Unrestrained plethysmography is an unreliable measure of airways responsiveness in BALB/c and C57BL6 mice

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Abstract

There has been significant utilization of the technique described by Hamelmann *et al.* (1997) in which a parameter, *Penh*, related to airways responsiveness is non-invasively measured using unrestrained plethysmography (UP). We investigated this technique, seeking to answer these questions: 1) How do changes in *Penh* compare with changes in traditional plethysmographic and lung mechanical parameters? 2) How do UP parameters perform in two different mouse strains? Awake immunized and control BALB/c (N=16) and C57BL6 (N=14) mice were placed in the UP chamber and exposed to doses of aerosolized methacholine, while the following parameters were measured at each concentration: inspiratory time (T_I), expiratory time (T_E), total time (T_{Tot}), T_I/T_{Tot}, peak inspiratory pressure (P_I), peak expiratory pressure (P_E), *Pause*, *Penh*, tidal volume (V_T), V_T/T_I, V_T/T_E, and V_T/T_{Tot}. The next day, lung resistance and compliance (R_L, C_L), were invasively measured in the same animals. For the BALB/c, the parameters with the highest magnitude of correlation coefficient vs. R_L are (in order): 1) C_L, 2) *Pause* and *Penh*, 3) parameters of breathing frequency (T_E, T_{Tot}, T_I) and 4) parameters related to tidal volume (P_I, P_E, V_T). Flow parameters (V_T/T_{Tot}, V_T/T_E, V_T/T_I) and duty cycle parameters (T_I/T_{Tot}) had insignificant correlations. This ordering is significantly different in the C57BL6, where the parameters with the largest correlations are: 1) C_L, 2) parameters of breathing frequency, 3) flow parameters. *Pause*, *Penh*, tidal volume and duty cycle parameters had insignificant correlations. This data shows that *Penh* is problematic in the sense that it is strain specific; it behaves very differently in BALB/c and C57BL6 mice. We suggest that UP parameters largely originate as part of reflex control of breathing processes, rather than in the lung mechanics, and conclude that it is inappropriate to use UP parameters in general, and *Penh* specifically, as substitute variables for invasive mechanical indices such as R_L.

Introduction

An animal breathing in a closed chamber induces breathing related changes in chamber air pressure. This observation has subsequently been used in many experimental setups to non-invasively monitor breathing frequency and tidal volume (5,7,9,10,13,17,18). Recently, Hamelmann *et al*. (14) have suggested use of unrestrained plethysmography (UP) as a measure of bronchoconstrictive response to inhaled agonist in mice. This work described an heuristic parameter, *Penh*, calculated from the chamber pressure signal. BALB/c mice were exposed to doses of aerosolized methacholine (MCh) and the measured *Penh* was shown to correlate with the subsequently measured lung resistance. The current popularity of this technique is clearly due to its ease of use (as the animals are unanesthetised) and ability to obtain data rapidly and non-invasively.

However, recent studies suggest there are serious problems with this approach. Albertine *et al.* (1) and Petak *et al.* (27) have shown an inconsistent relationship between Penh and invasive measurements, especially in C57BL6 mice. Mitzner *et al.* (21,22), Lundblad *et al.* (19), and Enhorning *et al.* (8) have shown that the relationship between chamber pressure and airways mechanics is limited. Chamber pressure changes are caused by the heating and humidification, and compression and decompression of inspired gas. While only compression is physically related to bronchoconstriction, the signal is largely dominated by the heating and humidification effect. While these theoretical and experimental problems are significant, we felt that the potential usefulness of this technique warranted further investigation. After all, many times in the past, parameters without solid theoretical support have been shown to very useful (for example, FEV_1). In this study, we began our consideration with the premise that barometric

plethysmographical parameters are related to mechanical ones, even though these theoretical studies (19,21) suggest the link is tenuous. We sought to answer these questions: 1) How do the changes in *Penh* compare with changes in traditional UP and lung mechanical parameters? 2) How well do UP parameters perform in two different but widely used mouse strains?

Methods

Animals

All experimental protocols were reviewed and approved by the institutional animal care and use committee. Female BALB/c and C57BL6 mice (Jackson Laboratories, Bar Harbor, ME) were separated into immunized (BALB/c, N=9, 26.5±4.1g; C57BL6, N=7, 21.1±1.2g) and control (BALB/c, N=7, 29.3±2.6g; C57BL6, N=7, 18.0±1.4g) treatment groups. The sensitization and challenge protocol was as previously described (14); the immunized group received a sensitization treatment of I.P. OVA/alum on days 1 and 14, and a challenge treatment of aerosolized OVA (1% for 30 minutes) on days 28, 29, and 30. The experimental protocol began on day 32. The control group received the same treatment schedule but were sensitized and treated with saline. This protocol was chosen in order to maximize the difference between treatment and control groups.

UP measurements were made using a 181 mL cylindrical plexiglas chamber. The chamber had a small controlled leak with time constant 1.8 s, and a port into which aerosolized methacholine (MCh) could be pumped. Chamber pressure (P_b) was measured using a pressure transducer (Validyne, Northridge, CA) and recorded with an analog-digital converter (NB-MIO16x, National Instruments, Austin, TX) at a sampling frequency of 100 Hz after filtering with an appropriate anti-aliasing filter.

