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Original article

Comparison between pulmonary resistance and Penh in anaesthetised rats with tracheal diameter reduction and after carbachol inhalation

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Abstract

Introduction: Single-chambered barometric whole-body plethysmography is frequently used as a noninvasive lung function test. However, the validity of the enhanced Pause (Penh), an index of airflow limitation, remains controversial. We compared Penh with pulmonary resistance (R_L) to test whether Penh detects tracheal subobstruction and carbachol-induced airflow limitation in spontaneously breathing, anaesthetised rats. **Methods:** Fourteen male Sprague–Dawley rats underwent tracheal catheterisation, followed by measurements of R_L and Penh. Six rats underwent tracheal subobstruction by the consecutive insertion into the lumen of the tracheal tube of two catheters of decreasing diameter. Eight rats received an inhaled saline challenge, followed by two noncumulative nebulizations of carbachol (1.25 mg/mL, 1 min). **Results:** In rats with tracheal calibre reductions, R_L significantly increased at each reduction (0.218±0.052 vs. 0.417±0.058 vs. 0.820±0.258 cm H_2O/mL s, $p<0.05$), whereas Penh only increased after the last reduction (1.88±0.25 vs. 2.47±0.26, $p<0.05$). Increases (Δ) of R_L and Penh were not correlated. In comparison to postsaline values, carbachol induced a significant increase of Penh (1.93±0.44 vs. 4.05±1.45, $p<0.005$) and R_L (0.137±0.04 vs. 0.284±0.084 cm H_2O/mL.s, $p<0.005$). ΔPenh and ΔR_L were significantly correlated ($r=0.80$, $p<0.05$). **Discussion:** This study showed, by comparing Penh with R_L, that single-chambered plethysmography measuring Penh allows to detect carbachol-induced airflow limitation in spontaneously breathing, anaesthetised Sprague–Dawley rats, but poorly detects an increase in R_L due to tracheal calibre reductions. These findings suggest that Penh might be only be used as an index of airflow limitation under well-defined experimental conditions.

Keywords: Tracheal subobstruction; Carbachol; Diagnostic; Method; Lung resistance; Pulmonary function; Rat; Single-chamber barometric whole-body plethysmography

1. Introduction

The evaluation of respiratory function has become a key point in pharmaceutical and toxicological studies in which laboratory animals are used as models of human airway and lung diseases, such as asthma, chronic obstructive pulmonary disease, fibrosis, ozone toxicity, etc.

Most of the pulmonary function tests available at present are invasive tests, such as pulmonary mechanics (Hamelmann et al., 1997; Martin, Chu, Honour, & Harbeck, 2001; Palecek, 1969) or forced oscillation techniques (Hantos, Daroczy, Suki, & Nagy, 1987; Petak, Hantos, Adamicza, Asztalos, & Sly, 1997), which require anaesthesia and artificial ventilation, implying surgical intervention followed by euthanasia of the animal. One recent study describes repetitive measurements of pulmonary mechanics in anaesthetised, spontaneously breathing mice (Glaab et al., 2004). The major advantage of these tests is their capability to provide well-known variables [pulmonary resistance

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(R_L), dynamic compliance (C_{dyn}), elastance (E), reactance (X_L), etc.], giving precise information about the localisation and the nature (i.e., central or peripheral airway or lung parenchyma) of a functional modification present within the respiratory system. On the other hand, as these procedures are mostly terminal, they rarely allow a repeated assessment using the same animal and therefore require a large number of individuals.

These restrictions have led to the development of less invasive methods allowing a repeated investigation of the respiratory system, such as double- and single-chamber body barometric plethysmography (Chong, Agrawal, Romero, & Townley, 1998; DeLorme & Moss, 2002; Flandre, Leroy, & Desmecht, 2003; Pennock, Cox, Rogers, Cain, & Wells, 1979).

