Noninvasive measurement of midexpiratory flow indicates bronchoconstriction in allergic rats

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Departments of ¹Respiratory Medicine and ⁴Anatomy, Hannover Medical School, and ²Fraunhofer Institute of Toxicology and Aerosol Research, Drug Research and Clinical Inhalation, 30625 Hannover, Germany; and ³Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15238

Received 8 November 2001; accepted in final form 8 May 2002

Glaab, Thomas, Heinz G. Hoymann, Jens M. Hohlfeld, Regina Korolewitz, Matthias Hecht, Yves Alarie, Thomas Tschernig, Armin Braun, Norbert Krug, and Helmut Fabel. Noninvasive measurement of midexpiratory flow indicates bronchoconstriction in allergic rats. J Appl Physiol 93: 1208–1214, 2002. First published May 10, 2002; 10.1152/japplphysiol.01121.2001.—This study was designed to evaluate the value and applicability of tidal breathing pattern analysis to assess bronchoconstriction in conscious rats. Using noninvasive, head-out body plethysmography and the decrease in tidal midexpiratory flow (EF50), we measured airway responsiveness (AR) to inhaled acetylcholine and allergen in conscious Brown-Norway rats, followed by invasive determination of pulmonary conductance (Gl) and EF50 in anesthetized rats. Dose-response studies to acetylcholine showed that noninvasively recorded EF50 closely reflected the dose-dependent decreases observed with the invasive monitoring of simultaneously measured Gl and EF50. After sensitization and intratracheal boost to ovalbumin or saline, rats were assessed for early and late AR to aerosolized ovalbumin. Ovalbumin aerosol challenge resulted in early and late AR in allergen-sensitized rats, whereas controls were unresponsive. The allergen-specific AR, as measured noninvasively by EF50, was similar in degree compared with invasively recorded EF50 and Gl and was associated with enhanced IgE and airway inflammation. We conclude that EF50 is a noninvasive and physiologically valid index of bronchoconstriction in a rat model of asthma.

animal models; allergy; immunology; respiratory function test; asthma

ANIMAL MODELS OF ASTHMA HAVE been developed in various animal species and have provided new insights into the mechanisms underlying the pathophysiology of airway hyperresponsiveness in asthma. The highly inbred Brown-Norway (BN) rat model has been widely used to study respiratory mechanics in allergen-induced airway inflammation because of its well-developed airway smooth musculature, within-strain homogeneity of airway reactivity, and the development of early and late airway responsiveness (AR) to allergen (6, 7, 27). Recent advances in measuring respiratory mechanics in intact, spontaneously breathing animals have allowed simple, noninvasive, routine analysis of pulmonary function in animal models (11, 12, 22, 23, 26). Results of our previous studies suggest that midexpiratory tidal flow (EF50), as measured by head-out body plethysmography, can be used as a noninvasive measure of bronchoconstriction in mice (9, 21). The assessment of EF50 in conscious animals has a number of advantages. First, the measurements are technically easy to perform. Second, because this method does not require anesthesia or surgical interventions, the same animal can be studied on multiple occasions. Until now, only few data on the validity of EF50 in mice have been available. However, it is unclear whether this parameter can simply be transferred to other species with a different breathing pattern and whether this index is suitable to detect changes associated not only with ACh-related bronchoconstriction but also with allergen-specific early and late AR. Accordingly, the reliability of EF50 has to be studied more extensively, in direct comparison with conventional standard parameters, before it can be established as a surrogate for bronchoconstriction in experimental asthma models.

The aim of this study was to provide first experimental evidence that the noninvasive determination of EF50 can be used as a valid physiological measure of nonspecific and allergen-specific AR in conscious rats. To investigate the applicability of EF50 in a rat model of early and late AR, dose-response studies to aerosolized ACh were performed with noninvasive measurement of EF50 in conscious rats by head-out body pleth-

¹A year 2000 compatible DOS software version of the noninvasive EF50 method can be obtained free of charge to interested researchers from Y. Alarie.

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ysmography, followed by invasive monitoring of simultaneously measured \( EF_{50} \) and pulmonary conductance (GL) in orotracheally intubated, spontaneously breathing rats. The sensitivity of \( EF_{50} \) to detect early and late AR in allergic rats was compared with the “gold standard” parameter, GL. In addition, we examined the potential effects of induced hyper- and hypventilation on simultaneously measured \( EF_{50} \) and GL. This approach represents a significant advance in accuracy over previous validation experiments that have failed to demonstrate simultaneous measurements of noninvasive and invasive indexes of bronchoconstriction or that have included upper airway resistance (9, 11, 12, 21).