Mice were placed into the chamber and allowed to move freely. Saline and MCh solutions were aerosolized (Aerosonic Nebulizer, DeVilbiss Heath Care, Somerset, PA) and pumped into the chamber at a flow of 1.9 L/min for two minutes. The pump was then stopped, its connecting tube

occluded, and P_b was recorded continuously for two minutes. Measurements were made at concentrations of 0 (saline), 1.6, 3.1, 6.2, 12.5, 25, and 50 mg/ml MCh.

Twenty four hours after the UP measurements, lung resistance (R_L) and compliance (C_L) were measured in the same mice. The measurements were made under anesthesia while the animals were mechanically ventilated using a previously described plethysmographic method (20). R_L and C_L were determined following challenge with 10 breaths of MCh aerosol at concentrations of 0 (saline), 1.6, 3.1, 6.2, 12.5, 25, and 50 mg/ml. At the end of this protocol, broncho-alveolar lavage fluid (BAL) was collected to verify the inflammatory status of the animal. One control and one immunized C57BL6 and one control and four immunized BALB/c mice did not survive to the end of this protocol.

Data analysis

The 2 min. P_b records obtained after each MCh exposure were analyzed to extract candidate end expiration and end inspiration points. Each breath was tested against acceptance criteria (see below), and the average plethysmography parameters calculated for the first 30 s after each exposure. Signals were processed using the following algorithm. Low frequency drift was removed using a Butterworth digital high-pass filter (cutoff frequency of 1.5 Hz). Candidate breath start points were identified from a low-pass filtered signal (cutoff frequency 15 Hz) at points where the slope began to exceed a threshold, selected to be 20 % of the median slope magnitude. Candidate start points were then corrected with respect to the original, unfiltered signal. For each breath start point, the corresponding inspiratory and expiratory segments were identified, and the breath compared to the following acceptance criterion: 1) inspiratory and expiratory times within 0.3 to 3.0 times the median times, 2) inspiratory and expiratory ΔP_b

within 0.3 to 3.0 times the median value, 3) spurious signal noise within the breath below a threshold, and 4) P_b below the candidate end inspiratory point. For each accepted breath, T_I (inspiratory time), T_E (expiratory time), P_I (pressure change during inspiration), and P_E (pressure change during expiration) were directly calculated. The parameter values for each dose level were calculated to be the average value for all accepted breaths.

Tidal volume (V_T) was calculated from P_b using the approach of Epstein *et al.* (9) according to the equation

$$V_T = P_{I,corr} K T_{alv} \frac{P_{atm} - P_{v,alv}}{T_{alv}\left(P_{atm} - P_{v,box} + P_{I,corr}\right) - T_{box}\left(P_{atm} - P_{v,alv} + P_{I,corr}\right)} \quad (1)$$

where $P_{v,alv}$ and $P_{v,box}$ are water partial pressure (kPa) at alveolar and chamber conditions, P_{atm} is absolute atmospheric pressure (kPa), T_{alv} is the alveolar temperature (°K), and T_{box} is the chamber temperature (°K). K is a box calibration constant (V_K / P_K) calculated by measuring the increase in box pressure (P_K) due to a calibration volume (V_K) injected into the chamber. $P_{I,corr}$ is the inspiratory pressure corrected by taking into account the pressure drift in the chamber. We use the approach of Epstein *et al.* (10), using average chamber temperature and humidity values obtained by us in previous experiments (19), as well as the nasal and alveolar temperature data from Jacky (18).

We analyzed our records of P_b following the scheme outlined by Hamelmann *et al.* (14), even though their algorithm is proprietary so its precise details were not given. Hamelmann *et al.* used a plethysmographic chamber with a large leak, and thus an extremely low time constant (0.02s). We simulated the effects of their apparatus by subjecting our measurements of P_b to a frequency domain digital filter with a pole at f=1/0.02s and a zero at f=1/1.8s. The following values were

calculated from the filtered P_b (Fig. 1): P_{EP} (the maximum ΔP_b during expiration), P_{IP} (the maximum ΔP_b during inspiration), and T_R (the time interval which encompasses 63% of the integrated expiratory pressure signal). Linear interpolation was used to estimate T_R values which did not occur on sample boundaries. We also calculated the following additional parameters for each breath: T_{tot} (T_I + T_E), T_I / T_{Tot}, V_T / T_I, V_T / T_E, V_T / T_{Tot}, *Pause*, and *Penh*. Following (14), *Pause* and *Penh* are defined as:

$$Pause = \frac{T_E - T_R}{T_R} \tag{2}$$

$$Penh = \frac{P_{IP}}{P_{EP}} \times Pause \tag{3}$$

Statistical analysis

For each experimental group, the mean and standard error of each parameter at each MCh concentration level was calculated. For each mouse strain, the Pearson correlation coefficient (r) was calculated for each parameter with respect to R_L and C_L. Correlations with magnitudes greater than 0.427 for BALB/c (with N=16) and 0.459 for C57BL6 (with N=14) were determined to be significant at p<0.05, using the Fisher Z transformation. In order to analyze the effect of treatment (immunized vs. control) and strain on parameters values, a 2 way ANOVA test was calculated for each parameter. ANOVA yields the effect of treatment, strain and the interaction between treatment and strain, and a result was considered significant at p<0.01.

Results

BAL fluid was analyzed to determine the total cell and eosinophil counts. Average ±SE total cell counts were 187,000±12,300 (control) and 344,000±29,800 (immunized) in the BALB/c, and 319,000±27,000 (control) and 448,000±45,300 (immunized) in the C57BL6 mice. The percentage ±SE of eosinophils was 0 (control) and 34.8±3.2% (immunized) in the BALB/c, and 0 (control) and 53.7±3.3% (immunized) in the C57BL6. This indicates the presence of a substantial inflammatory response in the lungs of the antigen immunized and challenged mice at the time of assessment, and is similar to previous reports (14,31).

