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Compensatory effects following unilateral diaphragm paralysis

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ABSTRACT

Injury to nerves innervating respiratory muscles such as the diaphragm muscle results in significant respiratory compromise. Electromyography (EMG) and transdiaphragmatic pressure (Pdi) measurements reflect diaphragm activation and force generation. Immediately after unilateral diaphragm denervation (DNV), ventilatory behaviors can be accomplished without impairment, but Pdi generated during higher force non-ventilatory behaviors is significantly decreased. We hypothesized that 1) the initial reduction in Pdi during higher force behaviors after DNV is ameliorated after 14 days, and 2) changes in Pdi over time after DNV are associated with concordant changes in contralateral diaphragm EMG activity and ventilatory parameters. In adult male rats, the reduced Pdi during occlusion (~40% immediately after DNV) was ameliorated to ~20% reduction after 14 days. Contralateral diaphragm EMG activity did not significantly change immediately or 14 days after DNV compared to the pre-injury baseline for any motor behavior. Taken together, these results suggest that over time after DNV compensatory changes in inspiratory related muscle activation may partially restore the ability to generate Pdi during higher force behaviors.

1. Introduction

The final common pathway of neuromotor control is the motor unit, which consists of an α-motoneuron and the group of muscle fibers it innervates (Liddell and Sherrington, 1925). Recruitment of additional motor units (Fournier and Sieck, 1988; Sieck and Fournier, 1989) and/or an increase in the discharge frequency of recruited motor units (Fournier and Sieck, 1988; Iscoe et al., 1976; Seven et al., 2014; Sieck et al., 1984) increase the force generated by skeletal muscles including respiratory muscles such as the diaphragm. Indeed, orderly recruitment of diaphragm motor units allows for a broad range of motor behaviors from lower force ventilatory behaviors (requiring only 10–30% of the total force generating capacity of the diaphragm across species) and less frequent higher force behaviors that are necessary for maintaining airway patency (e.g. coughing, sneezing, sighing) (Mantilla et al., 2014; Mantilla et al., 2010; Sieck and Fournier, 1989). While recruitment of only fatigue resistant motor units is sufficient to accomplish lower force ventilatory behaviors, recruitment of more fatigable motor unit types is necessary to accomplish higher force behaviors (Mantilla et al., 2010; Mantilla and Sieck, 2011; Sieck, 1991, 1994; Sieck and Fournier, 1989). This large reserve capacity for force generation by the diaphragm muscle results in the ability to sustain ventilatory behaviors despite substantial loss of motor units (Alvarez-Argote et al., 2016; Gill et al., 2015; Mantilla and Sieck, 2011; Rana et al., 2017).

Unilateral phrenic nerve denervation (DNV) induces unilateral diaphragm paralysis, effectively inactivating 50% of the motor unit output of the diaphragm (Miyata et al., 1995; Zhan et al., 1995). We previously showed that immediately after unilateral DNV, transdiaphragmatic pressure (Pdi; an indirect measure of diaphragm force) decreases for behaviors requiring greater than 50% of the maximal Pdi in rats (Gill et al., 2015). In the same study, we showed that Pdi amplitude during ventilatory behaviors is unimpaired after DNV and ventilation is unimpaired as assessed by blood gas levels. Similarly, in humans, while maximal Pdi amplitude is reduced after unilateral diaphragm paralysis, Pdi amplitude necessary to accomplish quiet breathing (eupnea) is generally unimpaired although relative contributions from the gastric and esophageal components of Pdi may change (Hart et al., 2002; Hillman and Finucane, 1988; Lisboa et al., 1986). In dogs, Pdi measurements across respiratory motor behaviors after unilateral diaphragm paralysis is not available, but there is some evidence that tidal volume does not change following unilateral diaphragm paralysis (Katagiri et al., 1994) and thus impairment in Pdi during eupnea is unlikely. Accordingly, the purpose of the present study was to determine whether compensation for reduced Pdi during higher force behaviors occurs over a period of 14 days in rats, and the role of the contralateral diaphragm muscle in this compensation. We hypothesized that 1) the initial reduction in Pdi during higher force behaviors after DNV is ameliorated after 14 days, and 2) changes in Pdi over time post-

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DNV are associated with concordant changes in contralateral diaphragm electromyographic (EMG) activity and ventilatory parameters.

2. Materials and methods

2.1. Animals

All experiments were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. A total of 29 adult, male Sprague-Dawley rats (280–380 g) from Envigo (Indianapolis, IN) were used for this study. Anesthesia was achieved via intramuscular injections of ketamine (100 mg/kg) and xylazine (10 mg/kg) for all surgical procedures, Pdi and EMG recordings. Unilateral diaphragm paralysis was verified by the absence of EMG activity in the ipsilateral (right) diaphragm at all time points in the DNV group (n = 14). Control rats did not receive either DNV or exposure of the phrenic nerve (n = 15).

2.2. Unilateral diaphragm denervation

The right phrenic nerve was isolated in the lower neck and a 10–20 mm length of the nerve was sectioned as previously described (Geiger et al., 2003; Gill et al., 2015; Gosselin et al., 1994; Miyata et al., 1995; Zhan et al., 1995, 1997; Zhan and Sieck, 1992). Briefly, rats were laid in a supine position and a 2 cm incision was made starting from the middle of the clavicle in a rostromedial direction. Blunt dissection was performed to isolate the phrenic nerve, using the jugular vein, carotid artery, and brachial plexus as anatomical landmarks. Complete DNV was verified by absence of EMG activity in the ipsilateral hemidiaphragm.