The double-chambered system has been shown to provide accurate and repeatable measurements of physiological variables, such as respiratory rate (RR), inspiratory and expiratory times (T_i and T_e), tidal volume (V_T), minute ventilation (V_E), inspiratory and expiratory peak flows (PIF and PEF), and airway resistance (sRaw) (Chong et al., 1998; DeLorme & Moss, 2002; Flandre et al., 2003; Pennock et al., 1979). However, due to the presence of a neck collar separating the nasal and thoracic chambers, prolonged measurements imply discomfort and stress for the animal, potentially leading to decreased air tightness of the neck collar and reduced reproducibility of the measured variables (DeLorme & Moss, 2002).

A system for lung function assessment that requires even less restraint of the animal is the single-chambered whole body plethysmograph, where the animal can move freely and has access to food and water, allowing measurements over extended time periods (Chand et al., 1993; Dohi et al., 1999). This instrument bears an advantage in terms of animal well-being, and its use is less time consuming, especially in comparison to invasive tests. However, there are some drawbacks to this technique, which have lead to several criticisms (Enhorning, van Schaik, Lundgren, & Vargas, 1998; Petak, Habre, Donati, Hantos, & Barazzone-Argiroffo, 2001). Indeed, inspiratory and expiratory flows are indirectly determined by measuring pressure changes in the single-chamber plethysmograph, which are due to the heating and humidification of the air entering the animal's lung (Drorbaugh & Fenn, 1955). Consequently, PIF, PEF, V_T, and V_E partially depend on ambient temperature and humidity conditions and need to be interpreted with caution (Enhorning et al., 1998). The variable allowing the quantification of airflow limitation is the enhanced pause or Penh, a unitless index calculated as follows: Penh=[PEF/PIF]×Pause, where Pause=[T_e−RT]/RT, RT being the relaxation time (ms), i.e., the time when area under the expiratory curve equals 35%. In several studies measuring Penh during intrathoracic airway obstruction, this index has been shown to be correlated with airway resistance (sRaw) or pulmonary resistance (R_L; DeLorme & Moss, 2002; Chong et al., 1998; Halloy et al., in press; Hamelmann et al., 1997). However, as other investigations did not confirm this correlation between Penh and sRaw or R_L (Adler, Ciesewicz, & Irvin, 2004; Lundblad, Irvin, Adler, & Bates, 2002; Petak et al., 2001), the validity of Penh for the quantification of airflow limitation remains controversial (Bates, Irvin, Brusasco, Drazen & Fredberg, 2004). Furthermore, the question as to whether Penh might also be used as an estimator of tracheal airway obstruction has not yet been addressed.

The aim of the present study was, therefore, to compare Penh with R_L in spontaneously breathing, anaesthetised rats undergoing either tracheal subobstruction or by inhaling the bronchoconstrictive agent carbachol.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (n=14) weighing 350±29 g were used. The animals were housed in appropriate cages on wood shavings and received food and water ad libitum. The study was approved by the Animal Ethical Committee of the University of Liège.

2.2. Study design

The rats were divided into two groups: Six rats were used for protocol 1, whereas eight rats underwent protocol 2. For both protocols, the animals were anaesthetised and underwent tracheotomy and tracheal catheterisation. When the animals were breathing regularly through the tracheal catheter, they underwent (in random order) measurements of R_L and Penh. The animals were allowed to breath spontaneously throughout protocols 1 and 2.

Rats selected for protocol 1 (n=6) underwent a stepwise reduction of upper airway calibre by the consecutive insertion of two catheters of decreasing inner diameter into the tracheal tube. After each catheter insertion, R_L and Penh were recorded in a random order during 5 min.

Rats selected for protocol 2 (n=8) underwent an inhaled placebo challenge of sterile saline (NaCl 0.9%), followed by two nebulizations of a single dose of carbachol, allowing randomised recording of a single measurement of R_L or Penh during the 5 min after nebulization.