Figure 1 shows representative P_b signals for immunized BALB/c (top) and C57BL6 (bottom) mice at a MCh concentration of 25 mg/ml. The expiratory signal in the BALB/c shows a delay between the end of one expiration and the beginning of the next, while the C57BL6 does not show any clear delay. As the MCh concentration increased, the ratio between T_R and T_E decreased, resulting in an increase in *Pause* (Eq. 2). This example shows how sensitive T_R is to any variability in the identification of the end-expiratory point. If this point is erroneously identified 10 ms too early (lower dotted line), the baseline level is dramatically reduced with a commensurate increase in T_R.

Figure 2 shows R_L and C_L as a function of inhaled MCh concentration. The BALB/c mouse shows large changes in both parameters with concentration, while the C57BL6 has relatively little response. Because not all BALB/c survived to the highest concentration (9 of 16 animals survived), the contribution from the most responsive animals is not seen at this concentration level.

Table 1 shows the correlation coefficients for each parameter vs. R_L and C_L, as well as the percentage difference in baseline values between the immunized and the control group. For the BALB/c, the parameters with the highest magnitude of correlation coefficient vs. R_L are (in rank order): 1) C_L, 2) *Pause* and *Penh*, 3) parameters of breathing frequency (T_E, T_{Tot}, T_I) and 4) parameters related to tidal volume (P_I, P_E, V_T). Flow parameters (V_T/T_{Tot}, V_T/T_E, V_T/T_I) and duty cycle parameters (T_I/T_{Tot}) do not have significant correlations. This ordering is quite different in the C57BL6, where the rank order of the parameters is: 1) C_L, 2) parameters of breathing frequency, 3) flow parameters. *Pause*, *Penh*, tidal volume and duty cycle parameters do not have significant correlations. All correlation coefficients are lower in the C57BL6.

ANOVA analysis showed a significant effect of strain for all parameters except T_I, T_E, T_{Tot} and T_I/T_{Tot}, and a significant effect of treatment for all parameters except R_L, C_L, T_R, T_I/T_{Tot}, V_T/T_I, V_T/T_E, and V_T/T_{Tot}. The interaction between treatment and strain was not significant for any parameters except R_L, C_L, V_T/T_I, V_T/T_E, and V_T/T_{Tot}. These results are broadly consistent with the correlation coefficients in table 1. For example, T_{Tot} does not have a significant correlation for either strains, and does not show a significant effect of strain or treatment (ANOVA). The lack of significant effect of strain on breathing frequency parameter responses is consistent with the results figure 4. The paradoxical lack of treatment effect for this last group of parameters (R_L, C_L, V_T/T_I, V_T/T_E, and V_T/T_{Tot}) is explained by the interaction between treatment and strain; the changes in parameter values due to immunization are in opposite directions for each strain, as is shown for R_L and C_L in figure 2.

Figures 3 and 4 show *Penh* and T_E, the parameters with the highest correlation coefficients in the BALB/c. Figure 3 shows the response in these parameters vs. MCh concentration, while figure 4

shows their relationship to R_L. *Penh* is significantly different between control and immunized BALB/c mice at MCh concentrations of 3.1 mg/ml and above, while for C57BL6 mice, *Penh* is not significantly different between treatment groups at any concentration. T_E, on the other hand, shows a significant difference for both strains. Both *Penh* and T_E are clearly correlated with R_L for the BALB/c (figure 4), while a similar relationship for the C57BL6 does not appear to exist.

Discussion

Hamelmann *et al.* (14) introduced a parameter, *Penh*, as a non-invasive measurement related to lung mechanics. A mouse is placed into a closed chamber and allowed to move freely while aerosolized agonist is introduced, and *Penh* is calculated from the measured chamber pressure. The advantage of this technique is its simplicity and non-invasive nature. However, recent studies (1,8,12,15,19,21,22,24,27) have shown theoretical and practical problems with this approach. Albertine *et al.* (1) showed that UP measurements in C57BL6 mice correlate with invasive measurements at the beginning (days 1,2) of the OVA challenge protocol, but not later (days 5,9). Petak *et al.* (27) studied C57BL6 mice with hyperoxia-induced lung damage and showed an inconsistent relationship between UP measurements and low frequency forced oscillation measurements. Mitzner *et al.* (21,22), Lundblad *et al.* (19), and Enhorning *et al.* (8) showed that, while P_b reflects both conditioning of inspired air (proportional to V_L) and thoracic gas compression (proportional to P_L), only the latter is related to lung mechanics. Furthermore, the former process generally dominates P_b to an extent that depends on the absolute lung volume, which can vary significantly with agonist concentration (19). Anecdotal observations also indicate that *Penh* does not appear to "work" as well in the C57BL6 as the BALB/c strain. Interestingly, a large species dependence of UP had been noted as early as 1868 (3); chamber pressure was large in the duck, dog, cat, guinea pig, and tortoise, and small in the rabbit, pigeon, snake and frog. In spite of these issues, however, we considered that the virtues of UP, in terms of non-invasiveness and ease of use, were such that further investigation was warranted.

