Exaggerated airway narrowing in mice treated with intratracheal cationic protein


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Submitted 22 August 2005; accepted in final form 17 October 2005

Bates, Jason H. T., Scott S. Wagers, Ryan J. Norton, Lisa M. Rinaldi, and Charles G. Irvin. Exaggerated airway narrowing in mice treated with intratracheal cationic protein. J Appl Physiol 100: 500–506, 2006. First published October 20, 2005; doi:10.1152/japplphysiol.01013.2005.—Airway hyperresponsiveness in mice with allergic airway inflammation can be attributed entirely to exaggerated closure of peripheral airways (Wagers S, Lundblad LK, Ekman M, Irvin CG, and Bates JHT. J Appl Physiol 96: 2019–2027, 2004). However, clinical asthma can be characterized by hyperresponsiveness of the central airways as well as the lung periphery. We, therefore, sought to establish a complementary model of hyperresponsiveness in the mouse due to excessive narrowing of the airways. We treated mice with a tracheal instillation of the cationic protein poly-L-lysine (PLL), hypothesizing that this would reduce the barrier function of the epithelium and thereby render the underlying airway smooth muscle more accessible to aerosolized methacholine. The PLL-treated animals were hypersensitive to methacholine: they exhibited an exaggerated response to submaximal doses but had a maximal response that was similar to controls. With the aid of a computational model of the mouse lung, we conclude that the methacholine responsiveness of PLL-treated mice is fundamentally different in nature to the hyperresponsiveness that we found previously in mice with allergically inflamed lungs.

respiratory impedance; computational model; airway hyperresponsiveness; asthma

AIRWAY HYPERRESPONSIVENESS is a characteristic feature of asthma (25) and is thought to involve exaggerated narrowing of both central and peripheral airways (19–21). Our laboratory recently reported, however, that hyperresponsiveness in the most commonly used animal model of asthma, the mouse with allergic airway inflammation, can be attributed entirely to accentuated airway closure, without any evidence to support an increase in the degree of airway smooth muscle shortening (29). The allergically inflamed mouse thus appears to manifest only a limited set of the various functional abnormalities that may be present in clinical asthma. Indeed, asthma appears to be a particularly human disease, and attempts to recreate all of its attributes in a single animal model have been rather unsuccessful, despite many attempts (2, 5, 15, 30).

It may thus serve us better to pursue the development of a set of animal models that collectively encompass the full spectrum of abnormalities seen in asthma but that individually represent only part of this spectrum. This would allow us to examine the effects of each abnormality individually and perhaps even to study the interactions between abnormalities, if more than one can be implemented simultaneously in the same animal. In this sense, the acutely allergically inflamed mouse is a useful representation of one kind of hyperresponsiveness, namely that which occurs when an inflamed mucosa encroaches into the airway lumen. Such encroachment amplifies the increases in airway resistance and airway closure caused by a normal degree of smooth muscle shortening. This is by no means the only mechanism for producing hyperresponsiveness, however, and significant gaps remain in the currently available set of animal models embodying other mechanisms. The goal of the present study was, therefore, to establish a mouse model, complementary to the allergically inflamed preparation, in which hyperresponsiveness is due to enhanced narrowing of the conducting airways.

On the basis of previous work, we hypothesized that increased airway narrowing would occur in mice treated with poly-L-lysine (PLL), an artificial analog of native cationic protein. Our laboratory has previously shown that PLL permeabilizes epithelial cells (28) and that rats treated with intratracheal PLL are hypersensitive to methacholine that is delivered as an aerosol but not injected intravenously (16). These observations support the notion that PLL increases bronchial responsiveness by an epithelium-dependent mechanism, apparently making the underlying smooth muscle more accessible to an inhaled agonist. Such an effect would be expected to lead to increased contraction of the smooth muscle, and hence exaggerated airway narrowing, when the doses of methacholine are submaximal. By contrast, when the methacholine dose is supramaximal, the airway smooth muscle should contract maximally, regardless of how quickly the agonist is able to traverse the epithelium. Furthermore, at all doses, we would expect airway narrowing to be more rapid than normal, due to enhanced penetration of agonist to the underlying smooth muscle. The PLL-treated mouse should, therefore, constitute a model of airways hypersensitivity. To test these notions, we examined the time course of respiratory impedance following administration of increasing doses of methacholine aerosol to mice pretreated with intratracheal PLL. As in our laboratory’s previous study in allergically inflamed mice (29), we interpreted the results by performing equivalent virtual experiments on an anatomically based computational model of the mouse lung.

