

Noninvasive Measurement of Airway Responsiveness in Allergic Mice Using Barometric Plethysmography

E. HAMELMANN, J. SCHWARZE, K. TAKEDA, A. OSHIBA, G. L. LARSEN, C. G. IRVIN, and E. W. GELFAND

Divisions of Basic Sciences and Pulmonary Medicine, Department of Pediatrics; and Department of Medicine, National Jewish Medical and Research Center, Denver, Colorado

To study the mechanisms and kinetics underlying the development of increased airway responsiveness (AR) after allergic sensitization, animal models have been invaluable. Using barometric whole-body plethysmography and increases in enhanced pause (Penh) as an index of airway obstruction, we measured responses to inhaled methacholine in conscious, unrestrained mice after sensitization and airway challenge with ovalbumin (OVA). Sensitized and challenged animals had significantly increased AR to aerosolized methacholine compared with control animals. AR measured as Penh was associated with increased IgE production and eosinophil lung infiltration. In a separate approach we confirmed the involvement of the lower airways in the response to aerosolized methacholine using tracheotomized mice. Increases in Penh values after methacholine challenge were also correlated with increased intrapleural pressure, measured via an esophageal tube. Lastly, mice demonstrating AR using a noninvasive technique also demonstrated increased pulmonary resistance responses to aerosolized methacholine when measured using an invasive technique the following day in the same animals. The increases in Penh values were inhibited by pretreatment of the mice with a β_2 -agonist. These data indicate that measurement of AR to inhaled methacholine by barometric whole-body plethysmography is a valid indicator of airway hyperresponsiveness after allergic sensitization in mice. The measurement of AR in unrestrained, conscious animals provides new opportunities to evaluate the mechanisms and kinetics underlying the development and maintenance of airway hyperresponsiveness and to assess various therapeutic interventions. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *AM J RESPIR CRIT CARE MED* 1997;156:766-775.

Airway hyperresponsiveness (AHR), airway inflammation, and reversible airway obstruction are the hallmarks of bronchial asthma (1). In the development of AHR, neurogenic abnormalities (2) and airway inflammation (3), characterized by eosinophil infiltration (4), and the release of inflammatory mediators and cytokines (5) have been implicated. Animal models have been developed to investigate the pathogenetic mechanisms involved in the development of AHR, providing a means for *in vivo* manipulation and *in vitro* study of easily accessible cells and tissue (6). These models also permit the testing of different protocols and reagents for the prevention of AHR and airway inflammation, approaches that are unsuitable or impossible to perform in patients. Because of the advanced

understanding of the immune system in mice and the availability of reagents and genetically altered mice, murine models of AHR have become increasingly important in defining which cells and factors are involved (7). Several reports have described the roles that interleukin-4 (IL-4) (8), IL-5 (9, 10), and eosinophil lung infiltration (11, 12) play in the development of AHR in allergen-sensitized mice, but additional studies are needed to better define the kinetics and mechanisms underlying AHR.

To date, three different approaches have been used to measure altered airway function in mice: *in vitro* measurement of tracheal smooth muscle contractility after electrical field stimulation (13), *in vivo* measurement of lung resistance or compliance after intravenous injection of bronchoconstrictive agents such as methacholine and serotonin (14, 15), and *in vivo* measurement of peak airway opening pressure (16). Each of these methods have their limitations. The *in vitro* technique correlates well with allergic airway sensitization (13) and appears to reflect increased acetylcholine release caused by M_2 receptor dysfunction in sensitized animals (17). However, the influence of mucus production, mucosal edema, or other changes in the lower airways after allergic sensitization are not reflected in monitoring airway responsiveness with this technique. The *in vivo* techniques (14-16) perform measurements of AHR in tracheotomized and ventilated animals. The influence of anes-

(Received in original form June 7, 1996 and in revised form February 4, 1997)

Supported by Grants AI-29704 and HL-36577 (E.W.G.) from the National Institutes of Health and an ALA Asthma Research Center Award (C.I.).

Dr. Hamelmann is a fellow of the Deutsche Forschungsgemeinschaft (Ha 2162/1-1) and recipient of the 1996 Jenssen Pharmaceutica Award of the American Academy of Allergy, Asthma and Immunology.

Correspondence and requests for reprints should be addressed to Erwin W. Gelfand, M.D., Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

Am J Respir Crit Care Med Vol. 156, pp. 766-775, 1997

thetia and of the operating procedures on the readings is not well defined. Furthermore, intravenous challenge of the mice with bronchoconstrictive agents might not solely reflect physiologic stimulation of airway smooth muscles. Finally, this method is technically demanding and time-consuming.

In this report, we present data from studies carried out using barometric whole-body plethysmography (WBP) for the measurements of AR in unrestrained and conscious mice after sensitization and airway challenge with allergen. WBP has several potential advantages when compared with the above-mentioned techniques: it is technically not as demanding, allows measurements of AR to aerosolized stimulants, and provides a technique for repeated and long-term measurements of AR, as killing of the mice after the measurements is not required, thus allowing the evaluation of kinetics and treatment protocols of AHR. However, because of the indirect and noninvasive measurement of airway function, thorough evaluation of WBP is necessary before it can be accepted as a technique to measure AHR. The influence of upper airway responsiveness and changes in breathing pattern (respiratory rate, tidal volume) on the read-out of WBP need to be evaluated. We addressed these problems by measuring AR by WBP in tracheotomized animals, simultaneously measuring WBP and intrapleural pressure, and sequentially measuring WBP and lung resistance in the same animals. Further, we studied the effects of changes in the respiratory rate and of the response to a bronchodilator on WBP. The data shown in this study indicate that WBP in mice provides a valid assessment of AHR in allergen-sensitized mice.

METHODS

Animals

Female BALB/c mice 8 to 12 wk of age were obtained from Jackson Laboratories (Bar Harbor, ME). The mice were maintained on OVA-free diets. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Center for Immunology and Respiratory Medicine.

Sensitization and Airway Challenge

Groups of mice (three to four mice/group/experiment) receiving the following treatment were studied: (1) no treatment (N); (2) sensitization to OVA plus airway challenge with PBS (ip); (3) sham-sensitization with PBS plus airway challenge with OVA (Neb); (4) sensitization plus challenge with OVA (ipNeb). Mice were sensitized by intraperitoneal injection of 20 µg OVA (Sigma, St. Louis, MO) emulsified in 2 mg aluminum hydroxide (AlumInject; Pierce Chemical, Rockford, IL) in a total volume of 100 µl on Days 1 and 14. Mice were challenged via the airways with OVA (1% in PBS) or PBS for 20 min on Days 28, 29, and 30 by ultrasonic nebulization and assessed on Day 31 for AR. In selected mice, invasive methods to measure pulmonary resistance were employed on Day 32.

Determination of Airway Responsiveness

AR was measured in unrestrained animals by barometric plethysmography (18) using whole body plethysmography (WBP) (Figure 1) (Buxco, Troy, NY). Before taking readings, the box was calibrated with a rapid injection of 150 µl air into the main chamber. Measured were pressure differences between the main chamber of the WBP containing the animal, and a reference chamber (box pressure signal). This box pressure signal is caused by volume and resultant pressure changes in the main chamber during the respiratory cycle of the animal. A pneumotachograph with defined resistance in the wall of the main chamber acts as a low-pass filter and allows thermal compensation (Figure 1). The time constant of the box was determined to be approximately 0.02 s.