2.3. Animal preparation

Rats were sedated by a subcutaneous injection of 0.5 mg/kg diazepam (Valium, Roche, France) 20 min prior to anaesthesia induction by intraperitoneal (i.p.) injection of 40 mg/kg sodium thiopental (Pentothal, Abott, Belgium). Tracheotomy was performed just below the larynx, and a tracheal catheter was carefully inserted as far as the bronchial carina (BD Insyte I.V. Catheter 14G, internal diameter 1.74±0.4 mm). The catheter was tightly fixed to

the trachea by several ligatures to prevent air leakage at the site of tracheotomy. A water-filled polyethylene catheter (Intramedic, ID 0.86 mm, OD 1.5 mm) connected to a calibrated differential transducer (Buxco Electronics, Sharon, USA) was inserted through the oral cavity into the oesophagus. The tip of the catheter was pushed to the distal portion of the oesophagus where recorded transpleural pressure variations reached their maximum.

2.4. Single-chambered barometric whole-body plethysmography

A system of barometric whole-body plethysmography for rats was used (PLY3114, Buxco Electronics). After tracheostomy, the anaesthetised rats were placed in dorsal decubitus in the plethysmograph, which was ventilated by a continuous bias flow of 2 L/min (Bias Flow Regulator, Vent2, Emka Technologies, France). The air inlet was via a screen pneumotachograph positioned on the upper side of the chamber, whereas the air outlet (1.5 mm) was at one lateral end of the chamber. A differential pressure transducer was connected on one pole to the main chamber and on the second pole to a reference chamber equilibrated with atmospheric pressure by a small channel (1.5 mm). Pressure signals were amplified, digitised, and sampled at 100 Hz by use of the IOX software version 1.530, which provided a breath-by-breath analysis of waveforms from which Penh is derived as a measure of airflow limitation. Further respiratory variables, such as RR, T_i, T_e, peak inspiratory and expiratory pressures [expressed as pseudo-flows: PIF and PEF (mL/s)], estimated tidal volume [V_T (mL)], and relaxation time (RT) were also recorded. The chamber pressure signal was calibrated daily by the dynamic injection of 5 mL of room air via a syringe into the main chamber of the plethysmograph.

2.5. Pulmonary mechanics

The same plethysmograph chamber used for the measurement of Penh was used for pulmonary mechanics, but with the bias flow disconnected. Anaesthetised rats were placed in dorsal decubitus, but in this protocol, the tracheal catheter was connected via a 2-mm adaptor to the external environment of the plethysmograph. A differential pressure transducer (SN115810, Buxco Electronics) calibrated daily by the injection of a 10 ml volume of air was used to measure the changes of inspiratory and expiratory flow rates on a breath-by-breath basis and the calculation of PIF, PEF, V_T, and RR (Biosystem XA 2.5, Buxco Electronics). Total pulmonary resistance (R_L) was calculated by the integration of pleural pressure and flow signals.

2.6. Protocol 1: Tracheal calibre reduction

After a 5-min baseline measurement of both R_L and Penh, a first catheter with an internal diameter of 0.98 ± 0.04 mm (BD Insyte I.V. Catheter 18G) was inserted into the tracheal cannula, leading to a ~0.76-mm reduction of tracheal diameter. Both R_L and Penh were recorded in a random order over 5 min. The same procedure was repeated after the insertion of a second catheter with an internal diameter of 0.80 ± 0.04 mm (BD Insyte I.V. Catheter 20G), leading to a reduction of ~0.18 mm. After the measurement of R_L and Penh, the rats were killed by a lethal i.p. injection of 200 mg/kg pentobarbital (Dolethal, Vetoquinol, France).

2.7. Protocol 2: Inhaled carbachol challenge

Because preliminary tests had shown that inhalation challenges performed with acetylcholine did not allow to reach a stable and repeatable bronchoconstriction during at least 3 min, carbachol was used as a bronchoconstrictive agent. Anaesthetised rats were exposed to nebulized (UltraNeb 2000, Devilbiss Health Care, Somerset, USA) sterile saline (NaCl 0.9%) for 1 min in a separate chamber. Rats were placed in the plethysmograph chamber, and baseline measurements of R_L and Penh were performed in a random order within 1 min after inhalation. Following this, rats were exposed for 1 min to nebulized carbachol (1.25 mg/mL solution of carbachol; Sigma, St. Louis, USA), and R_L or Penh was recorded within 1 min after the challenge over a period of 5 min. The animals were allowed to recover for 20 min, after which they were exposed to a second carbachol challenge (1.25 mg/mL for 1 min), and Penh or R_L was recorded for 5 min. At the end of the protocol, the rats were killed by a lethal i.p. injection of 200 mg/kg pentobarbital (Dolethal, Vetoquinol).