METHODS

Animal experiments. All protocols were approved by the University of Vermont Institutional Animal Care and Use Committee. We studied a group of 6- to 10-wk-old BALB/c (Jackson Laboratories, Bar Harbor, ME) mice (n = 8, 20.8 ± 2.2 g) treated with intratracheal PLL and a corresponding control group of mice (n = 7, 20.2 ± 0.8 g) treated with intratracheal PBS. A preliminary analysis of the data...
showed that one animal in each group had poor impedance spectra that were not fit well by a model of impedance (see below). One additional animal in the PLL group had a dose-response curve that was substantially different from the others in the group. The remaining animals (n = 6 in each group) were tightly grouped and were selected for consideration in this study. The mice were anesthetized with an intraperitoneal injection of pentobarbital sodium at a dose of 90 mg/kg and then tracheostomized. A length of polyethylene tubing was passed into the trachea to allow instillation of 50 μl of PLL in PBS at a concentration of 2 mg/ml, followed by two or three 0.3-ml aliquots of air to push the PLL into the lung, as previously described (6, 16). Next, a 19-gauge metal cannula was secured in the tracheal opening and connected to a mechanical ventilator (FlexiVent, Scireq, Montreal, QC). Mechanical ventilation was administered for 30 min before the start of the experiment, at which point one-fourth of the original dose of pentobarbital was given. Baseline mechanical ventilation was applied at 180 breaths/min with a tidal volume of 0.25 ml against a positive end-expiratory pressure of 3 cmH2O applied by a water trap.

The experimental protocol began with the normalization of lung volume history by the delivery of two deep lung inflations of 1.0 ml followed by 2 min of regular ventilation. Mice were then challenged with an aerosol of PBS for 40 s, achieved by channeling the inspiratory flow from the ventilator through an ultrasonic nebulizer containing PBS. During the challenge, the piston of the ventilator was programmed to deliver a tidal volume of 0.8 ml at a rate of 30 breaths/min. Due to the shunt compliance of the nebulizer chamber, however, the tidal volume actually delivered to the lungs was ~0.6 ml. Following cessation of aerosol delivery, the ventilatory rate and tidal volume were returned to baseline, and measurements of the complex input impedance of the respiratory system (Zrs) were made at regular intervals for the next 3 min. Finally, two more deep lung inflations were given. The above protocol was then repeated three more times with aerosols containing methacholine in PBS at sequentially increasing concentrations of 3.125, 12.5, and 50 mg/ml. Responses were obtained for all concentrations in all animals studied, with the exception of one of the animals in the saline group from which data at 50 mg/ml were not obtained.

During the 3 min following delivery of each aerosol challenge, every 10 s of regular ventilation were terminated in a 1-s passive expiration followed by a 2-s broadband (1–19.625 Hz) volume perturbation, after which ventilation was immediately resumed. The peak-to-peak excursion of the ventilator piston during delivery of these perturbations was 0.17 ml above functional residual capacity, a peak-to-peak excursion of the ventilator piston during delivery of which data at 50 mg/ml were not obtained.

The model consists of a single airway having a Newtonian resistance (Rn) that serves a uniformly ventilated tissue compartment that has a constant-phase impedance. The model is described by the equation

\[ Z_{rs}(f) = R_n + i2\pi f Iaw + \frac{G - iH}{2\pi f^2} \]  

where Iaw is the inertance of the gas in the central airways, G reflects viscous dissipation of energy in the respiratory tissues, H reflects elastic energy storage in the tissues, f is frequency, \( i = \sqrt{-1} \), and \( \alpha \) couples G and H. Following the approach of Ito et al. (18), we consider f in the above equation to be normalized to the frequency at which \( 2\pi f = 1 \), so that G and H both have the same units as Rn, namely cmH2O·s·ml⁻¹. This model has been shown to accurately describe Zrs between 0 and 20 Hz under control conditions and during mild bronchoconstriction (12, 13, 24). Our laboratory has found previously (29) that Iaw has a negligible effect in the mouse lung <20 Hz, no doubt because the mass of the gas in the mouse trachea is so small. Also, the tracheal cannula bypasses a significant fraction of the trachea, and the impedance of the cannula itself is removed in the calculation of Zrs. Consequently, Iaw cannot be estimated reliably from Zrs <20 Hz and so will not be considered further.

**Virtual experiments.** As previously described (29), we also performed virtual experiments using a computational model of the mechanics of the mouse lung based on the asymmetrical airflow branching scheme of Horsfield (11). We assumed Poiseuille flow in each airway to calculate its flow resistance. Together with the mass of gas contained in the lumen, this gives airway impedance (Zaw) as:

\[ Z_{aw}(f) = \frac{8\mu L}{\pi r^4} + i\frac{2\mu L}{r} \]  

where \( r \) is airway radius, \( L \) is airway length, \( \mu \) is the viscosity of air, and \( \rho \) is the density of air. We neglected the influence of airway wall shunting on Zrs by assuming that the airways are rigid during the application of the oscillatory volume perturbations used to determine Zrs.