Inspiration and expiration are recorded by establishing start-inspiration and end-inspiration as the box pressure/time curve crosses the

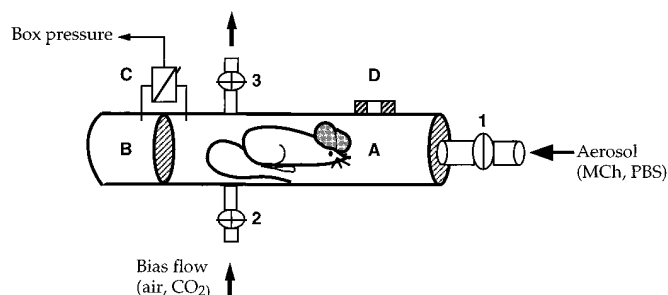


Figure 1. Schematic diagram of the whole-body plethysmograph. (A) Main chamber containing the mouse. (B) Reference chamber. (C) Pressure transducer connected to analyzer. (D) Pneumotachograph. (1) Main inlet for aerosol closed by valve. (2) Inlet for bias flow with four-way stopcock; (3) Outlet for aerosol with four-way stopcock.

zero point (see Figure 2). Start of an inspiration is determined by extrapolating from a straight line drawn from two levels of the rising inspiratory phase of the box pressure signal. Time of inspiration (T_i) is defined as the time from the start of inspiration to the end of inspiration; time of expiration (T_e) as the time from the end of inspiration to the start of the next inspiration (Figure 2). The maximum box pressure signal occurring during one breath in a negative or positive direction is defined as peak inspiratory pressure (PIP) or peak expiratory pressure (PEP), respectively (Figure 2). Recordings of every 10 breaths are extrapolated to define the respiratory rate in breaths per minute. The relaxation time (T_r) is defined as the time of pressure decay to 36% of the total expiratory pressure signal (area under the box pressure signal in expiration). This may thus serve as a correlate to the time constant (RC) of the decay of the volume signal to 36% of the peak volume in passive expiration. During bronchoconstriction, the main alteration in the signal occurs during early expiration and leads to changes in the waveform of the box pressure signal (19, 20). This

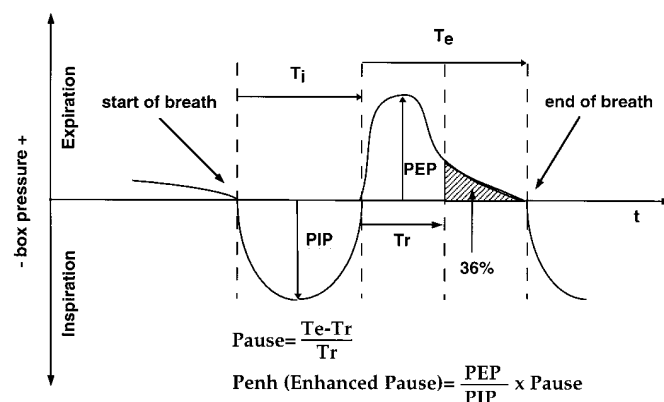


Figure 2. Computation of the parameters measured by barometric plethysmography. Schematic figure of a box pressure wave in inspiration (down) and expiration (up) explaining the computation of the parameters measured by WBP. T_i = inspiratory time (s), time from start of inspiration to end of inspiration; T_e = expiratory time (s), time from end of inspiration to start of next inspiration; PIP = peak inspiratory pressure (ml/s), maximal negative box pressure occurring in one breath; PEP = peak expiratory pressure (ml/s), maximal positive box pressure occurring in one breath; f = frequency (breaths/min), respiratory rate; T_r = relaxation time (s), time of the pressure decay to 36% of total box pressure during expiration.

change in the waveform can be quantified comparing the mean expiratory box pressure during early expiration (MP1) with the mean expiratory box pressure during late expiration (MP2) by measurement of Pause (Figure 2) where MP1 = mean expiratory box pressure 1; MP2 = mean expiratory box pressure 2; P = expiratory box pressure:

$$\begin{aligned} \text{MP1} &= \frac{0.65 P}{\text{Tr}} \\ \text{MP2} &= \frac{0.35 P}{\text{Te} - \text{Tr}} \\ \text{Pause} &= \frac{\text{Te} - \text{Tr}}{\text{Tr}} = \frac{0.35 P}{0.65 P} \times \frac{\text{MP1}}{\text{MP2}} \sim \frac{\text{MP1}}{\text{MP2}} \end{aligned}$$

During bronchoconstriction, the changes in box pressure during expiration (PEP) are more pronounced than during inspiration (PIP) (20) (see Figure 3). This is reflected by the formula for enhanced pause (Penh), a dimensionless value used in this study to empirically monitor airway function:

$$\text{Penh} = \text{Pause} \times \frac{\text{PEP}}{\text{PIP}}$$

Penh reflects changes in the waveform of the box pressure signal from both inspiration and expiration (PIP, PEP) and combines it with the timing comparison of early and late expiration (Pause). Penh is not a function of the absolute box pressure amplitude or the respiratory rate, but rather a junction of the proportion of the pressure signal from

inspiration and expiration and of the timing of expiration. An example of the box pressure waveform from a normal mouse before and after challenge with aerosolized methacholine is shown in Figure 3, demonstrating the changes in the waveform as well as in Pause and Penh after agonist inhalation.

Mice were placed in the main chamber, and baseline readings were taken and averaged for 3 min. Aerosolized PBS or methacholine in increasing concentrations (3 to 50 mg/ml) were nebulized through an inlet of the main chamber for 3 min, and readings were taken and averaged for 3 min after each nebulization. Airway reactivity was expressed as a fold increase for each concentration of MCh (Penh_{MCh}) compared with Penh values after PBS challenge (Penh_{PBS}).

For the quantification of the dose-response to methacholine, the linear regression of Penh on log base 2 was calculated for individual mice. The log dose corresponding to an increase in Penh of 100 or 200%, respectively, was determined, and the average log doses of the different groups were compared by analysis of variance. The data are reported as the geometric mean with the lower and upper limit of 95% confidence interval.

Studies with Mechanical Ventilation

In order to determine the influence of breathing frequency and tidal volume as well as bronchoconstriction under controlled conditions,

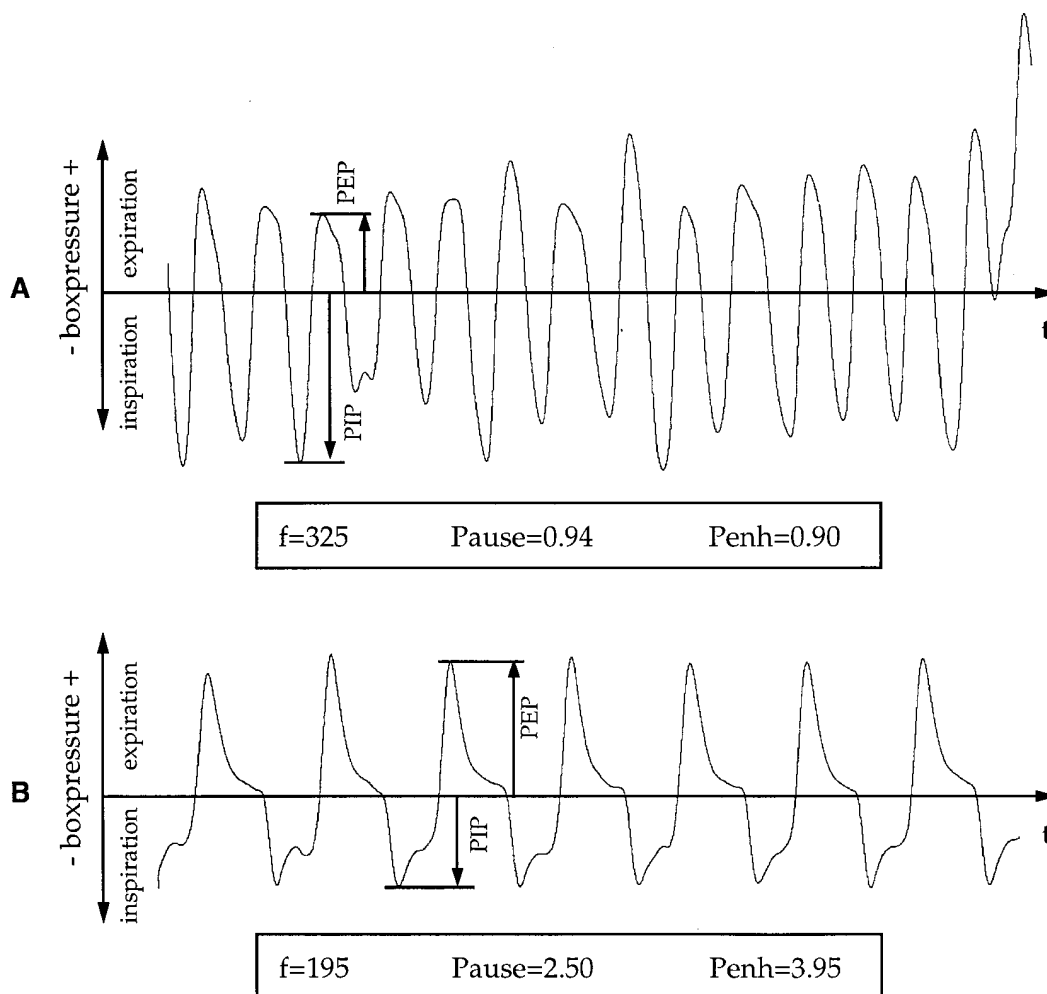


Figure 3. Changes in box pressure waveform after methacholine challenge. Waveform of the box pressure signal derived from a normal mouse after 3 min of nebulization with aerosolized PBS (A) or aerosolized methacholine (50 mg/ml in PBS) (B). f = respiratory rate (breaths/min); Pause, Penh (enhanced pause), PIP and PEP: see Figure 2 for description.