2.8. Statistical analysis

Respiratory variables (R_L, Penh, and RR) were normally distributed and are shown as mean values$\pm$S.D. Data were analysed by paired Student's *t*-test. Correlation analyses were performed by linear regressions. A *p*-value lower than 0.05 was considered significant.

3. Results

3.1. Protocol 1: Tracheal calibre reduction

As shown in Fig. 1, R_L increased significantly by around 100% after each reduction step (G18 and G20), whereas Penh only increased by ~30% after the last diameter reduction (G20). The increase in Penh after the last diameter reduction was mainly due to an increase of the PEF/PIF ratio (Fig. 2a), whereas the Pause did not increase significantly (Fig. 2b).

To test whether the variations recorded for R_L were correlated to those recorded for Penh, linear regressions were performed between ΔR_L[G18-baseline] and

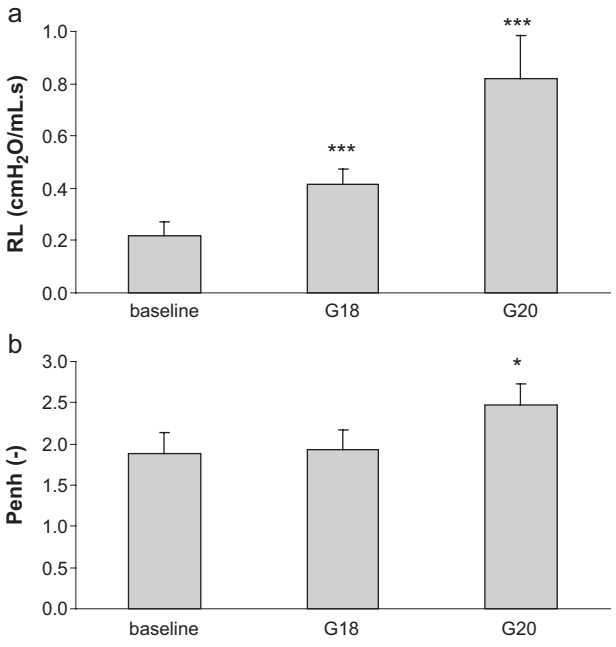

Fig. 1. (a) Pulmonary resistance (R_L) and (b) corresponding enhanced pause (Penh) recorded at baseline and after tracheal calibre reduction by the insertion of a G18 and G20 catheter into the tracheal tube of anaesthetised and tracheotomised rats (n=6). * Indicates a significant difference from baseline, with *p<0.05, **p<0.005, and ***p<0.0005.

ΔPenh[G18-baseline] and between ΔR_L[G20-baseline] and ΔPenh[G20-baseline]. R_L and Penh variations, as well as the corresponding correlation analyses, are shown in Table 1 and indicate that the increase of R_L was not significantly correlated with that of Penh for any reduction step.

Respiratory rate recorded during measurement of R_L and Penh was not significantly different at baseline (97±11 vs. 94±10, p>0.05). Tracheal calibre reductions significantly decreased RR at each reduction step (for R_L: 97±11 vs. 84±4 vs. 75±7, p<0.005; for Penh: 94±10 vs. 84±9 vs. 77±8, p<0.005); but differences in RR between R_L and Penh records were not significant.

3.2. Protocol 2: Inhaled carbachol challenge

Fig. 3 illustrates the effect of carbachol exposure in anaesthetised and tracheotomised rats (n=8) on R_L (a) and Penh (b). Both R_L and Penh were significantly increased by approximately 100%. The increase in Penh was due to a significant increase of the Pause (Fig. 4b), whereas the PEF/PIF ratio remained unchanged (Fig. 4a).