The purpose of our study was to determine how changes in *Penh* relate to changes in traditional invasive measures of lung mechanics, and whether the nature of this relationship varies between mouse strains. We chose to study BALB/c and C57BL6 mice because both strains are commonly used as models of lung disease; the BALB/c strain mounts a strong immune response with high IgE levels (16) while the C57BL6 strain is largely used for environmental exposure and production of some types of transgenic animals, such as knockout and constitutive overproducers of cytokines. We chose to compare the parameters of UP to independent measurements of R_L because this parameter has been widely used to characterize lung mechanical function in laboratory animals. The robustness of R_L as a general measure of mechanics is supported by the fact that C_L, another widely used index of lung mechanical function, exhibited a very similar relationship to the UP parameters (Table 1).

V_T is calculated from P_b using the approach of Epstein *et al.* (10) (equation 1), which adds corrections for signal drift to the equations of Drorbough *et al.* (7). This approach assumes that P_b originates solely from heating and humidification of inspired gas. These formulae are widely used (9,13,17,18,21,32), although they have been shown to be inaccurate under conditions of increased R_L where compressive changes in P_b may be significant (10,19,21,30). This compressive contribution to P_b depends on alveolar pressure and absolute lung volume, variables we were unable to measure non-invasively. Thus, in our calculations, no correction has been made for compressive contributions to P_b, so V_T may be overestimated at higher MCh concentrations. The contribution from this effect is small (19,21,22) and is commonly ignored (26,32). Another potential inaccuracy in our calculation stems from the assumed values (from Jacky (18)) for nasal and alveolar temperature and humidity. These formulae assume complete heating and humidification of inspired air, which had been shown to be inaccurate for humans

(25,26), although it is probably a valid assumption in mice because of their much smaller lung volumes. Thus, while our estimated values of V_T may be somewhat inaccurate, the observed trends are unlikely to be artifacts.

Figure 3 shows the response of T_E and *Penh* to increasing concentrations of MCh. T_E is significantly different for treatment groups for both strains, although the difference is reduced at the higher concentrations. *Penh*, on the other hand, is significantly different between treatment groups in the BALB/c but not in the C57BL6. These results are similar to those of table 1, where *Penh* is the UP parameter with the highest correlation to lung mechanics in the BALB/c, but does not correlate to lung mechanics in the C57BL6 mouse. On the other hand, other UP parameters, such as T_E, have similar correlations in both strains. This strain difference in behavior of *Penh* has also been observed by other workers (personal communications). The sensitivity of *Penh* at low MCh concentration originates largely in its dependence on T_R, which shows a marked early response to agonist but returns to baseline values at the highest concentration (figure 5). T_R also behaves very differently in the different strains and appears to account for most of the differences in *Penh* response between the C57BL6 and BALB/c. The calculated value of *Penh* is sensitive to the value of T_R, since T_R is in the denominator of the *Penh* equation (Eq. 2) which becomes very small at the highest concentrations of MCh. T_R is itself very sensitive to the value estimated for expiratory time. For example, as shown in figure 1, if the end of expiration is identified to be 10 ms too early, the resulting increase in T_R reduces *Penh* by a factor of four. Thus, *Penh* shows important experimental problems, in addition to the theoretical ones mentioned previously, which indicate that it cannot considered as a valid measure of airway mechanics.

In order to explain these observations, we hypothesize (as suggested by Petak *et al.* (27) and Morris *et al.* (23)) that *Penh* largely reflects factors related to control of breathing rather than airway mechanics. This is supported by the following arguments: *1) Differences in UP parameters correspond to differences in physiology.* Tidal volume parameters (and *Penh*) correlate well in BALB/c but not in C57BL6 mice, while flow parameters show the reverse effect. This observation corresponds closely with known physiological differences between these strains, as Takeda *et al.* (31) showed that eophinophils are distributed in peribronchial and peripheral lung tissue in BALB/c but were distributed diffusely in peripheral lung tissue in C57BL6 mice. This suggests that BALB/c mice have largely an airway response, and an adaptive response to the bronchoconstriction is to take slower and deeper breaths, as seen in the data (Fig. 3). In contrast, C57BL6 mice have largely a tissue response to MCh and so change flow with little decrease in tidal volume. The apparent decrease in responsiveness in the immunized C57BL6 mouse (Fig. 2) may be reflective of this peripheral response, possibly due to the strain-dependent spectrum of inflammatory mediators such as IL-6 (6). Also, if our data are interpreted in terms of the change with respect to baseline value (such as (31)), then there is no decrease of responsiveness with immunization. *2) It is known that, at least in larger mammals, part of the breathing pattern response to agonists such as MCh is due to reflex mechanisms.* Phillipson and coworkers (11,28,29) have shown the importance of the vagal reflexes in the control of breathing pattern in dogs. For example, elimination of the stretch and vagal reflexes uncouples the mechanical response from the neural response and produces independent reductions in T_{Tot} (28). In unsedated exercising dogs, inhalation of histamine increases R_L, decreases C_L, and induces rapid shallow breathing, while cooling the vagus nerve abolishes these responses (4). Sectioning the parasymphatic vagal pathway in the cat increases the response in R_L to aerosolized MCh

compared to sham, symathectomy, or section of both vagal pathways (2), demonstrating that MCh elicits a vagal reflex. Recently, Wagner *et al.* (33) showed that MCh also causes reflex bronchoconstriction in sheep. 3) *UP parameters begin to diverge between treatment groups at lower concentrations of MCh than mechanical variables*. R_L shows a significant difference in response between treatment groups beginning at 6.2 mg/ml (Fig. 2), while *Penh* shows a significant difference at 3.1 mg/ml (Fig. 3). This is consistent with a non-mechanical (i.e. reflex) origin for the response at very low MCh concentrations.

