Respiratory Physiology & Neurobiology 188 (2013) 56–59

Contents lists available at SciVerse ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Short communication

Novel method for transdiaphragmatic pressure measurements in mice

Sarah M. Greising[a], Dylan C. Sieck[a], Gary C. Sieck[a,b], Carlos B. Mantilla[a,b,*]

[a] Department of Physiology and Biomedical Engineering, Mayo Clinic, College of Medicine, 200 First Street SW, Rochester, MN 55905, USA
[b] Department of Anesthesiology, Mayo Clinic, College of Medicine, 200 First Street SW, Rochester, MN 55905, USA

ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form 11 April 2013
Accepted 15 April 2013

Keywords:
Diaphragm muscle
Mouse strain
Phrenic nerve
Respiratory compliance

ABSTRACT

The diaphragm muscle (DIAm) is responsible for breathing and determines the ability to generate both ventilatory and non-ventilatory behaviors. Size limitations of the mouse make transdiaphragmatic pressure (Pdi) measurement using a dual balloon system untenable. Adult C57BL/6J mice ($n = 8$) and C57BL/6 × 129 ($n = 9$), underwent Pdi measurements using solid-state pressure catheters spanning the thoracic and abdominal surfaces of the DIAm. Measurements were conducted during eupnea, hypoxia (10% O_2)–hypercapnia (5% CO_2), chemical airway stimulation (i.e., sneezing), spontaneously occurring deep breaths, sustained tracheal occlusion, and bilateral phrenic nerve stimulation. There was a difference in the Pdi generated across the range of ventilatory and non-ventilatory behaviors ($p = 0.001$). No difference in Pdi across behaviors was evident between mouse strains ($p = 0.161$). This study establishes a novel method to determine Pdi across a range of DIAm behaviors in mice that may be useful in evaluating conditions associated with reduced ability to perform expulsive, non-ventilatory behaviors.

1. Introduction

The diaphragm muscle (DIAm) is the primary muscle of inspiration and is necessary to sustain ventilation throughout the lifespan. The DIAm is also essential in generating expulsive, non-ventilatory behaviors necessary for airway clearance. Transdiaphragmatic pressure (Pdi) measurements have been a useful tool used in a number of species to determine the DIAm force during both ventilatory and non-ventilatory behaviors. Such measurements are commonly performed using a dual balloon catheter system (ATS/ERS Statement on respiratory muscle testing, 2002), however the size of the mouse makes measuring Pdi difficult with this method. Recently the availability of solid-state pressure transducers has made this size limitation of the mouse less of an obstacle and thus Pdi measurements may be possible in mice.

To date Pdi has been used clinically as well as in pigs, cats, hamsters, and rats as a surrogate for DIAm force (Mantilla et al., 2010; Sieck, 1991; Watchko et al., 1986). Pdi measurements can be conducted across a range of DIAm behaviors, from eupneic breathing to maximal DIAm activation induced by sneezing (Mantilla et al., 2010) and gagging (Sieck and Fournier, 1989). Indeed, Pdi is significantly correlated to the peak root mean squared EMG amplitude across a range of ventilatory and non-ventilatory DIAm behaviors in rats (Mantilla et al., 2010). Substantial reserve capacity for generating maximal behaviors beyond quiet ventilation is evident in force, root mean squared EMG and Pdi. In humans, cats, rats and hamsters, quiet breathing can be accomplished by generating 10, 12, 21 and 27% of maximal Pdi (Mantilla et al., 2010; Sieck, 1994). The relationship between Pdi and ventilation is influenced by mechanical components of the respiratory system. Thus, in this study respiratory system, chest, and lung mechanics were measured in mice to aid in interpretation of Pdi results.

Characterizing the function of respiratory muscles and specifically Pdi in mice is important in determining how DIAm function is impaired by injury or disease. The purpose of this study was to develop a novel method to measure Pdi in mice across ventilatory and non-ventilatory behaviors. Two commonly used mouse models, C57BL/6J and C57BL/6 × 129 mice, were evaluated. Many studies use 129 mice to isolate embryonic stem cells for genetic manipulation, which are backcrossed and maintained on a C57BL/6 × 129 mixed background.