During carbachol-induced airflow limitation, the increase in R_L (ΔR_L: 0.146±0.09 cm H_2O/mL s) was positively and significantly correlated with the increase in Penh (ΔPenh: 2.117±1.223; r=0.80, p<0.05).

Regarding respiratory frequency, values recorded during the measurement of R_L and Penh were similar (86±14 vs. 89±13) but were significantly decreased after the inhalation challenge (for R_L: 73±18 vs. 86±14, p<0.05; for Penh: 75±13 vs. 89±13, p<0.05).

4. Discussion

The validity of Penh as an estimator of airflow limitation remains controversial (Bates et al., 2004), but on the other hand, the advantages of using single-chambered whole-body barometric plethysmography as a screening index of lower airway resistance has led to an increased application of this technique in respiratory research. Single-chambered plethysmography has not yet been frequently described in rats (Michielsen, Leusink-Muis, Vos, & Bloksma, 2001; Zhang, Fedan, Lewis, & Siegel, 2004), and potentially existing strain-related differences, such as described in mice (Adler et al., 2004; Duguet et al., 2000; Schulz et al., 2002), are unknown. Consequently, the results of our study need to be interpreted with care and might not necessarily apply to other rat strains. Moreover, our animals were investigated under anaesthesia although single-chambered plethysmography is intended for use in conscious animals. However, it has been shown that the simultaneously recorded modifications of Penh and R_L in anaesthetised piglets undergoing a cumulative bronchoconstrictive challenge were positively correlated, indicating that Penh might be used under well-defined circumstances in anaesthetised animals (Halloy et al., in press). To circumvent anaesthesia, a comparison between single- and double-chambered plethysmography performed in conscious rats might be an interesting alternative to our study design, at least for the inhalation challenge.

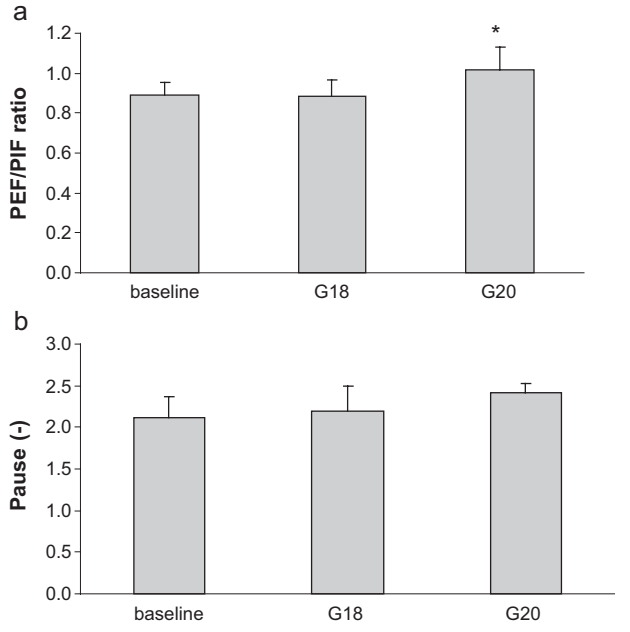

Fig. 2. Ratio between peak expiratory flow and peak inspiratory flow (PEF/PIF ratio) (a) and pause (b) assessed by double-chambered plethysmography at baseline and after tracheal calibre reduction by the insertion of a G18 and G20 catheter into the tracheal tube of anaesthetised and tracheotomised rats (n=6). *Significant difference from baseline and G18, with p<0.05.

Table 1
Variations of R_L and Penh recorded during tracheal diameter reductions in tracheotomised rats (n=6), with correlation analyses between corresponding ΔR_L and ΔPenh

Reduction of tracheal diameter	ΔR_L (cm H_2O/mL s)	ΔPenh (−)	Correlation analysis (x: ΔR_L; y: ΔPenh)	R-value	p-Value
G14 to G18	0.199 ± 0.046^a	0.053 ± 0.070^c	$y=-0.44x+0.14$	0.29	>0.5
G14 to G20	0.602 ± 0.160^b	0.589 ± 0.409^d	$y=1.56x-0.35$	0.61	>0.2

Data are shown as means±S.D.
G14: initial G14-sized tracheal catheter; G18: first tracheal calibre reduction using a G18-sized catheter; G20: second tracheal calibre reduction using a G20-sized catheter; ΔR_L: increase in pulmonary resistance recorded after tracheal diameter reduction; ΔPenh: increase in Penh recorded after tracheal diameter reduction. Within-cell comparisons with different superscripts differ significantly, $p<0.05$.