In summary, while the technique of unrestrained plethysmography promises an easy to use, non-invasive index of lung mechanics in mice, there has been considerable concern over its validity. Theoretical studies (8,21,22,24) have shown that only a small fraction of the chamber pressure changes measured by UP reflects lung mechanics, while practical problems (1,23,27) in the interpretation of UP parameters have also been described. Our study supports these concerns. We show that, although *Penh* correlates with R_L in the BALB/c mouse, there is no significant correlation in the C57BL6 strain. Our data also suggest that *Penh* largely reflects effects from irritant receptors affecting the control of breathing. Furthermore, *Penh* does not even appear to be the most useful parameter that can be calculated from the UP signal. For example, T_E correlates almost as well with R_L as *Penh* does in the BALB/c mice, and performs significantly better in the C57BL6 strain. Additionally, the T_E vs. R_L relationship is similar for both strains, while the *Penh* vs. R_L relationship is more variable between strains. We conclude, therefore, that *Penh* is not special compared to other more easily interpretable quantities derivable from P_b, and that it is inappropriate to use UP parameters in general, and *Penh* specifically, as substitute variables for traditional, valid measured of lung mechanics.

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Figure Captions

Figure 1:

Sample chamber pressure waveforms for an immunized BALB/c (top) and C57BL6 (bottom) mice after exposure to 25 mg/ml MCh. Parameters P_{EP} (maximum ΔP_b during expiration), P_{IP} (maximum ΔP_b during inspiration), and T_R are shown. T_R (dashed line) is the time before which 63% of the integrated expiratory pressure signal occurs. Any errors in estimation of the end expiratory point dramatically affect T_R. For example, an erroneous estimate of 10 ms too early results in the calculation shown by the dotted line.

Figure 2

R_L and C_L (± SE) (logarithmic scale) as a function of MCh concentration: Control BALB/c (○), Immunized BALB/c (●), Control C57BL6 (□), Immunized C57BL6 (■). Significant differences (p<0.05) between control and immunized animals at each dose are indicated (*).

Figure 3

Penh (± SE) and T_E (± SE) (logarithmic scale) as a function of MCh concentration: Control BALB/c (○), Immunized BALB/c (●), Control C57BL6 (□), Immunized C57BL6 (■). Significant differences (p<0.05) between control and immunized animals at each dose are indicated (*). *Penh* is significantly different between treatment groups for the BALB/c, but not for the C57BL6. T_E shows significant differences between treatment groups for both strains.

Figure 4

Penh (top) and T_E (bottom) values versus R_L (both axes logarithmic) measured the next day in the same animals: BALB/c (o), and C57BL6 (+). The best fit line is shown for each strain group. For both parameters, the fit versus R_L is better for the BALB/c than and the C57BL6.

Figure 5

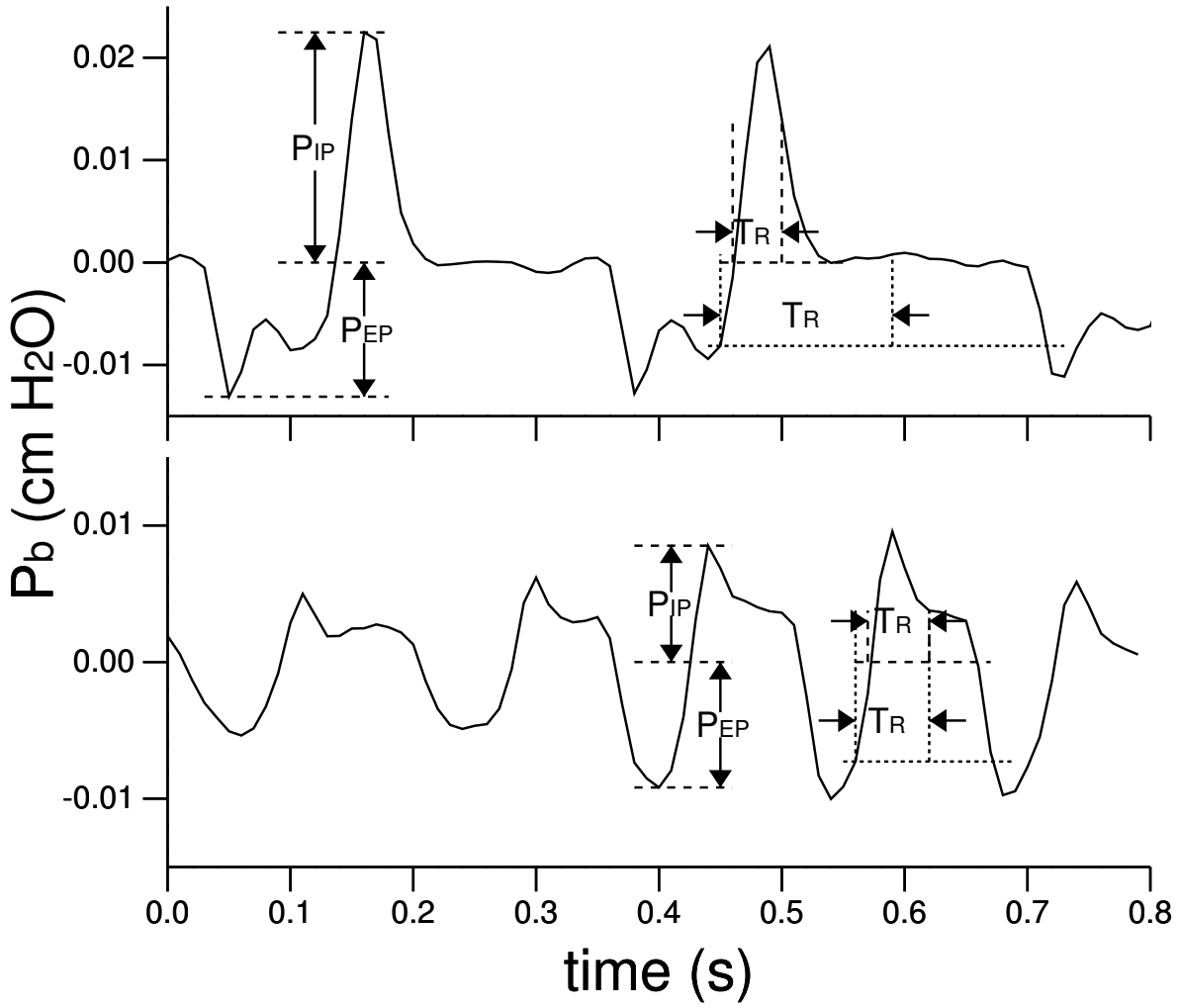
T_R (± SE) (logarithmic scale) as a function of MCh concentration: Control BALB/c (O), Immunized BALB/c (●), Control C57BL6 (▫), Immunized C57BL6 (■).Significant differences (p<0.05) between control and immunized animals at each dose are indicated (*). T_R responds differently between treatment groups at middle concentrations, but not at the highest concentrations.