we performed the following studies. Mechanical ventilation was achieved by using a computer-controlled high-speed volume ventilator (Flexivent; SCIREQ, Montreal, Quebec, Canada). The piston of this ventilator is connected to a linear pumping motor and a linear displacement transducer (21). By measuring the precise position of the shaft and accounting for gas compression, the volume delivered within the mouse or plethysmograph is defined and known. We then ventilated either the empty WBP or the live mice over a frequency (*f*) rate of 100 to 300 breaths/min and tidal volume ranges of 0.1 to 0.3 ml. We performed a tracheostomy and then connected the mice through the well of the plethysmograph to the ventilator. To investigate the effects of agonist inhalation in ventilated mice, the animals were challenged with MCh (25 mg/ml) given either intratracheally with *f* set to 60 breaths/min, tidal volume of 500 μ l for 30 s, or intraperitoneally after establishing PBS baselines, and Penh was measured under fixed ventilation settings (*f* = 300 breaths/min; tidal volume = 150 μ l).

Correlation to Respiratory Rate in Conscious Mice

To investigate if there is a relationship between Penh and respiratory rate in conscious, unrestrained, and spontaneously breathing mice, we investigated the effects of CO₂ inhalation. Normal mice were set in the main chamber of the WBP, and Penh baseline readings were taken for the first 3 min. A steady bias flow with normal air (1 L/min) was established through an additional inlet of the main chamber to allow long-term online readings. A second baseline reading of 3 min was measured after the mice were resting within the box for 30 min. Bias flow was then changed to 8% CO₂ in air (1 L/min), and respiratory rate and Penh were measured after 15 min.

Lower Airway Responsiveness

To document involvement of the lower airways in the measurements of Penh, mice were anesthetized intraperitoneally (0.3 ml 2.5% avertin in PBS) and 5-mm-long sterile plastic tubes were inserted into the tracheas and fixed by suture. PBS, MCh challenge, and measurements were performed in the spontaneously breathing mice in the WBP as described above.

Correlation to Pleural Pressure

In an attempt to directly correlate bronchoconstriction to the index derived by barometric plethysmography, intrapleural pressure changes were measured simultaneously with measurements of Penh. Saline-filled tubes were inserted into the esophagus of anesthetized mice and connected to a pressure transducer (Model MC1; Validyne, Northridge, CA). Mice were challenged with nebulized PBS and increasing concentrations of MCh as above. Changes in intraesophageal pressure (ΔP), which reflect changes in intrapleural pressure, were calculated for each MCh concentration. Changes in box pressure and respiratory frequency were measured by WBP. ΔP and Penh were recorded simultaneously and expressed as a fold increase over values after PBS challenge.

Correlation to Pulmonary Resistance

To correlate Penh with a measurement of lung resistance, *in vivo* pulmonary resistance (*R_L*) was measured in anesthetized, tracheostomized, and ventilated mice as previously described (14, 15). A four-way connector was attached to the tracheostomy tube, with two ports connected to the inspiratory and expiratory sides of the ventilator (Model 683; Harvard Apparatus, South Natick, MA). Ventilation was achieved with a rate of 160 breaths/min, tidal volume of 150 μ l during recording, and with a rate of 60 breaths/min, tidal volume of 500 μ l during MCh challenge. As a modification to previous work from our laboratory (15), MCh was administered as an aerosol for the period of 10 breaths for each concentration via the tracheostoma. Change in pressure, flow, and volume were recorded, and *R_L* was calculated from peak values after each challenge. Penh was first measured on Day 31 of the protocol and *in vivo* *R_L* was obtained in the same animals 1 d later.

Effects of a Beta Agonist

To study the effects of an inhaled β_2 -agonist on measurement of Penh in allergen-sensitized and challenged mice, albuterol was nebulized as an aerosol for 3 min into the main chamber followed by a 3-min read-

ing. The control group consisted of sham-treated mice aerosolized with PBS for 3 min instead of albuterol. Six minutes later, MCh was aerosolized at a 50 mg/ml concentration followed by a 6-min reading. In a different set of experiments, the effects of albuterol on repeated MCh challenge was investigated. After establishing PBS baseline values, mice were challenged with MCh (50 mg/ml) for 3 min and Penh was recorded for 6 min. The mice were divided into two groups receiving nebulization with either PBS or albuterol for 3 min followed 6 min later by a second MCh challenge (50 mg/ml) for 3 min.

Measurement of Anti-OVA Antibody and Total Ig Levels

Anti-OVA Ig serum levels were measured by ELISA as previously described (22). The antibody titers of the samples were related to pooled standards that were generated in the laboratory and expressed as ELISA units per milliliter (EU/ml). Total IgE and IgG levels were determined using the same method compared with known mouse IgE or IgG standards (PharMingen, San Diego, CA). The limits of detection were 100 pg/ml for IgE and 1 ng/ml for IgG.

Bronchoalveolar Lavage (BAL) and Lung Cell Isolation

Lungs were lavaged via a tracheal tube with Hank's balanced salt solution (HBSS, 3 $\times$ 0.5 ml), and the cells in the lavage fluid were counted. Lung cells were isolated as previously described (22). Cells from BAL or lungs were resuspended in HBSS and counted with a hemocytometer. Cytospin slides were stained with Leukostat (Fisher Diagnostics, Pittsburgh, PA) and differentiated in a blinded fashion by counting at least 300 cells by light microscopy.

Statistical Analysis

Analysis of variance was used to determine the level of difference between all groups. Single pairs of groups were compared by Student's *t* test. Comparisons for all pairs were performed by Tukey-Kramer HSD test; *p* values for significance were set to 0.05. Values for all measurements are expressed as the mean $\pm$ standard deviation (SD) except for values for airway reactivity (Penh, resistance, impedance), which are presented as the mean $\pm$ standard error of the mean (SEM).

RESULTS

Noninvasive AR Increases after Methacholine Challenge in Allergen-sensitized and Challenged Mice

We established a mouse model of systemic sensitization and airway challenge with allergen, monitoring airway responsiveness using WBP in unrestrained and conscious mice. Sensitization with OVA followed by airway challenge significantly increased serum levels of anti-OVA IgE and IgG₁ and enhanced production of total IgE in BALB/c mice compared with non-sensitized control mice receiving no treatment or OVA airway challenge on two consecutive days (Table 1). In addition, all of the sensitized and challenged mice developed allergen-specific immediate cutaneous responsiveness to intradermal injections of OVA; no responses were observed in nonsensitized control mice without or with airway challenge (data not shown).