MATERIALS AND METHODS

Animals. Pathogen-free inbred male BN rats (Charles River, Sulzfeld, Germany), 6–7 wk of age, were used in all experiments. Rats were housed for at least 1 wk before investigation. Rats were divided into four main groups: 1) ACh challenge (n = 8) (after noninvasive monitoring of \( EF_{50} \), animals were reused for invasive determination of \( EF_{50} \) and GL to ACh challenge 24 h later); 2) challenge experiments with saline, CO2, and halothane (n = 5); 3) allergen-sensitized and boosted rats for invasive (n = 8) and noninvasive (n = 8) measurements of early and late AR to inhaled ovalbumin (Ova); and 4) sham-treated controls for invasive (n = 6) and noninvasive (n = 6) measurements of early and late AR to inhaled Ova. All animal experimentation conformed to the guidelines of the National Research Council Guide.

Sensitization protocol. For allergic rats (Ova/Ova), on day 0, animals were sensitized by a subcutaneous injection of 1 mg Ova (grade V, Sigma Chemical, Deisenhofen, Germany) precipitated in 20 mg of aluminum hydroxide gel in 0.25 ml normal saline (n = 8). An adjuvant consisting of 6 × 104 heat-killed Bordetella pertussis organisms (Chiron-Behring, Marburg, Germany) was injected intraperitoneally at the same time. On days 7 and 14, the anesthetized animals were boosted intratracheally via an orotracheal tube with 0.1 ml of 0.3% Ova in normal saline (NaCl). Control rats (NaCl/NaCl) were sensitized and boosted to normal saline instead of Ova (n = 6). On day 21, all animals were challenged with aerosolized Ova as described below.

Noninvasive measurement of AR in conscious rats. AR was assessed as previously described in mice (9, 26) with a modified head-out body plethysmography system for rats (head-out body plethysmography system model 855, HSE-Harvard, March-Hugstetten, Germany). Briefly, rats were placed in the body plethysmographs while the head of each animal protruded through a neck collar (18–20 mm ID, dental latex dam; Roeko, Langenau, Germany) into the head-exposure chamber, which was ventilated by a continuous airflow of 0.5 l/min. Rats held in head-out body plethysmographs tolerated noninvasive measurements very well. Monitoring of respiratory function was started after 2–5 min when animals and individual measurements settled down to a stable level. For airflow measurement, a wire mesh pneumotachometer with defined resistance (6 layers of wire mesh cloth, linearity ±2% ranging from 20–300 breaths/min; HSE-Harvard) and a differential pressure transducer (Validyne DP 45-14, range ±2 cmH2O, HSE-Harvard) coupled to an amplifier were attached in midposition to the side wall of each plethysmograph. For each animal, the amplified analog signal from the pressure transducer was digitized via an analog-to-digital converter (DT 302, Data Translation) at a sampling rate of 250 Hz. Before each measurement, the plethysmograph was calibrated with an injection of 1 ml air into the body chamber. The pneumotachographic tidal flow signal was integrated with time to obtain tidal volume (VT). From these signals, all of the noninvasive parameters listed in Table 1 (\( EF_{50} \), time of inspiration (TI) and expiration (TE), VT, respiratory rate (f)) were calculated for each breath with commercial software (HEM 3.3, Notocord, Paris, France).

Inspiration and expiration were monitored by establishing start expiration and end expiration as the tidal flow/time curve crosses the zero point. During bronchoconstriction, the main alteration in the tidal flow signal occurs during the midexpiration phase. \( EF_{50} \) (in ml/s) calculates the tidal flow at the midpoint (50%) of expiratory VT and was used as an index of bronchoconstriction. An example of tidal flow and corresponding VT patterns from a naive rat before and after challenge with aerosolized ACh is shown in Fig. 1. Analysis of tidal flow values at 25, 50, and 75% VT during expiration had revealed that \( EF_{50} \) was the most appropriate value to detect airway obstruction (26). The degree of bronchoconstriction to inhalational ACh and Ova challenge was determined from minimum values of \( EF_{50} \) and was expressed as percent changes from corresponding baseline values.

