study we tested the bronchodilation hypothesis embodied in the model by comparing its predictions of post-DI airway resistance to corresponding experimental measurements made in mice. Our
goal was to determine if the simple mechanism embodied in the model is sufficient to explain the effects of the DI, or if we need to invoke some of the more complex mechanisms that have been previously proposed.

METHODS

Animal preparation. We studied female BALB/c mice, obtained from Jackson Laboratories (Bar Harbor, ME), at two different ages. The younger mice were 8–12 wk of age, while the older mice were maintained in the animal facility at the University of Vermont until they were ~6 mo of age.

Mice were anesthetized with pentobarbital sodium by intraperitoneal injection (90 ml/kg, diluted in PBS to 5 mg/ml) and tracheotomized, and an 18-gauge cannula was tied into the trachea. The mice were connected to a computer-controlled small animal mechanical ventilator (flexiVent, SCIREQ, Montreal, Quebec, Canada) for baseline mechanical ventilation at 200 breaths/min and a tidal volume of 0.2 ml against a PEEP of 25 cmH2O. The animals were paralyzed with an intraperitoneal injection of pancuronium bromide (0.8 μg/kg).

Our studies conformed to the National Research Council Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Effects of PEEP. To establish how PEEP affects the ability of the airways to narrow, we studied the effects of PEEP on methacholine responsiveness in a group of young mice (the Young PEEP group, n = 8) and a group of old mice (the Old PEEP group, n = 5). The experimental protocol began with the delivery of a deep inflation (DI) of the lungs to an airway pressure limit of 25 cmH2O. The animals were paralyzed with an intraperitoneal injection of pancuronium bromide (0.8 μg/kg).

Effects of DI. To establish how DI affects the ability of the ASM to shorten is affected differently if the ASM is mechanically loaded early vs. late in the contraction process, we studied the effects of timing of a DI after the onset of bronchoconstriction in a group of old mice (the DI Timing group, n = 6). The mice were subjected to a methacholine injection at a PEEP of 1 cmH2O exactly as described above, using the same volume perturbation (Fig. 1A). The injections were also given when the perturbation included a period of 1 s during which lung volume was increased by 1 ml at either 2–3 s, 5–6 s, or 8–9 s (Fig. 1B–D, respectively). The rate at which the volume was increased by 1 ml and then decreased again 1 s later was the maximum that could be delivered by the ventilator piston. Ten minutes were allowed to elapse between the challenges, the order of which was randomized in each animal.

Next, we assessed whether the ability of the ASM to shorten is affected by how quickly it is subjected to a mechanical load. To do this, we studied how the rate at which the lungs were inflated during a DI affected bronchial responsiveness in a group of young mice (the DI Rate group, n = 8). The mice were subjected to a methacholine injection at a PEEP of 1 cmH2O exactly as described above, using the same volume perturbation (Fig. 1A). The injections were also given

![Fig. 1. Examples of the various volume perturbations used in this study (left) and their corresponding pressure signals measured in the ventilator cylinder (right). Row A shows baseline volume perturbation (with no volume change) and the pressure signal it produces. Rows B–D exemplify the volume perturbations that contain 1-ml step volume changes applied from 2–3 s, 5–6 s, and 8–9 s, respectively. Row E shows the ramp volume perturbation. The methacholine injection was given at time t = 0.](http://jap.physiology.org/)
when the perturbation included a period of 1 s during which lung volume was increased by 1 ml between 5 and 6 s. These 1-ml lung volume increases were delivered at two different rates. In one case, the volume was delivered, as described above, in a step at the maximum rate of the ventilator piston (Fig. 1C), while in the other case volume was ramped up linearly over 0.5 s and then back down again at the same rate to produce a saw-tooth pattern (Fig. 1E). Ten minutes was allowed to elapse between the methacholine challenges, the order of which was randomized in each animal.

In a final group of old mice, we determined whether loading the ASM during shortening affected its ability to further shorten when stimulated with a subsequent dose of methacholine, to further test the responsiveness of the ASM. To do this, we delivered a second injection of methacholine 15 s after the first injection (the Second Injection group, n = 6). The mice were subjected to a methacholine injection at a PEEP of 1 cmH2O, which was immediately followed by a 24-s volume perturbation. The methacholine injections were repeated twice, once without changing lung volume (a 24-s version of the perturbation shown in Fig. 1A) and the other time with a 1-ml step increase in lung volume at 5 s that returned back to baseline again at 6 s (the perturbation used was identical to that shown in Fig. 1C except that it continued out to a duration of 24 s with the order of methacholine injection being given at 15 s). Ten minutes were allowed to elapse between the methacholine challenges, the order of which was randomized in each animal.