We compared the responses to inhaled MCh in the four groups of mice: untreated mice (N), sensitized and PBS-challenged mice (ip), nonsensitized and OVA-challenged mice (Neb), and sensitized and OVA-challenged mice (ipNeb). The control groups (N, Neb, ip) showed similar albeit shallow, dose-dependent increases in Penh in response to aerosolized MCh compared with the Penh values after PBS (Figure 4). In contrast, in mice that were sensitized and challenged with allergen via the airways (ipNeb), the increase in Penh in response to aerosolized MCh was significantly enhanced compared with the control mice. The MCh doses required for 100 and 200% increases in Penh were significantly reduced for sensitized and challenged mice by $\sim$ 3.5-fold and $\sim$ 5-fold, respectively, shifting the dose-responses leftwards compared

TABLE 1
OVA-SPECIFIC ANTIBODY AND TOTAL Ig LEVELS IN THE SERUM

Group	Sensitization	Challenge	OVA-specific (EU/ml) [†]		Total Ig (ng/ml)	
			IgE	IgG ₁	IgE	IgG
N	None	None	< 10	< 10	14.7 ± 1	243 ± 65
Neb	None	OVA	< 10	< 10	16 ± 3	235 ± 68
ipNeb	OVA	OVA	7,622 ± 736 [§]	1,029 ± 369 [§]	48.8 ± 10 [§]	265 ± 86

Definition of abbreviations: OVA = ovalbumin; N = serum titers for OVA-specific and total antibodies, which were determined by ELISA in untreated mice (n = 8); Neb = nonsensitized, OVA-challenged mice (n = 12); ipNeb = OVA-sensitized, OVA-challenged mice (n = 18). Presented are the means ± SD (OVA-specific in ELISA units/ml, total Ig in ng/ml) from three independent experiments.

* Presented are the means ± SD from the three independent experiments.

[†] ELISA units per milliliter.

[§] Significant (p < 0.01) differences compared with control group (N).

with nonsensitized control mice (Table 2). The responses peaked at 1.5 to 2 min after the challenge with aerosolized MCh, and Penh returned to prenebulization values after ~3 min for MCh doses ≤ 12 mg/ml and after ~5 to 7 min after higher doses. The Penh baseline readings after PBS were similar for all three control groups, but they were higher for sensitized and challenged animals (Figure 4). These data indicate that Penh values are increased in allergen-sensitized, airway-challenged animals. Furthermore, the response to MCh was greater in this group of mice.

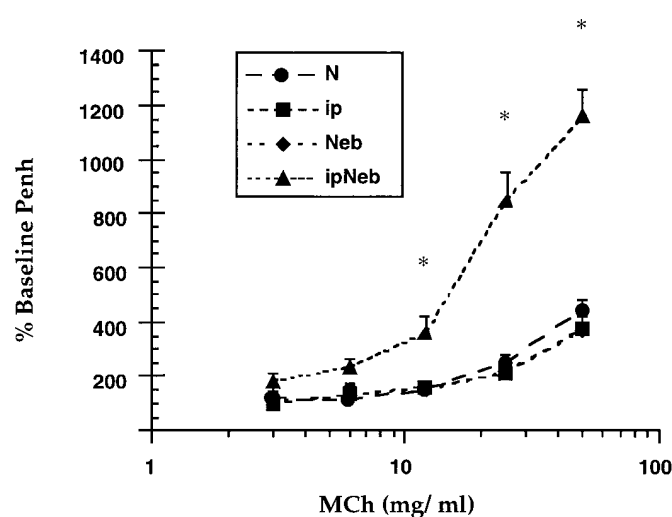


Figure 4. Penh increases in allergen-sensitized and challenged mice. Animals were sensitized with OVA/alum ip on Days 1 and 14 and challenged with OVA via the airways on Days 28, 29, and 30. Airway responsiveness to aerosolized methacholine was measured in unrestrained, conscious mice. Mice were placed into the main chamber of the WBP and were nebulized first with PBS, then with increasing doses (3 to 50 mg/ml) of methacholine for 3 min for each nebulization, followed by readings of breathing parameters for 3 min after each nebulization with PENH values determined. Compared are nontreated (N) (n = 8), nonsensitized, challenged (Neb) (n = 12), sensitized, nonchallenged (ip) (n = 12), and sensitized, challenged (ipNeb) (n = 12) mice. Expressed are the means ± SEM of the Penh values in percentages of Penh values after PBS nebulization of three independent experiments. Penh_{PBS}: N, 0.84 ± 0.03; Neb, 0.85 ± 0.03; ip, 0.81 ± 0.04; ipNeb, 1.04 ± 0.05; p < 0.05. *p < 0.01 compared with controls values.

Noninvasive AR Increases after Methacholine Challenge in Ventilated Mice

To study the impact of changes in breathing frequencies (f) and tidal volumes, we measured Penh under conditions where mice were mechanically ventilated. First, the empty WBP was connected to the ventilator and Penh was measured over the frequency range of 100 to 300 breaths/min and the tidal volume range of 100 to 300 µl. Both f and tidal volume were measured correctly by the WBP, and recorded values for tidal volume did not change under different f. Penh during mechanical ventilation changed less than 10% with various f in measurements performed in live, ventilated mice. Penh values increased nearly proportionally with increasing tidal volume in a range from 100 to 250 µl. The < 2-fold increase in Penh observed in ventilated mice (0.26 at 100 µl to 0.44 at 250 µl) was due to an increase in the Pause (0.34 to 0.54) resulting from a decrease in Tr (from 0.09 s at 100 µl to 0.07 s at 250 µl) and to a lesser extent by a decrease of T_E (from 0.12 s to 0.11 s). The decrease in Tr may be explained by the greater elastic recoil/smaller compliance of the lungs when ventilated with a higher tidal volume. These data further indicate that the volume dependency of Penh does not account for the much greater changes (> 10-fold increase versus baseline Penh) seen in sensitized challenged mice with similar changes in tidal volume (see Table 3).

Next, we measured the changes in Penh after challenge with MCh under ventilated conditions. Normal, anesthetized mice were ventilated and challenged with MCh (25 mg/ml) either intratracheally or intraperitoneally. Under both conditions, the mice developed a more than 200% increase in Penh

TABLE 2
DOSE-RESPONSE OF PENH TO MCh

Group	Sensitization	Challenge	MCh Dose (mg/ml) Required for an Increase in Penh of			
			100%	(LL; UL)	200%	(LL; UL)
N	None	None	17.7	10.5; 16	32	21; 48.5
Neb	None	OVA	18.7	11.5; 30.4	42	32; 105
ipNeb	OVA	OVA	5.2 [†]	4.4; 6.1	7 [†]	5.6; 8.3

Definition of abbreviations: Penh = plethysmography and increases in enhanced pause; MCh = methacholine; N = dose-response in Penh after challenge with aerosolized MCh in untreated mice (n = 8). For other definitions, see Table 1.

* Reported is the geometric mean and the lower (LL) and upper (UL) level of the 95% confidence interval in mg/ml of MCh required for an increase of Penh to 100 or 200% compared with PBS-baseline values.

[†] p < 0.01 versus control groups (N and Neb).

TABLE 3
EFFECTS OF METHACHOLINE OR AVERTIN ON
BREATHING PATTERNS AND PENH*

Group	Anesthesia	Challenge (nebulized)	Respiratory Frequency	Tidal Volume	Penh
ipN	None	PBS	320 ± 12 [†]	0.25 ± 0.03 [†]	1.01 ± 0.06 [‡]
ipN	None	MCh 50	165 ± 24	0.4 ± 0.04	11.4 ± 2.6
ipN	Avertin	PBS	189 ± 21	0.35 ± 0.04	1.04 ± 0.2

* Correlation between breathing patterns and Penh values. Expressed are the mean ± SD values of respiratory frequency (in breath/min), tidal volume (in μ l), and Penh after PBS and MCh (50 mg/ml) challenge for conscious (n = 8) and anesthetized (avertin, n = 8), sensitized, and challenged mice.

[†] p < 0.05 versus two other groups.

[‡] p < 0.05 versus MCh challenge.

values after MCh of PBS-baseline values. This suggests that the increase in Penh cannot be accounted for by changes in f or tidal volume after challenge with MCh as these variables are controlled in mechanically ventilated mice.