Invasive determination of AR. AR was assessed in anesthetized and orotracheally intubated rats as previously described (14–16). The spontaneously breathing animal was placed in the supine position in a thermostat-controlled whole body plethysmography, and slight anesthesia was maintained with a gas mixture of 0.8% halothane in 30% oxygen. Tidal flow was determined by a pneumotachometer

| Table 1. Baseline values for respiratory parameters from naive Brown-Norway rats |
|------------------|------------------|------------------|------------------|
| **Respiratory Parameters** | **Definition** | **Conscious** | **Anesthetized** |
| VT, ml | Amount of air inhaled or exhaled per breath | 1.58 ± 0.16* | 1.08 ± 0.12 |
| TE, s | Time from maximum to minimum VT | 0.39 ± 0.03* | 0.35 ± 0.04 |
| TI, s | Time from minimum to maximum VT | 0.20 ± 0.02* | 0.23 ± 0.03 |
| f, breaths/min | Number of breaths per minute | 116 ± 0.6 | 82 ± 14 |
| \( EF_{50} \), ml/s | Tidal flow during expiration at 0.5 VT | 8.42 ± 0.99 | 8.0 ± 1.1 |
| RT, cmH2O·s·ml⁻¹ | Relation of the integrated differences in transpulmonary pressure and tidal flow during the breathing cycle | 0.22 ± 0.04 | 0.22 ± 0.04 |
| GL, ml·cmH2O⁻¹·s⁻¹ | Reciprocal of pulmonary resistance, 1/RT | 4.73 ± 0.86 | 4.73 ± 0.86 |

Values are means ± SD obtained during a 5-min control period from both 46 conscious and 46 anesthetized, orotracheally intubated Brown-Norway rats. VT, tidal volume; TE, expiratory time; TI, inspiratory time; f, respiratory rate; \( EF_{50} \), tidal midexpiratory flow; RT, pulmonary resistance; GL, pulmonary conductance. In contrast to decreased values in VT and f with concomitant increases in TI and TE, \( EF_{50} \) values were not significantly affected by anesthesia. *P < 0.01 vs. anesthetized rats.
Fig. 1. Left: normal breathing pattern of an anesthetized, orotracheally intubated, spontaneously breathing Brown-Norway rat. Right: breathing pattern during bronchoconstriction due to inhalation of ACh aerosol, illustrating the simultaneous decreases in midexpiratory tidal flow (EF50) and pulmonary conductance (GL). A: tidal volume (VT) obtained from the integrated resistance (Rl) was calculated from the integrated differences in esophageal pressure and tidal flow (14–16). Rl and EF50, together with all of the other parameters listed in Table 1, were recorded simultaneously with commercial software (HEM 3.3, Notocord). For better comparability of data, Rl was expressed as GL (1/RL).

Challenge protocol. After recording of baseline values, AR to doubling doses of aerosolized ACh (ranging from 20 to 160 mg/m³; Sigma Chemical) in naive rats and AR to aerosolized Ova (grade V, 135 mg/m³ over 8.5 min) in sensitized and nonsensitized animals were assessed. Aerosols were generated by a computer-controlled aerosol generator system (Bronchly III, particle size 1.5–2 μm mass median aerodynamic diameter; Fraunhofer ITA, Hannover, Germany), as previously described (14–16). To characterize the potential effects of induced hyper- and hypoventilation on the parameters of bronchoconstriction, inhalational exposures to 10% carbon dioxide (CO2) and 3.5% halothane (Eurim-Pharma, Piding, Germany) were performed. The degree of AR to all of these challenges was determined from minimum values of GL and EF50 and was expressed as percent changes from corresponding baseline values, which were taken as 0%.

Determination of total IgE in serum. Plates were coated with mouse monoclonal antibody to rat IgE (MCA 193, Serotec, Wiesbaden, Germany) in a concentration of 0.1 μg/ml per well. The plates were incubated overnight, washed, blocked with Roti Block (Roth, Karlsruhe, Germany), and incubated with sera and standard for 1 h. Total IgE protein (rat IgE, PRP07, Serotec) was used as a standard in a concentration of 0.2 μg/ml. After washing, 50 μl of a second peroxidase-labeled anti-IgE antibody (MCA 187P, Serotec) were added to the plates and incubated for 1 h. After an additional washing step, 50 μl of the peroxidase substrate o-phenylenediamine dihydrochloride (Sigma Chemical) were pipetted to each well. The plates were developed for 10 min, and the reaction was stopped by adding 100 μl/well of 1 N HCl (Merck, Darmstadt, Germany). Plates were read with an ELISA plate reader (model MRX, Dynatech, Chantilly, VA) at 490 nm.