Calculation of impedance. The pressure and flow data sampled at 128 Hz during application of each volume perturbation were used to calculate the complex input impedance of the respiratory system (Zin) within a 2-s sliding window that moved across the 20-s data segment in steps of 0.125 s (41) after digital removal of the mechanical effects of the ventilator circuit, as previously described (13). This assumes stationarity of lung impedance within each 2-s window and thus gives an effective temporal resolution of 2 s. As we expect the mechanical properties of the lung to change smoothly and monotonically up to an effective temporal resolution of 2 s following methacholine injection, these limits on temporal resolution are unlikely to prevent us from gaining an accurate picture of how lung impedance evolves over this time period. Each estimate of Zin was fit to the equation of a lung model consisting of a single airway serving a constant-phase viscoelastic tissue unit, the so-called constant-phase model of Zin (16) described by the equation

\[ Z(\omega) = R + i\omega L + \frac{G - i\omega H}{\omega_0^2} \]  

where \( R \) is a Newtonian resistance composed mostly of the flow resistance of the conducting pulmonary airways (40), \( I \) reflects the inerterance of the gas in the central airways, \( G \) reflects viscous dissipation of energy in the respiratory tissues (tissue resistance), \( H \) reflects elastic energy storage in the tissues (tissue stiffness), \( \omega \) is angular frequency, \( i = \sqrt{-1} \), and \( \alpha \) couples \( G \) and \( H \) (16). Angular frequency in Eq. 1 is normalized to \( \omega_0 = 1 \text{rad/s} \) (so that \( R, G, \text{and} H \) all have units of cmH2O-s·m-1·l-1) (18). \( I \) has negligible effect in the mouse lung below 20 Hz, and so can be ignored (13). We thus obtained time courses for \( R, G, \text{and} H \) sampled at 8 Hz from 1 to 19 s after each injection of methacholine, with the exception of 3-s gaps any time a DI was given due to the exclusion of data measured while the DI was being delivered.

Computational model fitting. Our computational model of a contracting airway and the method we use to fit it to experimental data have been described in detail previously (5). For completeness, the following is a brief overview. We model an airway in two dimensions as a circular ring of ASM wrapped around an elastic airway wall embedded in homogeneously elastic lung parenchyma. When the ASM contracts it narrows the airway against the outward pull of the parenchyma that is attached to the outside of the airway wall. This outward pull comes from two sources: 1) the transpulmonary pressure (Ptp) that is transmitted across the parenchyma when it is undistorted (uniform and isotropic), which is determined by lung volume under the assumption of a constant tissue elastance; and 2) the local distortion of the parenchyma caused by narrowing of the airway, which is assumed to follow the relationship identified by Lai-Fook (24). The inward recoil of the airway wall is determined by its stiffness, which is assumed to arise from a fraction \( (1 - k) \) of the airway circumference that expands according to the one-third power of Ptp. The remaining fraction, \( k \), of the circumference is assumed to be inextensible, where \( 0 < k < 1 \). Once activated, the ASM follows the classic Hill force-velocity relationship (15). This relationship is hyperbolic when active force \( (F_A) \) is less than isometric force \( (F_0) \), and linear when \( F_A \approx F_0 \) with slopes matched at \( F_0 \) thus

\[ \frac{dF}{dt} = \frac{b(F_0 - F_A)}{a + F_A} \text{ when } F_A < F_0 \]

\[ = \frac{bF_0}{a + F_0} - \frac{bF_A}{a + F_A} \text{ when } F_A \approx F_0 \]  

where \( r \) is airway radius, and \( a \) and \( b \) are constants (see Fig. 1B of Ref. 4). Following experimental findings reported in rats (7), we set \( a = F_0/4 \). Equation 2 thus contains two free parameters, \( F_0 \) and \( b \). We also assume that \( a \) and \( b \) are independent of ASM length. This is not entirely true, of course, but ASM does have a rather flat peak on its force-length relationship (22) so we consider it a reasonable first approximation that greatly simplifies the modeling.