Noninvasive AR Does not Correlate with Breathing Patterns and Respiratory Rate

MCh challenge of mice induces increases in Penh and decreases in respiratory rate. To study the effects of changes in breathing patterns on Penh in spontaneously breathing mice and to investigate if the observed slowing of the respiratory rate itself causes an increase in Penh, sensitized and challenged mice were anesthetized intraperitoneally with an injection of avertin (2.5% in PBS) and compared with conscious animals. In anesthetized animals, frequency was decreased to ~60% compared with that in conscious mice. However, changes in these breathing patterns were not accompanied by increases in Penh

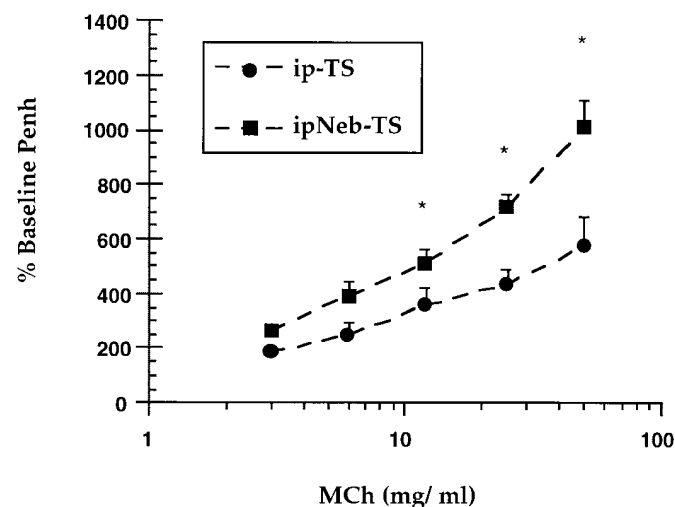


Figure 5. Penh increases after methacholine challenge of the lower airways. Mice were sensitized and challenged as described in Figure 1. Mice were tracheostomized, and airway responsiveness was measured as described in Figure 1. Compared are sensitized but not challenged (ip-TS) (n = 12) and sensitized and challenged tracheostomized mice (ipNeb-TS) (n = 12). Expressed are the means ± SEM of the Penh values in percentages of Penh values after PBS nebulization from three independent experiments. Penh_{PBS}: ip-TS, 0.94 ± 0.07; ipN-TS, 1.2 ± 0.15. *p < 0.01 compared with controls values.

(Table 3). MCh challenge (50 mg/ml) of conscious mice resulted in changes in breathing patterns similar to those observed in anesthetized animals, but they were followed by significant increases in Penh. Challenge of anesthetized mice with MCh resulted in a dose-response curve similar to those seen in the conscious animals (Figures 4 and 5), although the respiratory rate of each concentration of MCh challenge in the anesthetized animals was less than that in conscious animals (data not shown).

To further study the influence of the respiratory rate in spontaneously breathing mice receiving Penh, CO₂ was used as a respiratory stimulant. After 30 min resting in the box with a steady bias flow of normal air, mice showed a significant decrease in the average respiratory rate of 25% with virtually no changes in Penh readings (Table 4). Changing the bias flow from normal air to air containing 8% CO₂ induced a significant increase in respiratory rate of 46% accompanied with a nonsignificant drop of Penh by 10% (p = 0.2). These data indicate that decreases or increases in the respiratory rate are not necessarily accompanied with changes in Penh but rather are independently regulated.

Noninvasive AR Increases after Methacholine Challenge of the Lower Airways

To address the possibility that increases in Penh are simply due to reactions of the upper airways, e.g., swelling of the nasal mucosa or increased glandular activity, we bypassed the upper airways by performing a tracheostomy (TS) in mice before challenge with aerosolized MCh. As indicated in Figure 5, challenge of these mice with nebulized MCh resulted in dose-dependent increases in Penh. In sensitized and challenged mice (ipNeb-TS), increases in Penh values were significantly enhanced. Baseline Penh values were similarly higher in ipNeb-TS than in ip-TS animals (Figure 5). The higher Penh baseline values compared with those in nontracheostomized mice is most likely due to the fixed resistance of the tracheostomy. The magnitude of the dose-response in sensitized, challenged animals after challenge with higher doses of MCh (25 and 50 mg/ml) was very similar in tracheostomized and nontracheostomized mice (Figures 4 and 5). The MCh dose required for a 100% increase in Penh was significantly decreased and shifted to the left from 4.4 (3.3; 5.9) in ip-TS to 2.9 (2.3; 3.7) in ipNeb-TS (~1.5-fold, p < 0.05), and from 16 (8; 24) in ip-TS to 4.6 (2.9; 7.8) in ipNeb-TS (~3.5 fold, p < 0.01) for a 200% increase. The smaller magnitude of the shift in that dose-response to MCh in tracheostomized compared with nontracheostomized mice might be explained by the fact that the availability of MCh is higher in tracheostomized mice because the upper airways are by-passed, which may result in higher responses in nonsensitized mice. Secondly, the data suggest that at least

TABLE 4
EFFECT OF CO₂ ON RESPIRATORY RATE AND PENH*

Condition	Time in Box (min)	Air (bias flow)	Breathing Frequency	Penh
1	3	Air	306 ± 13	1.06 ± 0.17
2	30	Air	243 ± 15 [†]	1.07 ± 0.15
3	45	Air/8% CO ₂	356 ± 13 [‡]	0.95 ± 0.2

* Correlation between respiratory frequency and Penh values. Expressed are the mean ± SD values of respiratory frequency (in breaths per min) and Penh for mice (n = 4) 3 min (Condition 1) and 30 min (Condition 2) after start of online measurements and after additional 10 min of bias flow with 8% CO₂ in air (Condition 3).

[†] p < 0.01 versus condition 1.

[‡] p < 0.01 versus Condition 2.

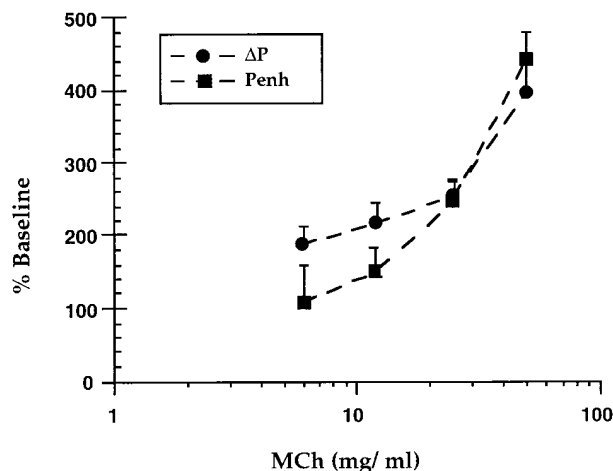


Figure 6. Increases in Penh and intrapleural pressure after methacholine airway challenge. Measurements of airway responsiveness in the WBP were performed as described in Figure 1. A saline-filled esophageal tube was connected to a second pressure transducer, and intraesophageal pressure was recorded simultaneously with PENH after each challenge with MCh. Expressed are the means $\pm$ SEM of Penh and ΔP (intraesophageal pressure differences) as percentages of baseline values after PBS nebulization from two independent experiments ($n = 8$).

part of the increased responsiveness measured by Penh may be related to altered responsiveness of the upper airways.

Noninvasive AR Correlates with Intrapleural Pressure

To directly correlate Penh values with changes in pleural pressure occurring in the lower airways after MCh challenge, a saline-filled esophageal tube was placed in the mice to reflect changes in intrapleural pressure. Simultaneously, Penh was measured using the WBP. Mice were then challenged with aerosolized PBS or increasing doses of MCh. As shown in Figure 6, nebulized MCh induced increases in Penh similar to those in intrapleural pressure (ΔP) compared with values after PBS exposure. Moreover, the increases in Penh correlated with increases in ΔP (Figure 7B). These data demonstrate that Penh values correlate with increases in intrapleural pressure differences in the lower airways after MCh challenge.