As in our laboratory’s previously study (29), each of the most distal airways terminated in an identical tissue unit with impedance Zti, given in analogy to Eq. 1 by

\[ Z_{ti}(f) = \frac{Gti - iHti}{(2\pi f)^\alpha} \]  

where Hti is the constant-phase elastic parameter for each individual tissue unit, and Gti is the corresponding dissipative tissue parameter. Gti is equal to the product of Hti and the unit hysteresivity, \( \eta \) (8).

The total impedance of the model, Zmod, was calculated by adding the individual Zaw and Zti in series or parallel, as appropriate, at each of the frequencies used to obtain Zrs experimentally. The following Monte-Carlo procedure was used. Sixteen independent determinations of Zmod were made, with the individual values of \( r \) in each case being made by random selection from a Gaussian distribution, having mean and standard deviation appropriate for the airway order in question, as determined by Gomes and Bates (11). The final Zmod was the average of the 16 individual Zmod values.

Zmod is thus constrained by the airway tree structure defined by Gomes and Bates (11) and by the forms of Eqs. 2 and 3. We adjusted Zmod to match a given set of experimental data by choosing the values of only three parameters; Hti and \( \eta \), and a scaling factor \( \beta \), which was simultaneously applied to all values of \( r \) to achieve a uniform relative narrowing or dilatation of all of the model airways.

Once the computational model was adjusted so that Zmod matched baseline Zrs, we made the model bronchoconstrict with a time course similar to a set of experimental data by having the radii of all airways in the model assume a time-varying fraction of their respective baseline values. This fractional time course was calculated as described below. The model also incorporated our previously described mechanism for lung derecruitment (29), whereby any airway narrowing to a specified threshold radius would be closed completely for the remainder of the simulation.

**Statistical analysis.** Differences in the magnitude or timing of responses to methacholine between the saline and PLL groups were compared by using unpaired t-tests. Differences in the coefficient of determination (a measure of goodness of fit of Eq. 1 to experimental data) between two points along the time course of the methacholine response within a group of animals (either saline or PLL) were compared by using paired t-tests. Differences in the coefficient of determination at the same time point between the two groups were compared by using unpaired t-tests. Statistical significance was taken as \( P < 0.05 \). We used the coefficient of determination, a standard measure of goodness of fit, to gauge how well Eq. 1 fit the experimental measurements of Zrs. The coefficient of determination is the fraction of the variance of a data set that is accounted for by the model.
RESULTS

The coefficient of determination obtained from fitting Eq. 1 to the experimental measurements of Zrs had values at baseline of 0.749 ± 0.356 for the saline group (the relatively large variation being due to a single animal that had an anomalously small value) and 0.890 ± 0.003 for the PLL group. These values were not significantly different. The between-group values were also not different at the peak of the response to 50 mg/ml methacholine and at the final plateau. However, the coefficient of determination decreased slightly but significantly at the peak to 0.729 ± 0.299 and 0.839 ± 0.064 in the saline and PLL groups, respectively, and was still significantly depressed at the plateau at 0.738 ± 0.286 and 0.850 ± 0.073, respectively.

Figure 1 shows the time courses of RN, G, and H following aerosolization of 3.125, 12.5, and 50 mg/ml methacholine. R N, Newtonian resistance; G, viscous dissipation of energy; H, elastic energy. ©, Saline-treated animals (n = 6); ●, animals treated with poly-L-lysine (PLL; n = 6). The three time courses are shown consecutively to save space, but there was actually a 40-s aerosol delivery period before each peak in the responses shown. The vertical dotted lines bracket parameter values obtained following deep lung inflations given to reestablish baseline conditions. *Significant differences in parameter values between groups at time points indicated, P < 0.05. †Significant differences in timing of the indicated response peaks between the two groups, P < 0.05.

Fig. 1. Time course of bronchoconstriction (means ± SE) in BALB/c mice following aerosolization of 3.125, 12.5, and 50 mg/ml methacholine. RN, Newtonian resistance; G, viscous dissipation of energy; H, elastic energy. ©, Saline-treated animals (n = 6); ●, animals treated with poly-L-lysine (PLL; n = 6). The three time courses are shown consecutively to save space, but there was actually a 40-s aerosol delivery period before each peak in the responses shown. The vertical dotted lines bracket parameter values obtained following deep lung inflations given to reestablish baseline conditions. *Significant differences in parameter values between groups at time points indicated, P < 0.05. †Significant differences in timing of the indicated response peaks between the two groups, P < 0.05.