Whilst tracheal calibre reductions induced a very significant increase of R_L and only a slight, but significant, change in Penh after the second reduction step (Fig. 1b), carbachol nebulization increased Penh and R_L to a similar extent (Fig. 3), suggesting that airflow limitations, which are induced by a cholinergic agent, are more likely to be detected by single-chambered plethysmography. Accordingly, the variations of both ΔPenh and ΔR_L were significantly correlated (r=0.80) after the carbachol challenge. To strengthen the comparison between catheter- and carbachol-induced airflow limitations, two different or cumulative inhalation challenges in each rat would have been interesting. This was, however, not possible because 2×2 nebulization challenges followed by 5-min records, instead of two inhalations within an anaesthesia duration of maximally 90 min, did not allow a sufficient recovery between measurements.

By considering the two components influencing Penh, the Pause, and the PEF/PIF ratio, it appears that tracheal calibre reductions induced significant changes of the PEF/PIF ratio (Fig. 2a), whereas the Pause was increased in a nonsignificant manner (Fig. 2b). The opposite occurred after the carbachol challenge; that is, the Pause was significantly increased and the PEF/PIF ratio remained unchanged (Fig. 4). The increase of Pause after the inhalation challenge has also been reported in mice after an inhaled acetycholine challenge (Hamelmann et al., 1997). The different evolution of the PEF/PIF ratio and the Pause indicates that the breathing strategy adopted during the tracheal calibre reduction and in response to the carbachol challenge was not similar and differently affected Penh values. This difference in breathing strategy might be related to the fact that the first protocol exclusively induced a mechanical obstruction, whereas cholinergic agents, such as carbachol, induce bronchial smooth muscle contraction, mucus, and surfactant secretion (Mullol & Baranuik, 1999). Therefore, the measurement of pulmonary dynamic compliance (C_{dyn}), which is likely to be more affected by a carbachol

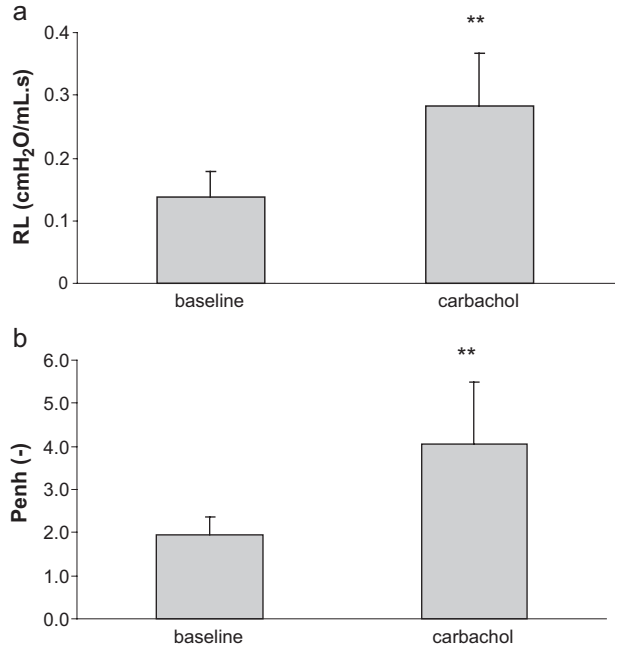

Fig. 3. (a) Pulmonary resistance (R_L) and (b) corresponding enhanced pause (Penh) recorded before (baseline) and after carbachol inhalation (carbachol) in anaesthetised and tracheotomised rats (n=8). * Indicates a significant difference from baseline, with *$p<0.05$ and **$p<0.005$.