2. Methods

2.1. Animals

Adult (6 month old) male mice were used for all experimental groups. The comparison of strains consisted of C57BL/6J (C57, Jax stock # 000664; $n = 8$) purchased from Jackson Laboratories (Bar Harbor, ME) and C57BL/6 × 129 mice (C57 × 129, $n = 9$) bred and maintained at the Mayo Clinic. Mice were group housed by genotype, maintained on a 12-h light cycle with free access to food and water. At the terminal experiment, mice were weighed (average

* Corresponding author at: 200 First Street SW, SMH Jo 4-184, Rochester, MN 55905, USA. Tel.: +1 507 255 7481; fax: +1 507 255 7300.
E-mail address: mantilla.carlos@mayo.edu (C.B. Mantilla).

1569-9048/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resp.2013.04.018

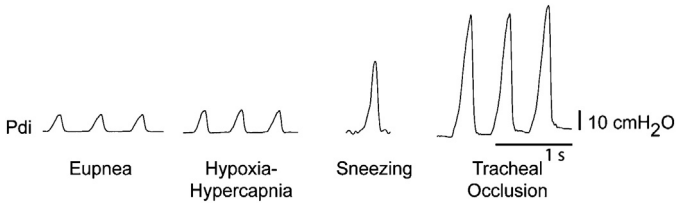

Fig. 1. Representative transdiaphragmatic pressure (Pdi) tracings from adult 6 month old mice during eupnea (breathing room air), hypoxia (10% O_2)–hypercapnia (5% CO_2), sneezing (induced by intranasal capsaicin), and sustained tracheal occlusion (15 s).

body mass: 32.0 ± 0.8 g, $p = 0.451$), anesthetized by an intramuscular injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) and euthanized by exsanguination. All protocols were approved by the Institutional Animal Care and Use Committee at the Mayo Clinic, in compliance with National Institute of Health guidelines.

2.2. Transdiaphragmatic pressure measurements

Methodology for Pdi measurements was adapted from previous studies (Mantilla et al., 2010; Sieck, 1994). Briefly, the abdomen was tightly bound and the trachea cannulated (19 G) while mice maintained spontaneous ventilation. Two 3.5 French Millar solid-state pressure catheters (SPR-524; Millar Instruments, Houston, TX) were then inserted through the mouth into the esophagus and stomach, spanning the thoracic and abdominal surfaces of the DIAm, respectively. Correct catheter position was determined based on the signal deflection and postmortem analysis.

Measurements were collected during the following conditions: (1) breathing of room air (eupnea) for 5 min, (2) exposure to hypoxia (10% O_2)–hypercapnia (5% CO_2) for 5 min, (3) sustained tracheal occlusion for 15 s, (4) bilateral phrenic nerve stimulation (0.5 ms duration pulses at 75 or 150 Hz in 300 ms trains repeated each s) using straight bipolar electrodes (FHC, Bowdoin, ME), and (5) stimulation of the nasal airway (i.e., sneezing) induced chemically by intranasal infusion of 10 μl of 30 μM capsaicin. Mice were allowed ~5 min intervals between interventions to allow for Pdi amplitude to return to eupneic values.

Intra-thoracic and -abdominal pressures were measured independently and recorded with a PowerLab 8/35 data acquisition system with an integrated amplifier following the manufacturer recommended calibration procedure. Data was analyzed using LabChart (Millar Instrumentation), band-pass filtered (0.3–30 Hz), and sampled at 100 Hz. Data from LabChart was exported to MATLAB for custom-designed automated analyses of peak amplitude and corresponding baseline. Baseline values were determined for each peak from the average of all inflection points in the segment preceding each peak, such that peak amplitude was the difference between the two values. Data was analyzed and averaged across behaviors for 1 min of eupnea, 1 min of hypoxia–hypercapnia, the 5 maximal breaths during occlusion, all spontaneous deep breaths, all sneezes, and the maximal value obtained during stimulation (representative tracings in Fig. 1). Deep breaths were defined as spontaneously occurring inspiratory events that were ~2× eupneic Pdi amplitude (Mantilla et al., 2010). Breathing frequency during both eupnea and hypoxia–hypercapnia was calculated.