At any point in time, \( F_A \) is the force that adds to the outward recoil of the parenchyma and the inward recoil of the airway wall to give a net force difference of zero. The explicit expression for \( F_A \) that this produces is derived in Ref. 5 and is given by

\[ F_A = \left[ \frac{r_{\text{TLC}}(P_{\text{Ptp}} - P_{\text{TLC}})}{P_{\text{TLC}}} \right]^\frac{1}{3} - 0.3r \left( \frac{P_{\text{Ptp}}}{P_{\text{TLC}}} \right)^{0.7} \]

\[ - r(P_{\text{TLC}} - \frac{1}{1 - k})^\frac{r}{(1 - k)r_{\text{TLC}} + k} \]  

where \( r_{\text{TLC}} \) is the radius that the virtual hole occupied by the airway would have at total lung capacity (TLC) if it expanded like the rest of the parenchyma, \( P_{\text{TLC}} \) is Ptp at TLC, and \( P_{\text{Ptp}} \) is the value of Ptp at which the uncontracted airway induces no distortion in the parenchyma surrounding it.

We used the above equations to calculate how \( r \) varies with time when the model is driven with a prescribed volume signal that, when multiplied by lung elastance, produces a time-varying Ptp(t) signal. Initially, the ASM was relaxed so that \( F_A = 0 \) and \( r \) was determined by the force balance between the inward recoil of the airway wall and the outward recoil of the surrounding parenchyma. Once the ASM in the model was activated, \( F_A \) was given by Eq. 3. A constant level of activation was then assumed so that, at each time step of 0.0625 s, Ptp(t) was used in Eq. 3 to determine F_A. The result was substituted into Eq. 2 to produce dF/dt, which was then used to determine r at the next time step using first-order Euler integration. This new value of r was then used in Eq. 3 again to determine the next value for F_A, and so on, until a complete time profile of r was produced. Finally, invoking the assumption of Poiseuille flow through the airway, a normalized airway resistance (R) profile was calculated by raising \( r_{\text{TLC}}/r \) to the fourth power.

The model was driven by a volume signal that varied sinusoidally above functional residual capacity (FRC) at a frequency of 1 Hz. The amplitude of the sinusoid was chosen so that it produced excursions in Ptp similar to those that would be predicted to occur in the mice if they were ventilated sinusoidally with the same peak-peak excursion of the ventilator piston as used to deliver the volume perturbations.
This neglects any loss of ventilator volume due to gas compression in the ventilator circuit, but as lung elastance was \( \sim 20 \text{ cmH}_2\text{O/ml} \) (see below) and the elastance of the gas in the ventilator circuit was \( \sim 140 \text{ cmH}_2\text{O/ml} \), this amounts to a volume of loss of \( \sim 15\% \), which, considering the simplistic nature of the model, is unlikely to have a significant bearing on our conclusions. FRC was chosen so that, using a nominal lung elastance of 5 (arbitrary units), the inflation pressures in the airway at the start of each simulation matched the experimental PEEP levels. Each model simulation was generated by choosing values for the parameters \( b \) and \( F_0 \) in Eq. 2 and \( k \) in Eq. 3, and then generating \( R \) signals at each of the three PEEP levels of 1, 3, and 6 cmH2O. The resulting \( R \) signals were scaled by a single factor so that they matched, in a least-squares sense, the corresponding experimental \( R \) signals. The model thus has four free parameters: the scale factor just described, together with \( b, F_0, \) and \( k \). We found previously (5) that the quality of the model fit is very insensitive to the value of the other wall stiffness parameter, \( P_0 \), so we fixed this parameter at 10 cmH2O. The best-fit values of the four free parameters were found using a grid search as previously described (5). We determined the sensitivity of each fitted parameter to the data by keeping the other parameters fixed at their best-fit values while adjusting the parameter in question either side of its best-fit value until the mean squared residual increased 5\% above its minimum value.

RESULTS

Figure 2 shows the time courses of \( R, G, \) and \( H \) obtained following methacholine injection at three different PEEP levels in the young and old mice (Young PEEP and Old PEEP groups, respectively). As expected, increasing PEEP from 1 to 6 cmH2O caused the rate of increase of \( R \) to decrease substantially (Fig. 2, top). PEEP also had a mitigating, although less pronounced, effect on \( G \) (Fig. 2, middle). By contrast, \( H \) was essentially unaffected by the injection of methacholine (Fig. 2, bottom).