Assessment of leukocyte distribution in bronchoalveolar lavage fluid and histology. Bronchoalveolar lavage (BAL) and histology were performed 16–20 h after allergen challenge, and histology were performed 16–20 h after allergen challenge, as previously described (25).

Statistics. A mixed model was fitted to the data by using the animal as random factor (4). Comparisons for multiple pairs were performed by the Tukey Kramer test and for single pairs by Student’s t-test. Correlations were calculated with Pearson’s correlation coefficient. Values for all measurements were expressed as means ± SD. The agreement between the methods was analyzed by the method of Bland and Altman (3). Agreement was expressed as the mean differences obtained by the different techniques (e.g., between the EF50 method in conscious and anesthetized rats and between EF50 and Gl in anesthetized animals). The limits of agreement were expressed as means ± 2 SD, and the 95% confidence intervals (CIs) of the mean as well as the lower and upper limits of agreement were calculated according to Bland and Altman. P values < 0.05 were considered significant. All statistical analyses were performed with SAS 8.01.

RESULTS

Baseline values for respiratory parameters in conscious and anesthetized BN rats. Table 1 presents the baseline values of respiratory parameters obtained from naive, conscious BN rats placed in head-out plethysmographs and from anesthetized, spontaneously breathing BN rats placed in whole body plethysmographs. Except for EF50, anesthesia significantly affected the respiratory parameters f, VT, TI, and TE compared with baseline levels of conscious animals.

Comparison of the noninvasive determination of EF50 with invasive measurements of Gl and EF50 in AR to ACh. Nonspecific AR to increasing doses of aerosolized ACh (20–160 mg/m³) was investigated in naive, conscious rats (n = 8) with nonspecific head-out body plethysmography. The same animals were anesthetized and invasively reexposed to inhaled ACh 24 h later. Figure 1 presents the breathing pattern of an anesthetized, spontaneously breathing rat that was breathing mixed air (room air with 30% oxygen) and the characteristic modifications to the normal airflow pattern after ACh aerosol exposure, illustrating the simultaneous decreases in VT, EF50, and GL during bronchoconstriction.

To determine whether decreases in EF50 correlate directly with decreased Gl values, EF50 was monitored simultaneously with Gl in the anesthetized animals.
ACh exposure induced a dose-related decline in Gl and EF50 values, with strong correlations between these parameters (Fig. 2). There was a close correlation between decreased EF50 and Gl values ($r = 0.93; P < 0.01$) in the anesthetized rats. In addition, a positive correlation was found between the decreased EF50 values in conscious animals and both the decreased Gl ($r = 0.85; P < 0.01$) and EF50 values ($r = 0.79; P < 0.01$) in anesthetized rats. The peak declines in EF50 recordings to nonspecific ACh challenge at 160 mg/m$^3$ were similar between invasively and noninvasively measured EF50, but were significantly less pronounced compared with Gl ($P < 0.01$).

Figure 3A shows the Bland-Altman plot of the differences between simultaneously measured EF50 and Gl against the mean of both values. EF50 measurements tended to underestimate the increase in bronchoconstriction by $-7.8 \pm 8.5\%$ (95% CI, $-4.7$ to $-10.9\%$) compared with Gl. The limits of agreement of invasive measurement of EF50 compared with Gl were between $-24.8\%$ (95% CI, $-19.5$ to $-30.1\%$) and 9.2\% (95% CI, 13.1$-$27.7\%). Figure 3B displays the Bland-Altman plot of the differences against mean for the invasively and noninvasively monitored EF50. The agreement between invasively and noninvasively monitored EF50 was good, with the mean difference being $-3.1\%$ (95% CI, $-7.3$ to $-1.2\%$). However, enhanced variation of single measurements was found at high-dose ACh challenge, and the upper and lower limits of agreement presented a variability of $-26.5\%$ (95% CI, $-19.2$ to $-33.8\%$) to 20.4\% (95% CI, 13.1$-$27.7\%). There was no significant difference between the agreement of invasively and noninvasively monitored EF50 in reflecting the ACh-induced change in airway constriction. Noninvasive EF50 recordings differed from the Gl method by $-10.8 \pm 12.6\%$ (95% CI, $-6.3$ to $-15.4\%$). The limits of agreement were $-36.1\%$ (lower limit; 95% CI, $-28.2$ to $-43.9\%$) and 14.4\% (upper limit; 95% CI, 6.5$-$22.3\%). After inhalation of each concentration of ACh, peak responses for Gl and EF50 were reached within 1 min and recovered to within 10% of the baseline during 1$-$3.5 min. EF50 and Gl values decreased proportionally with small increases in f and decreasing values for VT, whereas TE and TI remained nearly constant.