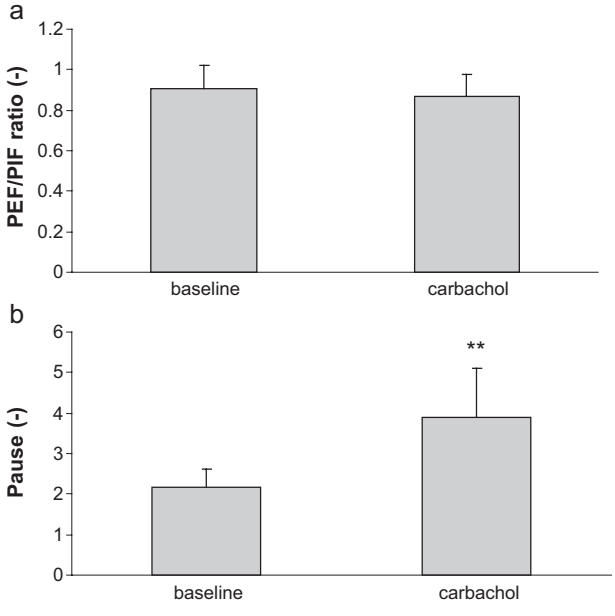

Fig. 4. Ratio between peak expiratory flow and peak inspiratory flow (PEF/PIF ratio) (a) and pause assessed by double-chambered plethysmography before (baseline) and after carbachol inhalation (carbachol) in anaesthetised and tracheotomised rats (n=8). * Indicates a significant difference from baseline, with *$p<0.05$ and **$p<0.005$.

nebulization than R_L is, and its correlation with Penh would also have been of interest in this study.

In conclusion, this study has shown that Penh poorly detected tracheal calibre modifications, whereas changes induced by pulmonary cholinergic stimulation were detected. However, this study has shown that Penh strongly depends on breathing pattern, which limits the use of Penh as screening index of airway resistance to well-defined and validated experimental settings.

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References

Adler, A., Ciesewicz, G., & Irvin, C. G. (2004). Unrestrained pelthysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *Journal of Applied Physiology*, *97*, 286–292.

Bates, J., Irvin, Ch., Brusasco, V., Drazen, J., Fredberg, J., et al. (2004). Correspondence: The use and misuse of Penh in animal models of lung disease. *American Journal of Respiratory Cell and Molecular Biology*, *31*, 373.

Chand, N., Nolan, K., Pillar, J., Lomask, M., Diamantis, W., & Sofia, R. D. (1993). Aeroallergen-induced dyspnea in freely moving guinea pigs: Quantitative measurement by bias flow ventilated whole body plethysmography. *Allergy*, *48*, 230–235.

Chong, B. T., Agrawal, D. K., Romero, F. A., & Townley, R. G. (1998). Measurement of bronchoconstriction using whole-body plethysmograph: Comparison of freely moving versus restrained guinea pigs. *Journal of Pharmacological and Toxicological Methods*, *39*, 163–168.

DeLorme, M. P., & Moss, O. R. (2002). Pulmonary function assessment by whole-body plethysmography in restrained versus unrestrained mice. *Journal of Pharmacological and Toxicological Methods*, *47*, 1–10.

Drorbaugh, J. E., & Fenn, W. O. (1955). A barometric method for measuring ventilation in newborn infants. *Pediatrics*, *16*, 81–87.

Dohi, M., Tsukamoto, S., Nagahori, T., Shinagawa, K., Saitoh, K., Tanaka, Y., et al. (1999). Noninvasive system for evaluating the allergen-specific airway response in a murine model of asthma. *Laboratory Investigation*, *79*, 1559–1571.

Duguet, A., Biyah, K., Minshall, E., Gomes, R., Wang, Ch., Taoudi-Benchekroun, M., et al. (2000). Bronchial responsiveness among inbred mouse strains. *American Journal of Respiratory and Critical Care Medicine*, *161*, 839–848.