Figure 3 shows the time courses of \( R \) from the Young PEEP and Old PEEP groups together with the fits provided by the computational airway model to the mean data. In both cases, the model is able to accurately describe the variation of \( R \) with both time and PEEP. The best-fit model parameter values and their sensitivity ranges are listed in Table 1.

Figure 4, top, shows the \( R \) time courses obtained in the DI Timing group. (Note that data are not available during those periods when the 1-ml inflation of the lungs were applied, including 1 s either side of these periods due to the analysis window width.) \( R \) always followed the same trajectory before a DI. However, the DI brought \( R \) down to a reduced level from which it then proceeded to increase again. However, post-DI \( R \) remained below \( R \) obtained without a DI for the duration of the measurement period. Furthermore, at the end of the measurement period, the rank ordering of \( R \) from the three post-DI maneuvers was not the same as the temporal order of the DIs. That is, the earliest DI (2–3 s) resulted in a final value of \( R \) that was greater than that from the latest DI (8–9 s) but less than from the intermediate DI (5–6 s). By contrast, DI had essentially no effect on \( H \) (Fig. 4, bottom), and only a modest effect on \( G \) (Fig. 4, middle), similar to the relative effects of PEEP on the three impedance parameters (Fig. 2).

Figure 5A shows the same \( R \) data as in Fig. 3, except here when a DI was given we do not show the pre-DI values of \( R \) in order to make the plot less cluttered. In Fig. 5B we show corresponding simulations obtained using the computational airway model. The values of the adjustable model parameters \( (F_0, a, b, \) and \( k) \) were set equal to those estimated from the PEEP trial applied to the old mice (Table 1), with an initial activation delay of 2 s to match the curves fitted to the PEEP data (Fig. 3, bottom). Four simulations with the model were performed. In the first simulation, the model was driven with a 1-Hz sinusoidal volume waveform with an amplitude that generated similar peak-peak pressures as observed in the experimental animals during the 16-s measurement period. The \( R \) signal produced by this simulation was scaled to match the corresponding experimental \( R \) signal. In the other three simu-

![Fig. 2. Time courses of the impedance parameters \( R \) (Newtonian resistance), \( G \) (tissue resistance), and \( H \) (tissue stiffness) (Eq. 1) in young (8–12 wk) and old (6 mo) mice following intravenous injection of a methacholine bolus given at time = 0 s. The lungs were inflated at the 3 different PEEP levels indicated before the injections. The different symbols indicate mean ± SE.](http://jap.physiology.org/)
lations, the volume waveform included a 1-s step inflation applied between 2–3, 4–5, or 8–9 s with an amplitude that generated a peak pressure of \(30 \text{ cmH}_2\text{O}\), which was similar to that produced by the volume steps in the experimental animals (Fig. 1, B–D, left). The resulting \(R\) profiles were also scaled by the same factor used to scale the first simulation. The control \(R\) profile generated from the first simulation, without a volume step, is very similar to that observed experimentally under similar conditions (compare closed circles in Fig. 5, A and B). The simulated \(R\) profiles obtained with the three volume steps are lower than the control profile, as was seen experimentally, but the simulated profiles exhibit two important differences compared with the experimental data. First, the amount by which \(R\) is reduced following a DI in the model is less than that observed experimentally by more than an experimental SE at virtually all time points. Second, the rank ordering of the reduction in \(R\) in the model is the same as the ordering of the timing of the DI (i.e., the earlier the DI, the closer is \(R\) to its control value at 15 s), in contradistinction to the situation with the experimental data.

Figure 6A shows the \(R\) time courses from the DI Rate group, which compares how the effect of rate of lung inflation affects the subsequent reduction in \(R\). Before either a fast or a slow DI from 5– 6 s, \(R\) followed a time course (not shown) that was identical to that of the control experiment in which a DI was not given (triangles). Following both the fast and slow DI, \(R\) was substantially reduced, although very slightly less so by the slow DI (closed circles) than the fast DI (open circles). Figure 6B shows the corresponding model simulations of \(R\) obtained when a DI was also given from 5– 6 s, either as a step or a ramp

![Fig. 3](image)

![Fig. 4](image)