Table 1

	BALB/c			C57BL6		
	Correlation vs. R_L	**Correlation vs. C_L**	**Baseline Difference (%)**	**Correlation vs. R_L**	**Correlation vs. C_L**	**Baseline Difference (%)**
R_L	1.000	–0.866 *	–3.6	1.000	–0.566 *	11.0
C_L	–0.866 *	1.000	9.1	–0.566 *	1.000	–52.3
T_I	0.714 *	–0.657 *	–16.9	0.418	–0.385	–1.3
T_E	0.745 *	–0.705 *	–9.6	0.703 *	–0.424	2.6
T_{Tot}	0.745 *	–0.698 *	–12.6	0.602 *	–0.457 *	0.8
T_I/T_{Tot}	–0.283	0.291	–5.2	–0.191	–0.006	–1.0
P_I	0.686 *	–0.566 *	–8.0	–0.070	0.302	–27.7
P_E	0.688 *	–0.566 *	–7.7	–0.066	0.307	–27.9
P_{IP}	0.165	–0.044	2.2	–0.385	0.536 *	–24.9
P_{EP}	0.720 *	–0.595 *	–7.1	–0.038	0.268	–31.4
T_R	0.097	–0.136	–10.6	0.211	–0.424	6.6
Pause	0.802 *	–0.729 *	3.1	0.290	–0.037	–7.7
Penh	0.809 *	–0.729 *	–7.6	0.288	–0.060	–16.9
V_T	0.671 *	–0.546 *	–9.0	–0.069	0.284	–28.2
V_T/T_I	–0.383	0.384	9.3	–0.472 *	0.609 *	–28.5
V_T/T_{Tot}	–0.463 *	0.489 *	3.5	–0.433	0.623 *	–28.6
V_T/T_E	–0.460 *	0.501 *	1.0	–0.397	0.594 *	–28.4

Correlation coefficients (r) for each UP parameter with respect to R_L and C_L for each mouse strain. Values indicated (*) are significant at $p<0.05$. Also shown is the average percentage difference in baseline parameter value of the immunized versus the control groups for each mouse strain.