Enhorning, G., van Schaik, S., Lundgren, C., & Vargas, I. (1998). Whole-body plethysmography, does it measure tidal volume of small animals? *Canadian Journal of Physiology and Pharmacology*, *76*, 945–951.

Flandre, T. D., Leroy, P. L., & Desmecht, D. J. (2003). Effect of somatic growth, strain, and sex on double-chamber plethysmographic respiratory function values in healthy mice. *Journal of Applied Physiology*, *94*, 1129–1136.

Glaab, Th., Mitzner, W., Braun, A., Ernst, H., Korolewitz, R., Hohlfeld, J. M., et al. (2004). Repetitive measurements of pulmonary mechanics to inhaled cholinergic challenge in spontaneously breathing mice. *Journal of Applied Physiology*, *97*, 1101–1111.

Halloy, D., Kirschvink, N., Hamoir, J. N., Vincke, G., Delvaux, F., & Gustin, P. (2004). Whole body barometric plethysmography: A screening method to investigate airway reactivity and acute lung injuries in freely moving pigs. *The Veterinary Journal*, *168*, 276–284.

Hamelmann, E., Schwarze, J., Takeda, K., Oshiba, A., Larsen, G. L., Irvin, C. G., et al. (1997). Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *American Journal of Respiratory and Critical Care Medicine*, *156*, 766–775.

Hantos, Z., Daroczy, B., Suki, B., & Nagy, S. (1987). Low-frequency respiratory mechanical impedance in the rat. *Journal of Applied Physiology*, *63*, 36–43.

Lundblad, L. K., Irvin, C. G., Adler, A., & Bates, J. H. (2002). A reevaluation of the validity of unrestrained plethysmography in mice. *Journal of Applied Physiology*, *93*, 1198–1207.

Martin, R. J., Chu, H. W., Honour, J. M., & Harbeck, R. J. (2001). Airway inflammation and bronchial hyperresponsiveness after *Mycoplasma pneumoniae* infection in a murine model. *American Journal of Respiratory Cell and Molecular Biology*, *24*, 577–582.

Michielsen, C., Leusink-Muis, A., Vos, J., & Bloksma, N. (2001). Hexachlorobenzene-induced eosinophilic and granulomatous lung inflammation is associated with in vivo hyperresponsiveness in the Brown Norway rat. *Toxicology and Applied Pharmacology*, *172*, 11–20.

Mullol, J., & Baranuik, J. N. (1999). Lung biology in health and disease: Vol. 134. Anticholinergic agents in the upper and lower airways. In S. L. Spector (Ed.), *Basics of muscarinic physiology* (pp. 3–22). New York: Marcel Dekker.

Palecek, F. (1969). Measurement of ventilatory mechanics in the rat. *Journal of Applied Physiology*, *27*, 149–156.

Pennock, B. E., Cox, C. P., Rogers, R. M., Cain, W. A., & Wells, J. H. (1979). A non-invasive technique for measurement of changes in specific airway resistance. *Journal of Applied Physiology*, *46*, 399–406.

Petak, F., Habre, W., Donati, Y. R., Hantos, Z., & Barazzone-Argiroffo, C. (2001). Hyperoxia-induced changes in mouse lung mechanics: Forced oscillations vs. barometric plethysmography. *Journal of Applied Physiology*, *90*, 2221–2230.

Petak, F., Hantos, Z., Adamicza, A., Asztalos, T., & Sly, P. D. (1997). Methacholine-induced bronchoconstriction in rats: Effects of intravenous vs. aerosol delivery. *Journal of Applied Physiology*, *82*, 1479–1487.

Schulz, H., Johner, C., Eder, G., Ziesenis, A., Reitmeier, P., Heyder, J., et al. (2002). Respiratory mechanics in mice: Strain and sex specific differences. *Acta Physiologica Scandinavica*, *174*, 367–375.

Zhang, X. D., Fedan, J., Lewis, D., & Siegel, P. (2004). Asthma-like biphasic airway responses in Brown Norway rats sensitized by dermal exposure to dry trimellitic anhydride powder. *Journal of Allergy and Clinical Immunology*, *113*, 320–326.