### Table 1. Best-fit values for the parameters of the computational airway model fitted to the mean R time-course data from the Young PEEP and Old PEEP groups shown in Fig. 3

<table>
<thead>
<tr>
<th></th>
<th>(F_0) cm·cmH(_2)O</th>
<th>(a) cm·cmH(_2)O</th>
<th>(b), cm/s</th>
<th>(k)</th>
</tr>
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<tbody>
<tr>
<td>Young PEEP group</td>
<td>5.06 (5.37, 4.81)</td>
<td>1.28 (1.41, 1.16)</td>
<td>0.039 (0.042, 0.037)</td>
<td>0.717 (0.721, 0.714)</td>
</tr>
<tr>
<td>Old PEEP group</td>
<td>3.81 (3.98, 3.64)</td>
<td>0.95 (1.03, 0.87)</td>
<td>0.049 (0.051, 0.046)</td>
<td>0.714 (0.717, 0.710)</td>
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The values of each parameter either side of the best-fit value that caused the mean squared fitting residual to increase by 5% are given in parentheses. PEEP, positive end-expiratory pressure.
to match the experimental protocol (Fig. 1, C and E, respectively). Here, the model was assigned the parameter values determined for the young mice (Table 1), since the mice in the DI rate group were also young. Again, the control simulation in which no DI was given is close to the corresponding experimental profile obtained without a DI (compare triangles). However, similar to what we see in Fig. 5, the model predicts a DI-induced reduction in \( R \) that is less than was actually observed experimentally by more than the experimental SE at all time points. Also, by 15 s, the post-DI \( R \) predicted by the model has almost caught up with the control value (Fig. 5B), whereas in the experimental data, \( R \) remains at \( \sim 60\% \) of control \( R \) at this time point.

Figure 7A shows the \( R \) time course from the Second Injection group in which a DI was given from 8–9 s and a second methacholine bolus was administered at 15 s. As before, the DI produced a substantial reduction in \( R \). The second bolus of methacholine caused the control (no DI) \( R \) profile to accelerate slightly after 15 s, instead of approaching an asymptote. A similar effect is observed in the post-DI \( R \) profile, which follows a lower but almost perfectly parallel course to the control \( R \). Figure 7B shows the corresponding model simulations. Here, the model received an extra activation at 16 s to correspond to the second methacholine bolus at 15 s and allowing for a 1-s circulation delay. The extra activation was achieved by suddenly increasing the three parameters controlling the activation of the ASM (\( F_0, a, \) and \( b \)). We found by trial and error that an increase of 50% in these parameters resulted in an increase in the simulated \( R \) similar to that observed experimentally. Although the simulated \( R \) profile obtained with no DI shows a series of two slightly concave sections that are not apparent in the experimental data, the overall scope of the changes are similar to those observed experimentally (compare closed circles). Immediately following a DI, \( R \) in the model is reduced by an amount similar to that observed experimentally. However, as time proceeds, \( R \) in the model virtually catches up to its control value (Fig. 7B), whereas it remains substantially reduced in the experimental data (Fig. 7A).

**DISCUSSION**

The overall goal of this study was to gain a better understanding of how DIs reverse bronchoconstriction by examining the extent to which a simple computational model of a dynamically narrowing airway is able to reproduce experimental findings. The ability of a DI to reverse bronchoconstriction is apparently different in normal individuals compared with at least some asthmatic subjects (1, 20, 21, 33, 34) for reasons that are not completely understood. Even less well understood is the phenomenon of bronchoprotection, whereby DIs given before a bronchial challenge have the capacity to mitigate...
the bronchoconstriction that caused the changes in $b$ and more modest changes in $g$. These were accompanied by qualitatively similar but $P E E P$ following intravenous methacholine injection (Fig. 2, marked negative dependence of the rate of change of $R$). The observed time courses of $R$ are therefore reflective of the way in which $P E E P$ affects the ability of ASM to narrow the airways. The computational airway model that we fit to these data is based on the notion that the ensemble behavior of all the conducting airways can be usefully represented in terms of what happens to a single effective airway. In other words, the dynamics of narrowing of this single airway is assumed to represent some kind of average of the behaviors of all the branches in the airway tree. Of course, it is inconceivable that all the individual airway branches would be following precisely the same narrowing profile as bronchoconstriction proceeds. On the other hand, the majority of the airways may well narrow at rates that, relative to their baseline diameters, followed a unimodal distribution and therefore could be fairly represented by a single airway narrowing at the mean rate. In any case, the model accurately accounts for the way in which $R$ varies with both time and $P E E P$ (Fig. 2). This mirrors our previous findings in rats (5), with the relative values of the model parameters (Table 1) reflecting the differences in species size. These findings, along with the plausible physiological bases for the various model components, gives us a degree of confidence that the model embodies the key mechanisms responsible for the $P E E P$ dependence of airways responsiveness in normal mice. This confidence is enhanced by the fact that the model was equally successful in describing the data from both young and old mice. Age-related differences in the rheological properties of lung tissue have been postulated to contribute to the relative prevalence of childhood asthma and its frequent resolution with age (39). The results of the present study show that any such age-related differences in the nature of the parenchyma did not affect the ability of the airway model to describe the effects of parenchymal tethering on airway caliber.