In our laboratory’s previous study (29), in which we initially established the computational model of the mouse lung and applied it to data from control and allergically inflamed BALB/c mice, we achieved a good fit to baseline data from normal animals with values for the three adjustable parameters β, Hti, and η of 0.83, 1,800 cmH2O·s·ml−1, and 0.1, respectively. To match the data from the present control animals at baseline as closely as possible, the values of β and Hti had to be changed slightly from those used in our laboratory’s previous study (29). Specifically, we increased β by 9%, and Hti was decreased by 11%. We also found that the simulated values of G were too high if we retained the value of 0.1 for η. In our laboratory’s previous study, the value of η was taken from a published study of dog lung strips that used single-frequency length oscillations, because data from mouse lung strips were not available at the time of our laboratory’s previous study. Recently, Fust et al. (9) fit the model in Eq. 1 to the mechanical impedance of isolated strips.
of mouse lung tissue subjected to uniaxial broadband length oscillations up to 20 Hz, and found a value for $\eta$ of 0.042. We, therefore, used this value of $\eta$ in the present computational model and obtained simulated time courses for $R_N$, $G$, and $H$ that reproduce the main features of the experimental measurements in both the saline group (Fig. 3) and the PLL group (Fig. 4).

The close matches between the experimental and simulated data shown in Figs. 3 and 4 demonstrate that the computational model of the normal BALB/c mouse our laboratory developed in our previous study for a single concentration of methacholine aerosol (29), with some minor adjustments to its parameter values, also accurately describes the time course of bronchoconstriction to a sequence of increasing methacholine concentrations. Furthermore, no alteration to the structure of this model is required to account for bronchoconstriction in PLL-treated mice. However, this does not prove that another model would not also describe the PLL data. Obviously, we cannot test every possibility, but we can determine how the simulated time courses of $R_N$, $G$, and $H$ are affected when we adjust the model to correspond to the allergically inflamed mouse, as in our laboratory’s previous study (29). In that study, we obtained an accurate reproduction of the parameter time courses measured in inflamed animals by keeping the degree of smooth muscle shortening the same as for control animals, while adjusting the model in two ways to account for inflammatory changes: 1) all of the airways of the model were lined with a uniform internal layer 18 $\mu$m in thickness, and 2) the threshold radius for airway closure was increased from 38 to 45 $\mu$m. We, therefore, applied these two modifications to the model and attempted to reproduce the parameter time courses of the PLL mice. To achieve a good match between simulated and measured $R_N$ time courses with these modifications, we had to reduce the degree of fractional airway narrowing to 60% of that used in the above PLL simulations. However, this resulted in the simulated $G$ and $H$ profiles being substantially elevated above their respective measured values (Fig. 4), indicating that the inflamed lung model is not appropriate for describing bronchoconstriction in the PLL-treated mouse.

**DISCUSSION**

Interpreting the results of our study first requires an understanding of what the impedance parameters $R_N$, $G$, and $H$ mean physiologically. Previous studies have shown that $R_N$ reflects the overall resistance of the airway tree (27). $G$ represents tissue viscance, a measure of energy dissipation within the tissue, but also seems to increase with regional heterogeneities throughout the lung (22). An increase in $R_N$ would thus be expected to occur when the conducting airways narrow, whereas the inevitable heterogeneities that accompany such narrowing would be expected to simultaneously elevate $G$. Regional heterogeneities also cause $H$ to increase, but to a lesser extent than $G$ (22, 29). Both $G$ and $H$, on the other hand, are equally sensitive to changes in intrinsic tissue rheology and to derecruitment of lung units (19, 22, 29), whereas $R_N$ is much less affected by such events because of its central airway component, which always contributes, regardless of what happens in the lung periphery.