Effects of CO2 and deep anesthesia on respiratory parameters. To investigate the influence of various agents with potential modifying effects on f, VT, EF50, and Gl recordings, a separate group of naive, anesthetized rats ($n = 5$) was exposed to 10% CO2 and deep anesthesia with 3.5% halothane (Table 2). Except for a stimulating effect on f and VT, CO2 exposure evoked no significant decreases in EF50 and Gl. Standard anesthesia with 2% halothane induced a reduction in f and VT (see Table 1). Changing halothane concentrations from 2 to 3.5% resulted in an additional decline in f and VT, as well as in significant decreases in EF50 and Gl values. These experiments demonstrate that EF50 was not decreased by CO2 exposure and that the changes in EF50 to 3.5% halothane exposure were quite similar to those simultaneously observed with Gl.

Early and late AR to Ova in allergic rats. The early and late AR to Ova was investigated on day 21 in two
groups divided into allergen- (Ova/Ova) and saline-treated (NaCl/NaCl) animals (Fig. 4). To avoid unbalanced challenges with Ova, each group was separated into two subgroups for invasive and noninvasive measurement of pulmonary function. All respiratory parameters as listed in Table 1 were continuously monitored during the aerosol challenge with Ova.

Invasive recordings of Ova/Ova rats showed significant declines in simultaneously measured EF50 and GL within 23 min, indicating an Ova-specific early AR. There was a strong correlation between decreased EF50 and GL values (r = 0.90; P < 0.01) in Ova/Ova rats. The mean difference between EF50 and GL was −10.6 ± 9.9% (95% CI, −4.9 to −16.3%) with upper and lower limits of agreement ranging from −9.2 (upper limit; 95% CI, −0.7 to 19.1%) to −30.4 (lower limit; 95% CI, −40.3 to −20.5%). Noninvasive measurements of allergen-specific AR in allergic animals also demonstrated a significant decline in EF50, which was similar in degree to the decline observed with invasively recorded EF50. Fig. 5 shows a characteristic time-response course of an early AR. Late AR peaked at 5–6.5 h after Ova challenge in allergic rats. No significant difference was found between the magnitude of the early AR and the late AR in the allergic rats. However, two of eight animals that showed an early AR demonstrated no late AR, as indicated by a recovery of GL and EF50 values close to baseline in these animals. There was a significant difference in the magnitude of both the early and late AR between the allergic animals (Ova/Ova) and the saline controls (NaCl/NaCl). These data suggest that EF50 measurement clearly indicates early and late AR in allergen-sensitized and -challenged rats. The allergic animals had enhanced serum levels of total IgE, compared with saline controls (Ova/Ova, n = 13 (pooled data from conscious and anesthetized rats), 1.61 ± 0.59 μg/ml vs. NaCl/NaCl, n = 11 (pooled data), 0.49 ± 0.06 μg/ml; P < 0.01). In addition, only Ova/Ova rats had significant increases in eosinophils and neutrophils in BAL fluid (Table 3). There was no statistical association between cellular content or IgE measurements and individual airway responses in dual responders and those rats that showed no late response.

**DISCUSSION**

In this report, we describe that the changes in EF50 closely reflected the changes observed in Gl during bronchoconstriction induced by aerosolized ACh and allergen in rats. We have characterized airway responses to ACh in naive animals and to allergen in a rat model that replicates the dual-phase response in asthma.