2.3. Respiratory system mechanics

Following Pdi measurements, mice were connected via tracheal cannula to the flexiVent computer controlled ventilator system (SCIREQ; Montreal, Canada). Analyses of respiratory system compliance were conducted following manufacturer recommendations, while mechanically ventilated (tidal volume: 10 ml/kg). Measurements were collected with the chest wall intact and subsequently in the isolated lung following a midline sternotomy. Thus, respiratory system, chest, and lung mechanics were obtained. Briefly, mechanics were assessed during a 1.2-s, 2.5 Hz forced oscillation maneuver (Snapshot-150 v5.2) followed by a 3-s low frequency forced oscillation containing mutually prime frequencies between 1 and 20.5 Hz (QuickPrime-3 v5.2). Data were fit with a constant phase model, and only included if a coefficient of determination >0.95 was achieved.

2.4. Statistical analysis

Data were analyzed by repeated-measures two-way ANOVA with Tukey–Kramer honestly significant difference post hoc tests, when appropriate, based on mouse strain and motor behavior (Pdi) and one-way ANOVA for mechanical measurement across strains. Correlation between eupneic Pdi and respiratory system resistances was assessed using Spearman's rank order test. Statistical analyses were conducted using JMP (Version 8.0; SAS Institute, Cary, NC); data are presented as mean ± standard error (SE). Significance was accepted at the $\alpha < 0.05$ level.

3. Results

3.1. Transdiaphragmatic pressure

There was no difference in the Pdi generated between mouse strains (two-way ANOVA, strain × behavior; interaction $p = 0.297$, strain $p = 0.161$; Fig. 2). However, there was a difference in the Pdi generated across behaviors (main effect $p = 0.001$; Fig. 2). During ventilatory behaviors the average (across mouse stains) Pdi generated was 9.9 ± 0.6 and 12.2 ± 0.9 cm H_2O for eupnea and hypoxia–hypercapnia, respectively although there was no difference between these two ventilatory behaviors. Of note, there was a trend for an increase in breathing frequency between eupnea and hypoxia–hypercapnia from 155 ± 7 to 165 ± 8 min^{-1}, with no differences across strains (two-way ANOVA, strain × behavior; interaction $p = 0.969$, strain $p = 0.824$, behavior $p = 0.344$).

Naturally occurring deep breaths occurred in all mice except two C57 mice; on average spontaneous deep breaths generate 37.3 ± 2.5 cm H_2O. On average sneezing generated 35.9 ± 2.2 cm H_2O, chemical stimulation to induce sneezing was only achieved in 11 of the 17 mice tested. There was no significant difference in the Pdi generated between deep breaths and sneezing, but the Pdi generated in these behaviors was significantly greater than that generated during eupnea and hypoxia–hypercapnia.

On average, tracheal occlusion generated 63.5 ± 7.0 cm H_2O and bilateral phrenic nerve stimulation at 75 Hz generated 71.0 ± 5.7 cm H_2O. Consistent and repeatable phrenic nerve stimulation could be accomplished at frequencies up to 75 Hz, but stimulation at higher frequencies in vivo was less reliable. Of note, maximum isometric force was elicited at 150 Hz stimulation in ex vivo DIAm strips tested in a force-frequency protocol of 1-s trains of stimuli between 5 and 175 Hz at 37 °C ($n = 4$ mice). The difference in force between 75 and 150 Hz was found to be 23.4% in these muscle strips. In a subset of mice ($n = 3$), bilateral phrenic nerve stimulation was conducted at both 75 and 150 Hz and indeed Pdi was 26.9% greater at 150 Hz vs. 75 Hz. The estimated Pdi with maximal bilateral phrenic nerve stimulation at 150 Hz was 89.0 ± 7.1 cm H_2O (Fig. 2).

3.2. Respiratory system mechanics

There were no differences in respiratory system resistances or compliances between mouse strains (Table 1). The isolated lung comprised ~80% of the resistance of the entire respiratory system