Noninvasive AR Correlated with *in vivo* Pulmonary Resistance

To determine if increases in Penh values correlate with increased *in vivo* pulmonary resistance, we monitored Penh and pulmonary resistance in the same animals on 2 consecutive days. Pulmonary resistance was measured in intubated and ventilated mice, administering aerosolized MCh via the tracheostomy. Data were calculated from the peak values after each MCh challenge and expressed as the increase compared with measurements after PBS nebulization. Aerosolized MCh increased pulmonary resistance in a dose-dependent manner; sensitized, OVA-challenged animals (ipNeb) showed significantly higher pulmonary resistance than did nonsensitized, OVA-challenged control animals (Neb) (Figure 8B). Increases in pulmonary resistance paralleled increases in Penh values monitored by WBP in the same animals the day previously (Figure 8A). A comparison of the responses of R_L and Penh for individual mice in the same experiment (Figure 7A) indicates the

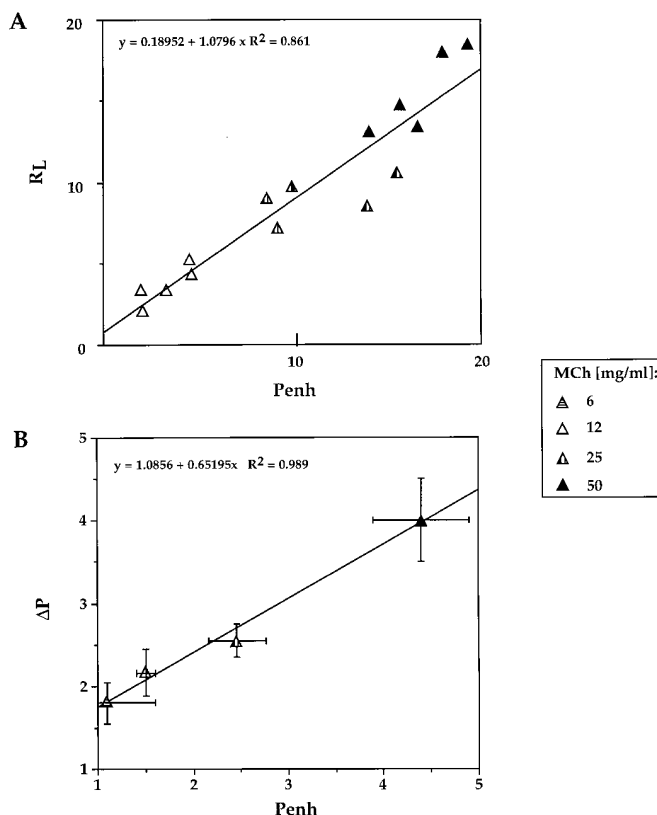


Figure 7. Penh correlates with pulmonary resistance and intrapleural pressure. Mice were sensitized and measurements of airway responsiveness were performed as described in Figure 4. Compared are the responses to aerosolized MCh measuring Penh and airway resistance (R_L) on 2 consecutive days in the same individual mice (A), or measuring Penh and ΔP simultaneously as described in Figure 3 (B). Shown are the results from one of two similar experiments (A) and the mean $\pm$ SEM of the results from two independent experiments (B), respectively.

strong correlation between Penh and increased pulmonary resistance in sensitized and challenged mice.

Noninvasive AR Increases Are Inhibited by Albuterol

To assess the effect of β_2 -agonist on Penh, albuterol was administered by nebulization to allergen-sensitized and challenged mice after obtaining a PBS baseline. Aerosolization of albuterol did not change baseline Penh values after PBS (data not shown). MCh was then aerosolized at 50 mg/ml, and Penh was recorded for 6 min after each nebulization. Sensitized and allergen-challenged animals receiving sham-treatment with aerosolized PBS instead of the β_2 -agonist showed a significant increase in Penh values after MCh challenge (Figure 9). Pretreatment with aerosolized albuterol significantly reduced the increases in Penh values after MCh challenge. Subsequent albuterol treatment of mice that showed a more than 10-fold increase in Penh after a first MCh (50 mg/ml) challenge resulted in significantly reduced Penh values after a second MCh challenge ($650 \pm 120\%$ of PBS baseline), whereas mice receiving PBS sham-treatment instead of albuterol after a first MCh challenge showed consistently higher Penh readings after repeated MCh challenge ($1,250 \pm 150\%$ of PBS baseline). These

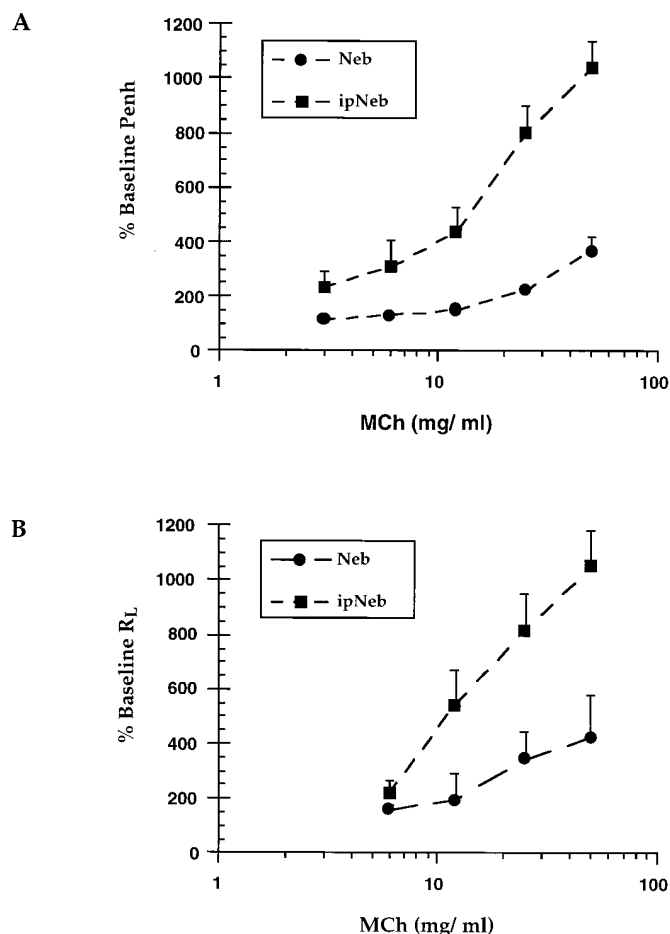


Figure 8. Increases in Penh and pulmonary resistance after methacholine airway challenge. Mice were sensitized and measurements of airway responsiveness were performed as described in Figure 1. Using the same mice, pulmonary resistance was measured in anesthetized, tracheostomized, and ventilated animals the following day. Aerosolized PBS and MCh were administered via the tracheostomy. Pulmonary resistance was calculated as $R_L = \Delta P / \Delta V$ (difference in tracheal pressure)/ ΔV (flow change) from peak values after each challenge. Compared are nonsensitized, challenged (Neb) ($n = 12$) and sensitized, challenged (ipNeb) ($n = 12$) mice. Expressed are the means $\pm$ SEM of Penh (A) and of R_L (B) as percentages of baseline values after PBS nebulization. Penh_{PBS}: Neb, 0.86 ± 0.03 ; ipNeb, 1.09 ± 0.08 from three independent experiments * $p < 0.01$ compared with control (N).

data indicate that increases in Penh values in response to MCh are at least partially preventable after pretreatment with the bronchodilator.

Increases in Penh Are Associated with Increased Eosinophils in Lung Tissue

To correlate AR with airway inflammation, total leukocyte counts and differential counts for BAL fluid cells and isolated lung cells of individual mice were compared in the different groups. Sensitization and challenge resulted in a significant increase in eosinophils in BAL fluid ($38 \pm 4\%$) and in lung cells ($13 \pm 2\%$) compared with naive animals ($1.2 \pm 0.4\%$ in BAL and $1.1 \pm 0.5\%$ in lung cells). The increase in total numbers of eosinophils of 12-fold in lung cells and 70-fold in BAL fluid

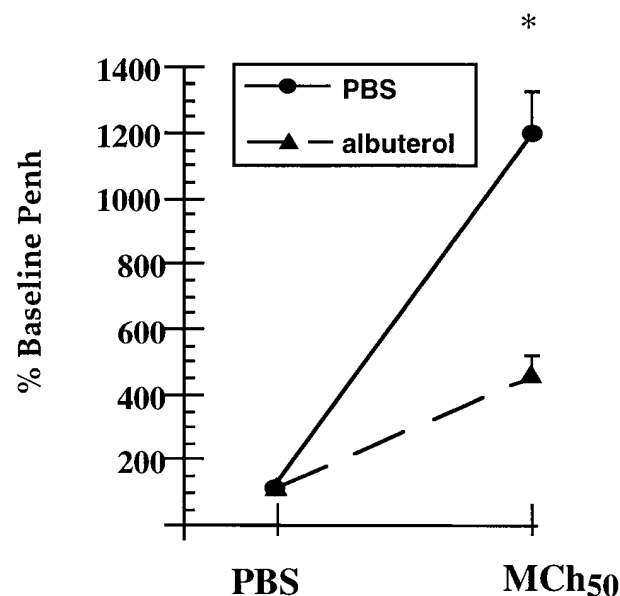


Figure 9. Penh increases are inhibited by nebulized albuterol. Mice were sensitized, and measurements of airway responsiveness were performed as described in Figure 1. After obtaining baseline Penh values after aerosolization of PBS, mice were treated with aerosolized albuterol or PBS for 3 min. After 6 min, mice were challenged with aerosolized MCh at 25 and 50 mg/ml for 3 min each, and Penh was recorded for 3 and 6 min after each nebulization. Compared are sensitized, challenged mice with albuterol ($n = 4$) or PBS ($n = 4$) treatment. Expressed are the means $\pm$ SEM of Penh values in percentages of Penh values after PBS nebulization. Penh_{PBS}, 1.06 ± 0.03 ; albuterol, 1.09 ± 0.08 . * $p < 0.01$ compared with PBS sham-treated mice.

was associated with a ~ 4 -fold increase in Penh after similar MCh challenge compared with the control animals (Table 5).