The effect of a DI on mouse lung mechanics also appears to be confined to the conducting airways (Fig. 4, top) because $H$ was not noticeably altered by DI (Fig. 4, bottom), and the modest effects on $G$ (Fig. 4, middle) can again be ascribed to increases in airway narrowing heterogeneity that are temporarily alleviated when the airways are stretched open. This led us to wonder how well the computational airway model might account for the manner in which a DI reverses bronchoconstriction, given that it had been so successful in describing the time and $P E E P$ dependence of $R$ when only small oscillatory volume changes were applied to the lungs (Fig. 3). We therefore applied similar DIs to the model as were applied experimentally. Figure 5 shows that while there are some similarities between the observed and predicted $R$ time courses, it is clear that post-DI reduction in responsiveness was greater in the subsequent airway narrowing, although again this effect is not the same in normal subjects vs. asthmatic subjects (37). While bronchoprotection has been difficult to demonstrate in animal models (17), bronchodilation in animals is readily invoked (17, 36, 42). Even so, the factors that determine how much bronchodilation occurs with a DI, and how rapidly the airways renarrow afterward, have not been fully characterized or explained. Our motivation for the present study was based on the notion that by making predictions with a computational model of a contracting airway, we would be able to infer the extent to which the mechanisms embodied in the model account for the observed effects of a DI, which would then help to elucidate gaps in our understanding of the mechanisms behind the phenomenon of bronchodilation.

Before considering how the model predictions compare with experimental data, however, we must consider further the nature of the data itself. First, as expected, we observed a marked negative dependence of the rate of change of $R$ on $P E E P$ following intravenous methacholine injection (Fig. 2, top). These were accompanied by qualitatively similar but more modest changes in $G$ (Fig. 2, middle). However, there was essentially no change in $H$ (Fig. 2, bottom), indicating that the bronchoconstriction that caused the changes in $R$ did not affect the intrinsic stiffness of the tissues and did not induce closure of small airways, both of which would have caused $H$ to rise (3, 42). The modest elevations in $G$ could possibly have been due to an effect on the tissues, although why such changes should be limited to the dissipative and not the elastic properties of the tissue is unclear. These changes in $G$ are, therefore, probably more likely to reflect nonuniform narrowing of airways leading to regional time-constant differences throughout the lung (3, 28). Taken together, these results thus indicate that intravenous methacholine in BALB/c mice causes a central airway constriction with little involvement of the lung periphery, in agreement with a recent previous study from our laboratory (43).
model than in the mice. Also, the experimentally observed suppression of responsiveness was greatest for the earliest DI (2–3 s following methacholine injection). The observed R time courses from the middle and late DIs (5–6 s and 8–9 s, respectively) bear the same relationship to each other as the model predictions but are both displaced downward significantly. Thus the airways of the mice did not respond to a DI precisely as the model predicts. In other words, the ASM in the mice did not undergo eccentric contraction simply according to a single force-velocity relationship that itself was unaffected by the DI.