Figure 1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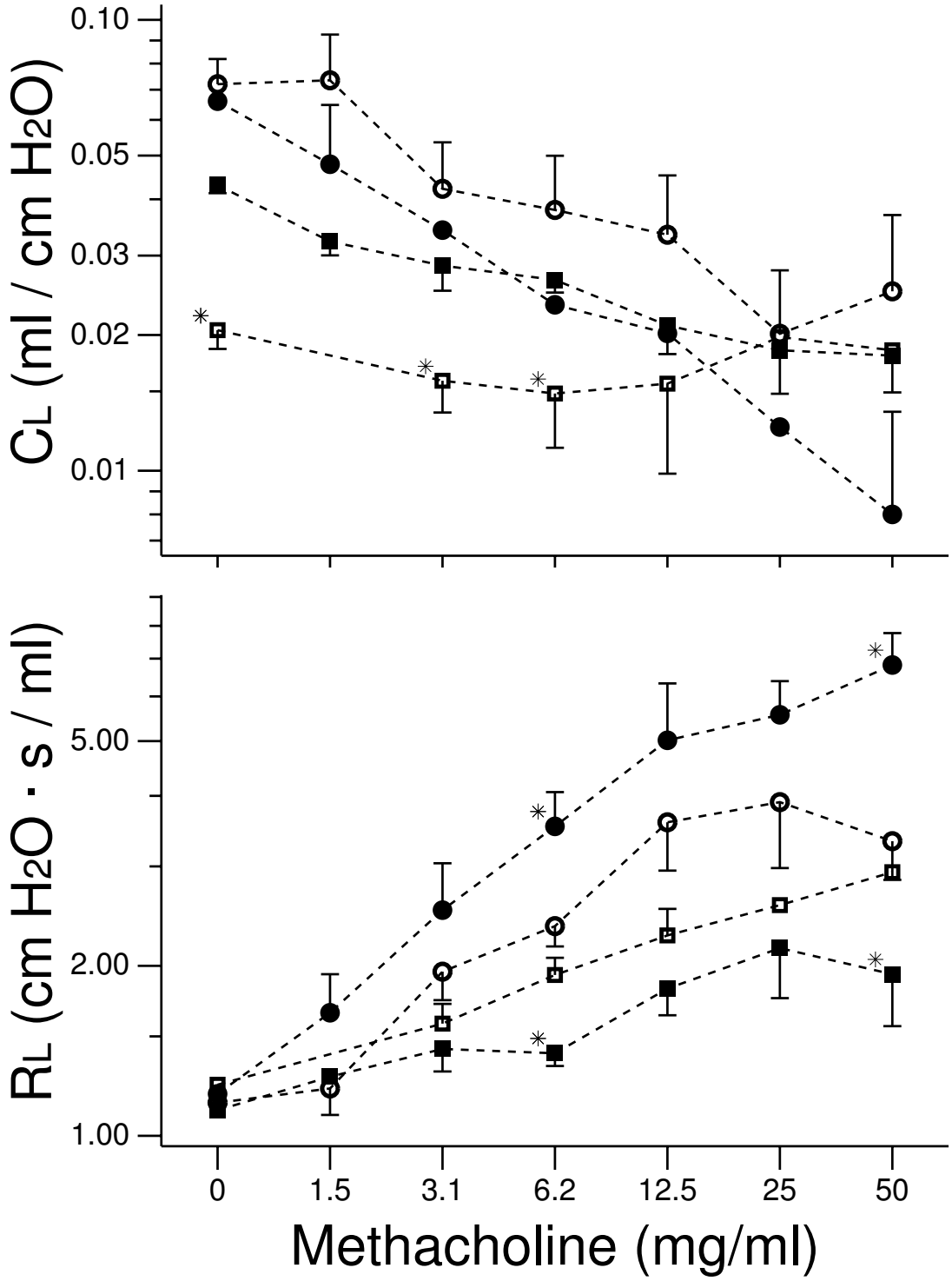
0.01
0.00
-0.01
PIP
PEP
TR
TR
0.01
0.00
-0.01
PIP
PEP
TR
TR
Pb (cm H2O)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
time (s)

Figure 2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0.05
0.03
0.02
0.01
C_L (ml / cm H_2O)
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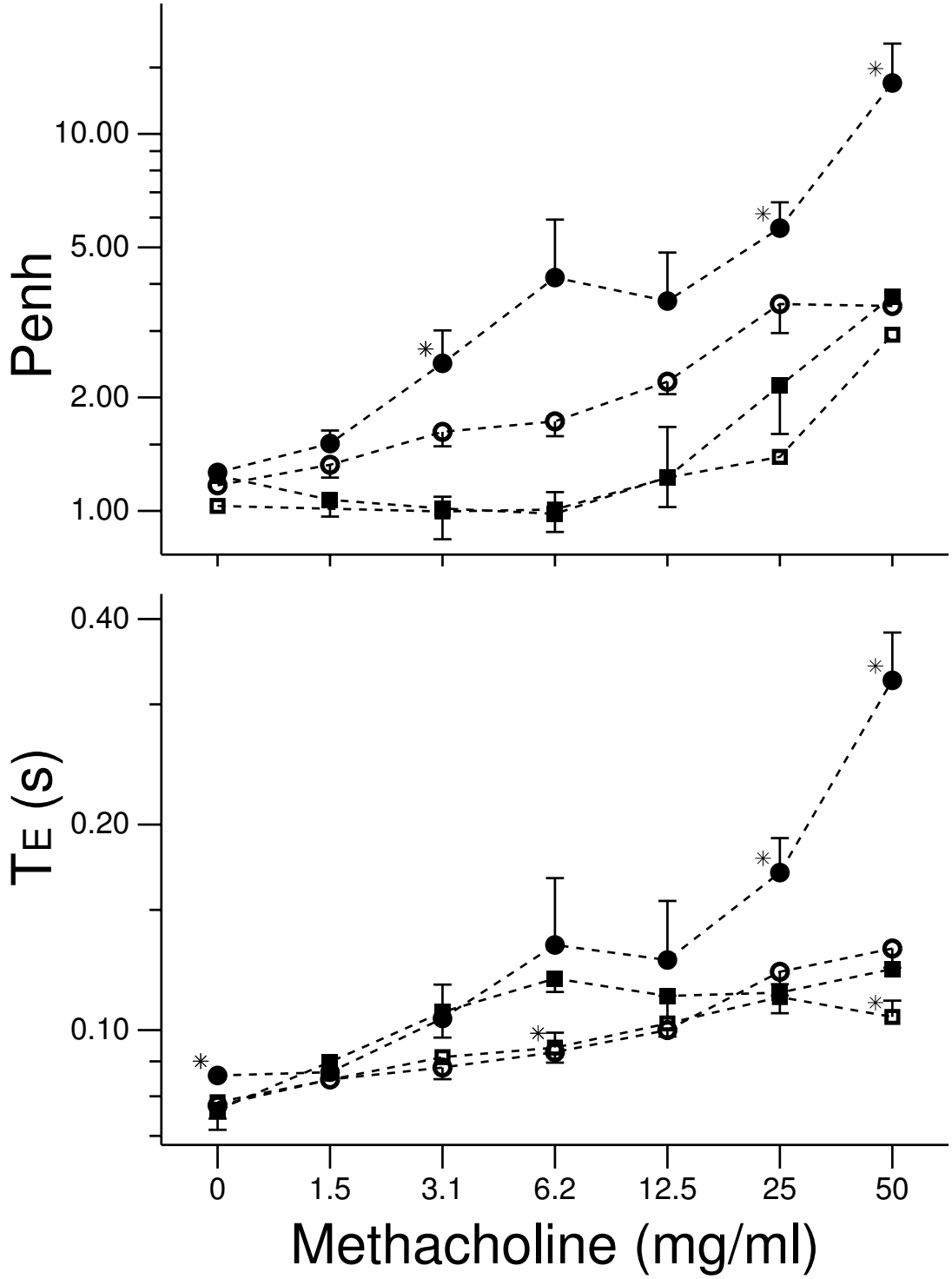
2.00
1.00
R_L (cm H_2O · s / ml)
0
1.5
3.1
6.2
12.5
25
50
Methacholine (mg/ml)