In the present study, we made a number of findings that are relevant to the foregoing. First, we found that intratracheal PLL caused elevations in $R_N$ and $G$ but only at the low and
The above results are compatible with the notion that intratracheal PLL causes airway hypersensitivity by compromising the barrier function of the epithelium, an ability shared by other cationic proteins (6, 7). Our laboratory introduced this notion previously to explain why intratracheal PLL causes exaggerated increases in lung resistance and elastance measured in rats at a single frequency when methacholine is delivered as an aerosol, but not when it is injected intravenously (16). PLL has been previously shown to lower the electrical conductivity of epithelial cell layers (28), which presumably reflects damage either to the cell membrane or to the tight junctions between cells. A permeabilized epithelium presumably allows more luminally applied agonist to make its way to the smooth muscle before being cleared or degraded, resulting in enhanced muscle shortening, excessive airway narrowing, and increased airway resistance. This notion is further supported by our finding that the peaks in RN and G from the PLL group occurred earlier than the corresponding peaks in the saline group (Fig. 1). Such an effect would be expected if the challenging agonist were able to reach the airway smooth muscle more easily, and hence more quickly, than normal. By contrast, our laboratory has previously argued (29) that the relatively delayed peak in RN seen in inflamed BALB/c mice reflects the increased transit time required for an agonist to cross a swollen, thickened epithelium. An effect of PLL solely on the epithelium, and not on the airway smooth muscle itself, would also mean that the maximum ability of the muscle to narrow the airways would be unchanged, which is indeed what we observed; the response of the PLL-treated animals to 50 mg/ml methacholine, which likely approached a supramaximal dose, was similar to that of the saline-treated controls.

To put the above interpretation in the context of our current understanding of lung structure, we now examine the data in light of our computational model of mouse lung mechanics (29). This model serves as a virtual laboratory for reproducing experiments in silico and allows us to determine the consequences of particular hypotheses about mechanisms of hyper-responsiveness. To use the computational model effectively, we must simulate data under conditions as close to those used experimentally as possible, because Eq. 1 does not fit the experimental spectra of Zrs(f) perfectly; small systematic deviations between Zrs(f) and the fit provided by Eq. 1 attest to the obvious fact that the lung is a much more complex system than that represented by Eq. 1. Furthermore, as bronchoconstriction develops, the adequacy of Eq. 1 as a description of lung mechanics is likely to change. However, although the goodness of fit provided by Eq. 1, as measured by the coefficient of determination, was significantly lower in both groups at the peak and plateau of the response to 50 mg/ml methacholine compared with baseline, the differences were relatively small. This suggests that Eq. 1 continued to describe the experimental data adequately, even during bronchoconstriction. Furthermore, by generating Zrs(f) data using the computational model at the same values of f as used experimentally, we are able, at least insofar as the model is a good representation of the real lung, to reproduce any variations in parameter values due to variations in the ability of Eq. 1 to describe the experimental data.

Using the computational model described above, we were able to accurately reproduce the data from both the saline (Fig. 3) and PLL (Fig. 4) groups, with the fractional airway

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*Fig. 4. Mean time courses of RN, G, and H from the PLL group, together with the parameter profiles simulated by the computational models of the normal mouse lung and of the lung of the allergically inflamed mouse (29). The experimental values represented by the solid circles are the same as the solid circles shown in Fig. 1.*

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intermediate concentrations of methacholine; at the highest concentration of 50 mg/ml, the responses of RN and G in the PLL and saline groups were similar (Fig. 1, top and middle). In other words, the dose-response curves for RN and G were shifted to the left, indicating an increase in the sensitivity of the airways to methacholine, but not in their peak responsiveness. A second and contrasting observation is that, although PLL also increased the methacholine responsiveness of H (Fig. 1, bottom), the effect became progressively more pronounced with increasing methacholine concentration. Thus our data indicate that, between 12.5 and 50 ml/ml methacholine, a progressively increasing degree of peripheral airway closure took place in the PLL group without a corresponding increase in the degree of airway narrowing. A possible explanation is that incomplete reopening of closed airways between challenges and the accumulation of airway secretions may have caused closure to increase over time. Indeed, the inability of agonist aerosol to fully penetrate a lung partially obstructed from a previous challenge has been postulated to account for the existence of a plateau in the methacholine responsiveness of a normal lung (3).
In summary, we have studied the time course of induced bronchoconstriction in BALB/c mice treated with intratracheal PLL. We found an increased response in the central airways at submaximal doses of methacholine aerosol, which we were able to accurately reproduce in an anatomically based computational model of the mouse lung simply by increasing the degree of airway narrowing. Moreover, we interpret these results as reflecting a reduced barrier function of the epithelium caused by the PLL, allowing for easier and more rapid access of aerosolized agonist to the underlying smooth muscle. This represents a completely different manifestation of enhanced bronchoconstriction to that found in the allergically inflamed mouse (29), underscoring the fact that airway hyperresponsiveness can occur by a variety of mechanisms (4). Which of these mechanisms has the most relevance for human asthma remains an open question, but studying different animal models that embody the various possibilities may help in finding the answer.

GRANTS

The authors acknowledge the financial support of National Institutes of Health Grants NCRR P20 RR15557, R01 HL62743, and R01 HL75593.

REFERENCES