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**Table 2. Effects of various airborne agents on respiratory parameters**

<table>
<thead>
<tr>
<th>Substance</th>
<th>f</th>
<th>Vt</th>
<th>EF50</th>
<th>GL</th>
</tr>
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<tbody>
<tr>
<td>CO2 10%</td>
<td>37.2 ± 9.4*</td>
<td>49.9 ± 18.4*</td>
<td>1.5 ± 4.8</td>
<td>−5.9 ± 10.8</td>
</tr>
<tr>
<td>Halothane 3.5%</td>
<td>−51.7 ± 12.8*</td>
<td>−25.9 ± 6.1*</td>
<td>−25.2 ± 9.1*</td>
<td>−15.2 ± 5.2*</td>
</tr>
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</table>

Values are means ± SD expressed as peak percent changes from baseline values (note: only minimum values for EF50 and GL were analyzed); n = 5 rats. Standard anesthesia was with 2% halothane, 30% oxygen. CO2 exposure induced significant increases in f, Vt, and the mean flow but evoked no decreases in EF50 and GL values. Deep anesthesia with 3.5% halothane resulted in parallel decreases in EF50 and GL with concomitant declines in f and Vt. Preexposure values for f, Vt, EF50, and GL were within the means ± SD of the baseline values for anesthetized rats as listed in Table 1. *P < 0.01 vs. baseline values.

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**Fig. 4. Assessment of early airway responsiveness (AR; A) and late AR (B) to ovalbumin (Ova) challenge (concentration = 135 mg/m³). AR to Ova was monitored in Ova/Ova rats (n = 8 each) and in NaCl/NaCl animals (n = 6 each). The EF50 responses were studied in separate groups of invasively and noninvasively monitored animals. Both early AR and late AR were related to prechallenge baseline values. Significant decreases in invasively and noninvasively recorded EF50 and GL within 23 min and at 5–6.5 h after Ova challenge were observed in Ova/Ova rats. AR is expressed as % change in EF50 and GL from corresponding baseline values, which were taken as 0%. Baseline values were within the means ± SD as listed in Table 1 and were similar in the Ova and saline group. **P < 0.01 (early AR) and ***P < 0.05 (late AR) for each parameter vs. NaCl/NaCl.

**Fig. 5. Characteristic time course of an early AR illustrating the parallel decreases in EF50 and Gl values with a concomitant slight decline in Vt during and after aerosolized Ova challenge of an allergic rat. There was an evident recovery of EF50 and Vt during the postchallenge period, which was less pronounced for Gl. AR is expressed as % change in Vt, EF50, and GL from baseline values, which were taken as 0%. Baseline values were within the means ± SD as listed in Table 1.**
Table 3. Cellular composition of BAL fluid

<table>
<thead>
<tr>
<th></th>
<th>Ova/Ova</th>
<th>NaCl/NaCl</th>
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<tbody>
<tr>
<td>Eosinophils, × 10^6</td>
<td>2.72 ± 1.99*</td>
<td>0.16 ± 0.12</td>
</tr>
<tr>
<td>Lymphocytes, × 10^6</td>
<td>0.38 ± 0.28</td>
<td>0.13 ± 0.15</td>
</tr>
<tr>
<td>Neutrophils, × 10^6</td>
<td>0.80 ± 0.48*</td>
<td>0.12 ± 0.07</td>
</tr>
<tr>
<td>Macrophages, × 10^6</td>
<td>11.72 ± 4.25*</td>
<td>4.55 ± 0.98</td>
</tr>
</tbody>
</table>

Values are means ± SD from 13–14 animals per group (pooled data). Eosinophils, neutrophils, lymphocytes, and macrophages recovered from bronchoalveolar lavage (BAL) fluid 16–20 h after ovalbumin (Ova) aerosol challenge were significantly increased in the Ova/Ova group. *P < 0.01 vs. NaCl/NaCl.

The increasing amount of genetic information from experimental asthma models has extended our understanding of the complex pathophysiology of asthma. However, there is a large discrepancy between the availability of the advanced tools of cell and molecular biology and the conventional invasive methods of assessing lung function in animal models (1). Accordingly, the development of an accurate and sensitive methodology that allows simple and repetitive determination of respiratory physiology in the intact organism is necessary to study the functional consequences of in vitro findings in the lung in vivo. Our strategy of noninvasive determination of EF_{50} with head-out body plethysmography enables both repetitive assessment and quantitative interpretation of bronchoconstriction in conscious, spontaneously breathing animals.