DISCUSSION

In this report, we characterize a method to measure *in vivo* airway responsiveness in conscious, spontaneously breathing mice. We used a barometric whole-body plethysmograph (WBP) that measures pressure differences between a main chamber containing the animal and a reference chamber. This box pressure signal potentially detects a number of different parameters (23). Among these are heat and humidity changes that occur in the inspired and expired air. However, as the respiratory rate of the animal is 300 breaths/min (or ~ 5 Hz) and stays at ~ 120 breaths/min (or ~ 2 Hz) in bronchoconstricted animals, the

TABLE 5
INCREASES IN PENH CORRELATE WITH EOSINOPHIL LUNG INFILTRATION*

Group	Sensitization (ip)	Challenge (nebulized)	Eosinophils In		Penh After	
			BAL ($\times 10^3$)	Lungs ($\times 10^5$)	MCh 25	MCh 50
N	None	None	2.4 ± 1.3	0.7 ± 0.2	1.4 ± 0.3	2.8 ± 0.2
Neb	None	OVA	2.2 ± 1.1	0.7 ± 0.3	1.5 ± 0.2	2.9 ± 0.4
ipNeb	OVA	OVA	$173 \pm 65^\dagger$	$8.6 \pm 2.2^\dagger$	$5.3 \pm 0.9^\dagger$	$9.06 \pm 1^\dagger$

* Correlation between eosinophil numbers in the BAL fluid and the lung tissue and Penh values after allergic sensitization. Compared are nontreated (N), nonsensitized, OVA-challenged (Neb), and sensitized, challenged (ipNeb) mice. Presented are the means $\pm$ SEM.

† Significant ($p < 0.05$) differences compared with control groups (N and Neb).

volume/flow changes are, in all likelihood, isothermal. The remaining contributors to the changes indicated by the changes of the box pressure signal are alterations in respiratory rate, tidal volume, or compression artifacts (24) (phase lags between nasal and thoracic flow). The avertin (Table 3) and CO₂ (Table 4) experiments suggest that changes in the respiratory rate are not causing similar changes in Penh as observed after agonist inhalation. Independence of Penh measurements from breathing frequency was determined in studies in mechanically ventilated mice where Penh is largely independent of frequency under ventilation with constant volumes. As some increase in Penh occurs with increasing tidal volume in mechanically ventilated mice, yet these changes cannot account for the more than 10-fold increase in Penh values observed after MCh challenge in allergen-sensitized and challenged mice. Taken together, these data suggest that this technique can be used to assess airway responsiveness in mice in a noninvasive fashion.

Bronchoconstriction is known to alter breathing patterns, and indeed changes in Pause and Penh are really due to alterations in the timing of breathing as well as a prolongation of the expiratory time. Airway constriction is further known to lead to an increase in the thoracic flow that is not synchronized with the nasal flow (25), thus resulting in an increase in the box pressure signal. The increase in the time lag between the nasal and the thoracic flow is proportional to total airway resistance and can be used to measure AR by barometric plethysmography (26–28). Penh is considered an empiric parameter that reflects changes in the waveform of the measured box pressure signal that are a consequence of bronchoconstriction. The data in this report show a close correlation between changes in indices derived from the box pressure signal (Penh) and changes in intrapleural pressure or lung resistance (R_L) to aerosolized MCh. Therefore, we conclude that under these conditions, this measurement (Penh) appears to be a valid indicator of bronchoconstriction in mice.

Several investigators have used barometric WBP to measure AHR in guinea pigs and rats (18, 26, 27). The use of this technique in mice enabled us to establish dose-response curves to an aerosolized bronchoconstrictive agent and to differentiate between normal levels of AR in control animals and hyperresponsiveness in allergen-sensitized and challenged mice. Sensitization without allergen challenge or airway challenge of nonsensitized mice was without effect on the Penh values when compared with nontreated animals. The response to MCh in sensitized and challenged animals was both shifted to the left (Table 2) and amplified (elevation of the maximal response, Figure 4) compared with that in control animals. This resembles *in vivo* AHR in patients suffering from bronchial asthma (29) and measurements of AHR with invasive methods in other animal models (30). The most likely mechanism underlying the increases in Penh is bronchoconstriction, mediated through the muscarinic receptors on smooth muscles of the airways. This is supported by the rapid but transient responses to aerosolized MCh. Further evidence for airway obstruction as the major mechanism underlying the increases in Penh was obtained using a β_2 -agonist: pretreatment of sensitized and allergen challenged animals with aerosolized albuterol significantly reduced the increases in Penh after MCh challenge. Albuterol treatment of mice that had already responded with high increases in Penh values after a first dose of MCh prevented similar responses after a second challenge with the same concentration of MCh. Importantly, changes in the respiratory rate in anesthetized mice or after CO₂-stimulation were not accompanied by changes in the Penh values, suggesting that Penh does not correlate simply with changes in breathing patterns.

One problem of measuring AHR with barometric WBP is the uncertainty of the site of obstruction (31) and the absolute value of airway resistance. We studied the effects of lower airway challenge on the development of AHR in the WBP. Lower airway challenge with MCh in tracheostomized mice resulted in a significant increase in airway responses and a shift to the left of the dose-response in allergen-sensitized and challenged animals compared with control mice. The somewhat smaller magnitude in the shift of the MCh dose-response when the upper airways are by-passed by the tracheostomy might suggest that at least a small part of the increased responsiveness as measured with Penh is related to altered upper airway responsiveness. Direct correlation between Penh and changes in lower AR was achieved in parallel measurements of Penh and intrapleural pressure after aerosolized MCh challenge. In order to correlate Penh values with pulmonary resistance, we compared the responses measured by WBP with measurements of pulmonary resistance in the same animals, obtained 1 d later. The responses monitored in the two systems were virtually identical, with comparable increases and a similar left-shift of the dose-response curve over baseline values. These data indicate that Penh correlates well with measurements of pulmonary resistance, and that WBP provides a valid measurement of AHR in allergen sensitized and challenged mice.

A number of studies have associated changes in AHR with increases in allergen-specific IgE (32, 23) and eosinophil airway infiltration (34–36). In our model of allergic sensitization, increases in specific IgE were observed. Further, after allergen challenge, increased numbers of eosinophils were detected in the BAL fluid and in isolated lung cells. Increases in Penh values were associated with production of antigen-specific IgE and the development of an eosinophil infiltration in the lungs after allergen challenge of sensitized mice. These findings confirm the association between AHR, measured by barometric WBP, and eosinophilic inflammation.

In summary, this report describes a method to monitor AHR to aerosolized MCh challenge in conscious, spontaneously breathing mice after allergen-sensitization and challenge. We have shown that changes in the box pressure signal (or the empirically derived parameter of Penh) track the changes in the respiratory system caused by bronchoconstriction. Because barometric WBP is a noninvasive technique the animals do not need to be killed once the measurements are finished, and several measurements on the same animals can be performed, allowing longitudinal studies and investigation of treatment protocols. AHR can be monitored over an extended period of time to mimic chronic allergen exposure, and the kinetics of restoration and secondary responses to allergen can be studied. In addition, this technique is potentially attractive in studying animals infected with various pathogens. Measurements of increased AHR obtained in the WBP correlated with increases in IgE serum levels, eosinophil lung infiltration, and increased lung resistance. We conclude that WBP provides a promising technique to investigate the mechanism and the kinetics underlying the development of AHR and will support the study of new approaches in the prevention of AHR.