This raises the question as to what could have accounted for the differences between the experimental and predicted post-DI R profiles shown in Fig. 5. First, we consider the exaggerated decrement in responsiveness caused by the early DI at 2–3 s. This DI was given just as R was just beginning to increase above baseline (Fig. 4), which presumably coincides with very early events in the process of ASM contraction. One possibility, therefore, is that the DI mechanically interfered with these early contraction events, which have been postulated to involve rapid cross-bridge cycling. This could have caused a greater decrement in subsequent ASM shortening than if the interference had occurred later in the contraction process when latch-bridges were maintaining stress but not contributing much to shortening. Li and Stephens (26) found evidence for such an effect in vitro, although we were unable to find similar evidence in a previous in vivo study in dogs (36). Another possible effect of the early DI was that it interfered with the delivery of the methacholine to the lungs by raising intrathoracic pressure and reducing blood flow at the critical point where the injected methacholine had just started to move into the pulmonary circulation. DIs given at later times, while no doubt still affecting pulmonary blood flow, would have been too late to affect the amount of methacholine reaching the lungs. On the other hand, one might expect methacholine delivery to be merely retarded by this process rather than reduced. Also, the chest wall of the mouse is highly compliant so the amount by which a DI could have raised intrathoracic pressure is limited.

The reasons for the enhanced reduction in responsiveness with the early DI are thus not entirely clear. Nevertheless, DIs given even at 8–9 s after methacholine injection still yielded R profiles (Fig. 5A) that were substantially lower than model predictions (Fig. 5B). Also, when a DI was given both quickly as a step change in lung volume from 5–6 s, and as a much slower change that ramped up to the same total volume change and down again in a linear fashion, R was significantly reduced in both cases and remained well below the control (no DI) R for the duration of the measurement period (Fig. 6A). By contrast, the computational model (Fig. 6B) predicts a somewhat smaller decrement in R that then accelerates after the DI and nearly catches up with the control R at the 15-s time point. Here, as in Fig. 5, we again see that the mice experienced a reduction in responsiveness following DI that is not explicable simply on the basis of the eccentric ASM contraction embodied in the computational model. Interestingly, however, the relationship between the step and ramp DI profiles predicted by the model is almost identical to that observed experimentally (compare the relative positions of the open and closed circle in Fig. 6, A and B). The reason for this difference in the model (Fig. 6B) is that when the DI is applied as a ramp, its descent begins 0.5 s earlier than when it is applied as a step. This allows for a small amount of ASM shortening to take place even as lung volume is in the process of decreasing, something that cannot happen when the DI is maintained right up to the instant of its removal as a step. Consequently, the airway is slightly more narrowed at the instant when the ramp DI is finished than when the step DI is finished. The same mechanism may have accounted for the similar differences in ramp and step DI profiles seen experimentally (Fig. 6A), which would indicate that any effect on the contractility of the ASM itself was the same for both fast and slow DIs. Interestingly, Skloot et al. (38) found that a rapid DI given before bronchial challenge in healthy humans was bronchoprotective against subsequent airway narrowing, as assessed by the nature of forced expiratory flow, while a slow DI was not. This suggests that the mechanism of bronchoprotection may be different from that responsible for bronchodilation in the mice of the present study.

As a further test of the idea that airway responsiveness was reduced, we performed a DI from 8–9 s as before, but this time followed it with a second intravenous injection of methacholine ~7 s later. Compared with R obtained with the second injection but without a DI, we again found that R was reduced, and this time it remained reduced even out to 24 s (Fig. 7A). By contrast, the model simulations of this experiment (in which we simulated the second methacholine injection by increasing the activation of the ASM) predict that the reduction in R caused by the DI will be all but made up for by 24 s (Fig. 7B). In other words, the mice continued to exhibit a decreased response to methacholine following the additional methacholine injection. Furthermore, this could not have been due to a DI-induced affect on pulmonary blood flow because the second methacholine bolus was given well after the DI.

Taken together, our experiments point to a transient reduction in airways responsiveness following a DI in mice that depends on the depth but not the rate of the DI and that is not affected by the maturity of the animals. This DI effect does not involve alterations in the delivery of the methacholine to the ASM, except possibly when the DI is given within ~2 s of the methacholine injection. The effect lasts for at least several seconds because it was still present at the end of our 16- to 24-s measurement periods. On the other hand, all mice in each experimental group underwent each DI intervention in random order, with a wait of 10 min between successive methacholine injections. As a further test of the idea that airway responsiveness was
can have a significant effect on bronchomotor tone (32) but is perhaps also unlikely to account for the reduction in responsiveness seen in the present study because lung volume was returned to baseline for the subsequent tracking of R, so one would expect that stretch receptor signaling would also have been returned to baseline following the DI.