The values for respiratory parameters monitored in conscious and in anesthetized rats were reproducible and paralleled those reported previously for male BN rats (Table 1) (28). Standard anesthesia with halothane did not influence baseline EF_{50} measurements compared with those in conscious animals (see Table 1). To evaluate the reliability of EF_{50} as a measure of bronchoconstriction, we performed dose-response measurements to inhaled ACh, ranging from 20 to 160 mg/m³, in both conscious and anesthetized animals. In these comparative studies, we demonstrated that EF_{50} accurately reflected the response to increasing doses of ACh observed with G_{L} as an outcome indicator. Direct comparison of invasively recorded EF_{50} and G_{L} with noninvasive measurement of EF_{50} revealed that G_{L} agreed closely with invasive and noninvasive EF_{50} recordings and showed comparable decreases of the dose-response curve over baseline values (Figs. 2 and 3). Consistent with data from previous studies (2, 6, 7, 27), we found that all BN rats immunized and boosted by Ova uniformly demonstrated an early AR followed by a late AR with a prevalence of 75%. This dual-phase AR was allergen specific, as the extent of both the early and late airway responses after provocation with Ova was significantly lower in control animals (NaCl/NaCl). It should be pointed out, however, that the post-Ova decrease in EF_{50} might relate to diminished lung compliance caused by heterogeneous airway closure and non-reopening after Ova-induced bronchoconstriction. Detection of early- and late-phase AR was based on corresponding decreases in G_{L} and EF_{50}. We found that, in response to ACh or allergen, invasively and noninvasively recorded EF_{50} tended to slightly underestimate the degree of bronchoconstriction and was sustained for a shorter period compared with the G_{L} method. A possible explanation for this observation might be that G_{L} rather than EF_{50} reflects changes in tissue resistance, known to largely contribute to the overall resistance to nebulized bronchoconstrictors in rats (10, 20). However, we have demonstrated that EF_{50} can significantly discriminate the degree of AR and showed acceptable overall agreement with G_{L} recordings. The range of variations from single measurements was not larger than those reported from other animal and human studies comparing invasive and noninvasive techniques (11, 13). In combination with the noninvasive monitoring of EF_{50} in conscious rats, this approach seems to be more appropriate than previous validation experiments of noninvasive technologies. Compared with the established noninvasive whole body plethysmography method (5, 11, 19) that uses the dimensionless variable enhanced pause (Penh) to empirically monitor bronchoconstriction in conscious animals, the absolute value of EF_{50} has physical meaning and enables a direct quantitative comparison from animal to animal (5). Consistent with reported data in mice (9, 18), we provide evidence that, in rats also, bronchoconstriction is clearly associated with a reduction in V_{T} (Fig. 5). In contrast, V_{T} has been shown to increase during bronchoconstriction with the Penh method (11). Although methodological differences exist between the Penh and the EF_{50} methods, including the limited quantitative and physiological interpretation of Penh, it has been shown recently in mice that both methods provide similar and reproducible results (8).

The independence of EF_{50} measurements from breathing frequency was examined in experiments with 10% CO_{2} and 2% halothane, which demonstrated that changes in breathing frequency induced no significant decrease in EF_{50} and G_{L} values (Tables 1 and 2). However, deep anesthesia with 3.5% halothane was associated with concomitant decreases in f, V_{T}, EF_{50}, and G_{L} values. This finding contrasts with the fact that volatile anesthetics such as halothane usually have direct relaxant effects on airway smooth muscle. The observed bronchoconstrictive response to high concentrations of halothane may be related to a pronounced increase in the tissue component of resistance caused by viscoelastic elements and lung inhomogeneity in the rat (24).

In our model of allergic airway inflammation, decreases in EF_{50} were linked with enhanced IgE serum levels and the development of neutrophil and eosinophil influx in the lungs after allergen challenge of allergic rats. The ability to measure pulmonary physiology repeatedly in a longitudinal study design would be valuable for the study of the time course of chronic lung and airway disorders. The recent development of simple testing methods in conscious, intact animals has contributed largely to a widespread and multidisciplinary use of pulmonary function tests in experimental asthma research (8, 17, 19).
In summary, EF50 measurements closely reflected the changes in invasively monitored EF50 and Gt. during challenges with inhaled ACh and allergen. We conclude that the broad applicability of noninvasive measurement of EF50 by head-out body plethysmography should provide a complementary methodology to measurement of EF50 by head-out body plethysmography.

We thank Prof. H. Hecker, Biometries of Hannover Medical School, for statistical support, as well as Gill Teicke for linguistic review of the manuscript.

This work was supported by Hochschulinterne Leistungsfoerderung of Hannover Medical School and in part by Deutsche Forschungsgemeinschaft Grant Fa 64/4-1.

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