Acknowledgment: The writers thank Dr. Peter Sly, Western University of Australia, Perth, Australia, for his help with the computer-controlled ventilation studies.

References

1. National Heart, Lung, and Blood Institute. January 1995. Global Initiative for Asthma. Global strategy for asthma management and prevention. NHLBI/WHO workshop report. NHLBI publication no. 95-3659.
2. Barnes, P. J. 1987. New concepts in the pathogenesis of bronchial hyper-

- responsiveness. *Agents Actions Suppl.* 21:225–237.
3. Djukanovic, R., W. R. Roche, J. W. Wilson, C. R. Beasley, O. P. Twentyman, R. H. Howarth, and S. T. Holgate. 1990. Mucosal inflammation in asthma (review). *Am. Rev. Respir. Dis.* 132:434–457.
 4. Bousquet, J., P. Chanez, J. Y. Lacoste, G. Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, and F. B. Michel. 1990. Eosinophilic inflammation in asthma. *N. Engl. J. Med.* 323:1033–1039.
 5. Robinson, D. S., Q. Hamid, S. Ying, A. Tscicopoulos, J. Barkans, A. M. Bentley, C. Corrigan, S. R. Durham, and A. B. Kay. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326:298–304.
 6. Wanner, A., W. M. Abraham, J. S. Douglas, J. M. Drazen, H. B. Richerson, and J. S. Ram. 1990. Models of airway hyperresponsiveness. *Am. Rev. Respir. Dis.* 141:253–257.
 7. Tu, Y. R., G. L. Larsen, and C. G. Irvin. 1995. Utility of murine systems to study asthma pathogenesis. *Eur. Respir. Rev.* 5:224–230.
 8. Corry, D. B., H. G. Folkesson, M. L. Warnock, D. J. Erle, M. A. Matthay, J. P. Wiener-Kronish, and R. M. Locksley. 1996. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperactivity. *J. Exp. Med.* 183:109–117.
 9. Foster, P. S., S. P. Hogan, A. J. Ramsay, K. I. Matthaei, and I. G. Young. 1996. Interleukin 5 deficiency abolishes eosinophilia, airway hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183:195–201.
 10. Hamelmann, E., A. Oshiba, J. Loader, G. L. Larsen, J. Lee, and E. W. Gelfand. 1997. Anti-interleukin-5 (IL-5) antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. *Am. J. Respir. Crit. Care Med.* (In press)
 11. Hamelmann, E., A. Oshiba, J. Paluh, K. Bradley, J. Loader, T. A. Potter, G. L. Larsen, and E. W. Gelfand. 1996. Requirement for CD8+ T cells in the development of airway hyperresponsiveness in a murine model of airway sensitization. *J. Exp. Med.* 183:1719–1730.
 12. Gavett, S. H., X. Chen, F. D. Finkelman, and M. Wills-Karp. 1994. Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am. J. Respir. Cell Mol. Biol.* 10:587–593.
 13. Larsen, G. L., H. Renz, J. E. Loader, K. L. Bradley, and E. W. Gelfand. 1992. Airway response to electrical field stimulation in sensitized inbred mice: passive transfer of increased responsiveness with peribronchial lymph nodes. *J. Clin. Invest.* 89:747–752.
 14. Martin, T. R., N. P. Gerard, S. J. Galli, and J. M. Drazen. 1988. Pulmonary responses to bronchoconstrictor agonists in the mouse. *J. Appl. Physiol.* 64:2318–2323.
 15. Renz, H., H. R. Smith, J. E. Henson, B. S. Ray, C. G. Irvin, and E. W. Gelfand. 1992. Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *J. Allergy Clin. Immunol.* 89:1127–1138.
 16. Levitt, R. C., and W. Mitzner. 1988. Expression of airway hyperactivity to acetylcholine as a simple autosomal recessive trait in mice. *FASEB J.* 2:2605–2608.
 17. Larsen, G. L., T. M. Fame, H. Renz, J. E. Loader, J. Graves, M. Hill, and E. W. Gelfand. 1994. Increased acetylcholine release in tracheas from allergen-exposed IgE-immune mice. *Am. J. Physiol.* 266:L263–L270.
 18. Jacky, J. P. 1978. A plethysmograph for long-term measurements of ventilation in unrestrained animals. *J. Appl. Physiol.* 45:644–647.
 19. Vijayaraghavan, R., M. Shaper, R. Thompson, M. F. Stock, and Y. Alarie. 1993. Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. *Arch. Toxicol.* 67:478–490.
 20. Dorsch, W., U. Waldherr, and J. Rosmanith. 1981. Continuous recording of intrapulmonary “compressed air” as a sensitive noninvasive method of measuring bronchial obstruction in guinea pigs. *Pflügers Arch.* 391:236–241.
 21. Schuessler, T. F., and J. H. Bates. 1995. A computer-controlled research ventilator for small animals: design and evaluation. *IEEE Trans. Biomed. Eng.* 49:860–866.
 22. Oshiba, A., E. Hamelmann, K. Takeda, K. L. Bradley, J. E. Loader, G. L. Larsen, and E. W. Gelfand. 1996. Passive transfer of immediate hypersensitivity and airway hyperresponsiveness by allergen-specific IgE and IgG1 in mice. *J. Clin. Invest.* 97:1398–1408.
 23. Epstein, M. A., and R. A. Epstein. 1978. A theoretic analysis of the barometric method for measurement of tidal volume. *Respir. Physiol.* 32:105–120.
 24. Ingram, R. H., and D. P. Schilder. 1966. Effect of gas compression on pulmonary pressure, flow, and volume relationship. *J. Appl. Physiol.* 21:1821–1826.
 25. Peslin, R., P. Jardin, and B. Hanhart. 1976. Modelling of the relationship between volume variations at the mouth and chest. *J. Appl. Physiol.* 41:659–667.
 26. Pennock, B. E., C. P. Cox, R. M. Rogers, W. A. Cain, and J. H. Wells. 1979. A noninvasive technique for measurement of changes in specific airway resistance. *J. Appl. Physiol.* 46:399–406.
 27. Johanson, W. G., Jr., and A. K. Pierce. 1971. A noninvasive technique for measurement of airway conductance in small animals. *J. Appl. Physiol.* 30:146–150.
 28. Silbaugh, S. A., and J. L. Mauderly. 1984. Noninvasive detection of airway constriction in awake guinea pigs. *J. Appl. Physiol.* 56:1666–1669.
 29. Woolcock, A. J., C. M. Salome, and K. Yan. 1984. The shape of the dose-response to histamine in asthmatic and normal subjects. *Am. Rev. Respir. Dis.* 130:71–79.
 30. Featherstone, R. L., P. A. Hutson, S. T. Holgate, and M. K. Church. 1988. Active sensitization of guinea-pig airways *in vivo* enhances *in vivo* and *in vitro* responsiveness. *Eur. Respir. J.* 1:839–845.
 31. Bellofiore, S., G. U. DiMaria, and J. G. Martin. 1987. Changes in upper and lower airway resistance after inhalation of antigen in sensitized rats. *Am. Rev. Respir. Dis.* 136:363–368.
 32. Rafferty, P., R. Beasley, and S. T. Holgate. 1987. The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine-5'-monophosphate in atopic asthma. *Am. Rev. Respir. Dis.* 136:368–373.
 33. Howarth, P. H., S. R. Durham, A. B. Kay, and S. T. Holgate. 1987. The relationship between mast cell-mediator release and bronchial reactivity in allergic asthma. *J. Allergy Clin. Immunol.* 80:703–711.
 34. Gleich, G. J. 1990. The eosinophil and bronchial asthma: current understanding. *J. Allergy Clin. Immunol.* 85:422–436.
 35. Van Oosterhout, A. J., A. R. Ladenius, H. F. Savelkoul, I. Van Ark, K. C. Delsman, and F. P. Nijkamp. 1993. Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. *Am. Rev. Respir. Dis.* 147:548–552.
 36. Mauser, P. J., A. M. Pitman, X. Fernandez, S. K. Foran, G. K. Adams, 3rd, W. Kreutner, R. W. Egan, and R. W. Chapman. 1995. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am. J. Respir. Crit. Care Med.* 152:467–472.