Figure 3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5.00
2.00
1.00
TE (s)
0.40
0.20
0.10
0
1.5
3.1
6.2
12.5
25
50
Methacholine (mg/ml)

Figure 4

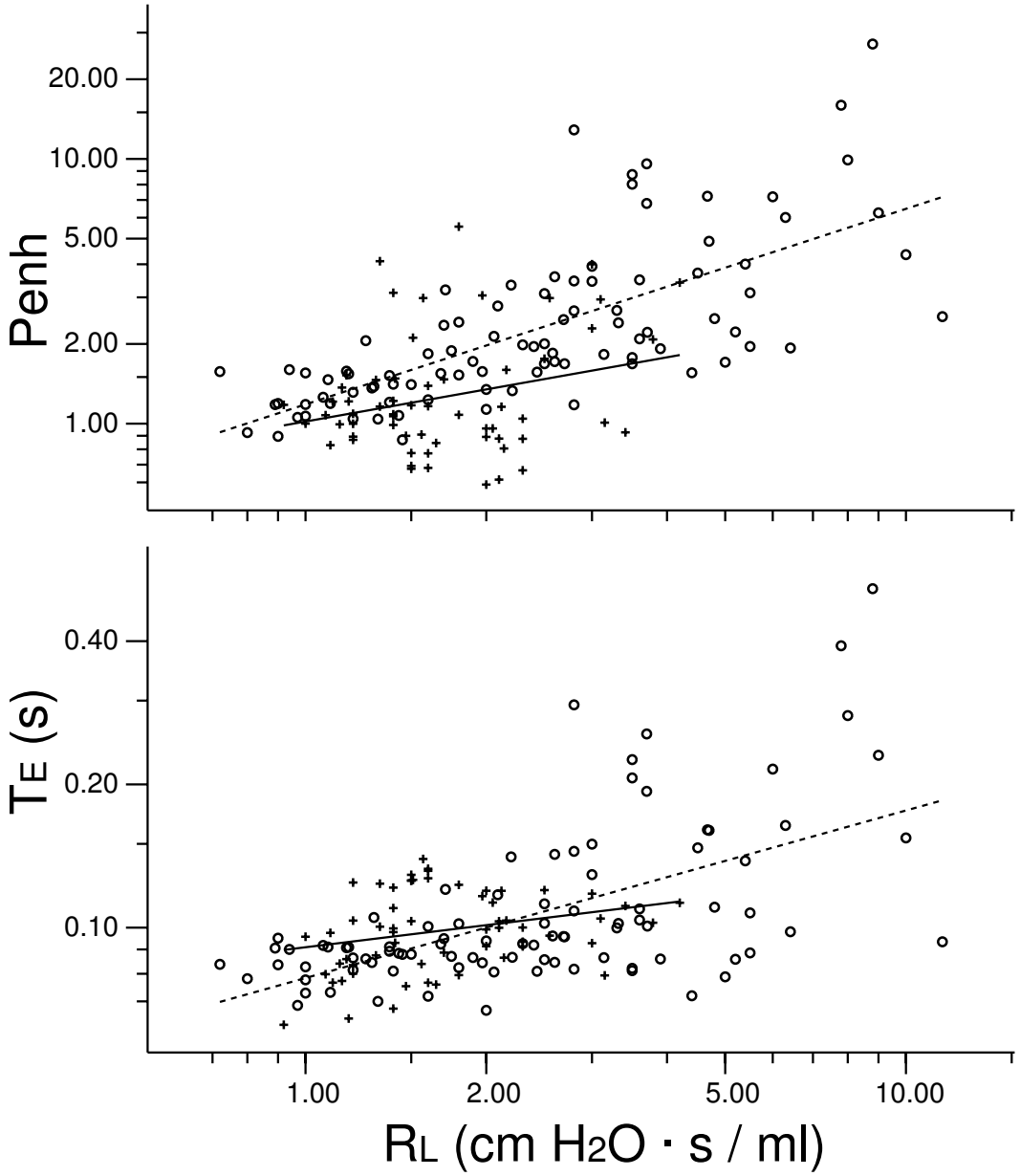
20.00
10.00
5.00
2.00
1.00
Penh
0.40
0.20
0.10
TE (s)
1.00
2.00
5.00
10.00
RL (cm H2O · s / ml)

Figure 5