It is possible, therefore, that the explanation for the transient effect of a DI on airways responsiveness in our mice is mechanical in nature. In support of this possibility is the fact that Noble et al. (31) found a time scale of recovery from stretch in isolated bronchial segments that was similar to that which seems to pertain to the data of the present study. Similar recovery dynamics were also found by Fabry et al. (9) in strips of activated ASM subjected to sudden stretch. Gunst et al. (14) postulated that reductions in ASM contractility could be caused by disruptions in the organization of cytoskeletal and contractile proteins. On the other hand, Fredberg et al. (12) found that disruption of cross-bridge binding could not account for all the effects of ASM stretch on contractility and so concluded that plastic changes in cell structure must also have occurred. Wang et al. (44) arrived at a similar conclusion in studying the reduced responsiveness of ASM caused by stretching before activation.

A direct effect on ASM contractility is not the only mechanical explanation for an effect of DI on airways responsiveness, however. As Noble et al. (30) point out, structures within the airway wall may exert a significant influence on the response to a DI. ASM consists of individual cells that are in some way physically attached to each other and to the other tissues of the airway wall. Ito et al. (19) noted that ASM cells need to maintain strong mechanical interactions with the extracellular matrix if they are to contribute effectively to airway responsiveness. Only then will any shortening accomplished by an individual ASM cell be transmitted to an equivalent degree of shortening of the airway circumference (assuming that the cells are arranged circumferentially). However, the structures that attach ASM cells to each other and to the airway wall are almost certainly viscoelastic, which means that they will respond to a large stretch by becoming temporarily slack. This may not necessarily affect the ability of the ASM to shorten, but it will temporarily decouple the ASM cells from the airway wall. Thus, when the ASM cells contract, their shortening will be taken up by the slackness in the attachments rather than being translated directly into narrowing of the airway. These attachments could also be intracellular, involving connections between contractile and structural proteins within a single ASM cell, but the effect on airway shortening would be similar. Indeed, Fabry et al. (9) studied the time course of recovery of force generation in activated strips of ASM and found evidence for multiple processes, which they interpreted as reflecting a disruption of both cross-bridge cycling kinetics and the cytoskeleton.

In any case, the mechanical explanation for the effects of a DI on airways responsiveness seems to invoke the notion of circumferential interdependence, either within individual ASM cells or between ASM cells and airway wall tissue. This concept stands in contradistinction to the usual notion of mechanical interdependence in the lung, which is viewed as a mechanism for radial force transmission between that airway wall and its parenchymal attachments. Radial interdependence appears to be mediated by elastic fibers that connect between the adventitial connective tissue and the epithelial basement membrane (25). Circumferential interdependence, by contrast, refers to elastic hoop stresses generated by the stretching of connective tissue within and between ASM cells in the airway wall. In the experiments reported here, it appears that we were only able to temporarily reduce these circumferential interdependence forces with a DI. One wonders, however, if they could have been further reduced, or even permanently disrupted, by a deeper or more rapid inflation of the lungs. Exploring this issue was beyond the capabilities of our experimental apparatus, and as it was we inflated the lungs from FRC to TLC in a small fraction of 1 s (Fig. 1), so it is not clear how much more vigorously we could have delivered a DI without causing lung injury. Nevertheless, we still have to wonder if it would be possible to irreparably damage the attachments of the ASM to each other and to the airway wall with a DI of suitable speed and depth, and if this might serve as a means of permanently alleviating airways hyperresponsiveness.

In summary, we have shown, with the aid of a simple computational model of a dynamically contracting airway embedded in elastic parenchyma, that a DI in normal mice administered while the ASM is in the process of contracting causes an immediate reduction in airway responsiveness. This effect persists for at least a matter of seconds but recovers within 10 min. Our data suggest that alterations in pulmonary blood flow are not involved in the response to DI except when the DI is given within 2–3 s of the instant of methacholine injection, corresponding to the time when agonist has just reached the lung from the injection site. We suggest that the most likely explanation for the post-DI reduction in airway responsiveness is a temporary reduction in the forces of circumferential interdependence between the ASM and the airway wall caused by the sudden stretching of the viscoelastic attachments involved. We speculate that inflammatory alterations in the nature of these attachments might play a role in the altered DI response seen in some asthmatic patients and that permanent disruption of the attachments might be a goal for treating airways hyperresponsiveness.

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