2.3. Transdiaphragmatic pressure measurements

Pdi was calculated as the difference in pressures measured between esophageal (P_{eso}) and gastric (P_{ab}) catheters as previously described (Gill et al., 2015; Greising et al., 2013a,b; Mantilla et al., 2010; Sieck and Fournier, 1989). Briefly, two 3.5 French Millar solid-state pressure catheters (SPR-524; Millar Instruments, Houston, TX) were inserted through the mouth into the esophagus and stomach. Correct catheter position was determined based on the direction of signal deflection during real-time measurements. Intra-thoracic and abdominal pressures were recorded and digitized (400 Hz) with PowerLab 4/35 and visualized in real-time with LabChart 8 (ADInstruments, Colorado Springs, CO). The Pdi signal was band-pass filtered between 0.3 and 30 Hz using a digital filter to remove offset and high-frequency noise. Data were exported for *post hoc* analysis using a custom-designed semi-automated script in MATLAB (MathWorks, Natick, MA). Peak amplitude, both instantaneous and average respiratory rate, inspiratory duration, and duty cycle were determined using previously described techniques (Medina-Martinez et al., 2015). Pdi measurements across all motor behaviors were obtained before, immediately after, and 14 days after unilateral DNV in the DNV group; in the time control group, measurements were made at two time points separated by 14 days. The abdomen was bound during Pdi measurements to approximate isometric conditions during diaphragm muscle contraction.

2.4. Diaphragm EMG measurements

Diaphragm EMG was recorded using chronically placed wire electrodes as previously described (Dow et al., 2006, 2009; Mantilla et al., 2011; Trelease et al., 1982). Briefly, pairs of multistranded fine wire stainless steel electrodes (AS631; Cooner Wire Inc., Chatsworth, CA) were stripped to expose an ~2 mm segment. A laparotomy was performed and a pair of electrodes with the exposed portion of the wire implanted into the mid-costal regions of both sides of the diaphragm with an inter-electrode distance of ~3 mm. The electrodes were tunneled and externalized in the dorsum of the animals for up to 19 days. Electrode implantation was performed 4 days prior to DNV. The ends of the fine-wire electrodes were connected via gold pin connectors to differential amplifiers (Model EMG100C, Biopac Systems Inc., Goleta, CA.). The EMG signal was amplified (2000×), band-pass filtered (100–5000 Hz) and digitally sampled at 10 kHz using Powerlab 4/35. During each recording session, the ECG signal present in the diaphragm EMG signal was isolated using a low-pass digital filter (f_c = 200 Hz) in LabChart 8 and used to determine the instantaneous heart rate.

Data were exported to MATLAB, downsampled to 2000 Hz, and analyzed using custom-made software based on previous work (Dow et al., 2006). For diaphragm EMG recordings, ECG contamination was removed by linearly correlating the average ECG tracing of each signal against each set of points within the signal. A threshold was set manually such that a cross correlation at a set of points greater than this threshold resulted in subtraction of the average ECG. The root mean square (RMS) of the EMG signal was calculated with a 50 ms window. The peak of the RMS EMG (RMS_{peak}) and central respiratory drive, estimated by measuring the RMS EMG value at 75 ms after the onset of activity (RMS_{75}), were determined for each behavior and time (Gill et al., 2015; Seven et al., 2014). All RMS EMG measurements were normalized to the pre-injury sigh RMS_{peak} for each animal. We have previously demonstrated that normalizing RMS values to near maximal behaviors such as sigh and sneeze reduces inter-animal variability over time (Mantilla et al., 2011). In addition, the tension-time index of the diaphragm was used as estimate of the efficiency of diaphragm activation before, immediately after, and 14 days after DNV was derived from the Pdi and EMG (Bellemare and Grassino, 1982). The tension-time index was calculated as Pdi amplitude (normalized to Pdi_{max})*Duty Cycle (Bellemare and Grassino, 1982). For this estimate, Pdi_{max} at each time point was assumed to be 37 cm H_2O before and 23 cm H_2O after DNV, as previously reported (Gill et al., 2015; Mantilla et al., 2010).

2.5. Motor behaviors

Data were collected during 1) breathing of room air (eupnea), 2) exposure to hypoxia (10% O_2)-hypercapnia (5% CO_2), 3) deep breaths ("sighs", defined as spontaneously occurring inspiratory events that were greater than 2 times eupneic Pdi amplitude at the baseline) and 4) sustained airway occlusion for ~45 s, as in previous studies (Mantilla et al., 2011, 2010; Seven et al., 2014). Rats were given sufficient time between behaviors to allow for acclimatization to normal breathing as determined by real-time calculation of Pdi amplitude and respiratory rate. For both eupnea and hypoxia-hypercapnia, 20 representative breaths that were uncontaminated by ECG, were selected to determine the RMS_{peak} and RMS_{75}. For airway occlusion, the 5 largest breaths within the last 10 s of the occlusion period were analyzed, as in previous studies (Gill et al., 2015; Mantilla et al., 2010; Seven et al., 2014).

2.6. Statistics

All statistical evaluations were performed using standard statistical software (JMP Pro 11, SAS Institute Inc., Cary, NC). Differences in Pdi, sigh normalized diaphragm EMG activity, and ventilatory parameters across experimental groups and motor behaviors were evaluated using a mixed linear model with behavior (eupnea, hypoxia-hypercapnia, sigh, occlusion), time (baseline, immediately after denervation, and 14 days afterwards), and the behavior*time interaction as fixed effects and with animal number as a random effect. When appropriate, *post hoc* analyses were conducted using the Tukey-Kramer Honestly Significant Difference (HSD) test, unless otherwise noted. Statistical significance was established at $p < 0.05$. A subset of rats were not included in the final analysis due to inability to chronically monitor EMG activity (n = 6; *e.g.*, resulting from electrode dislodgement) or based on *a priori* criteria on the proportional Pdi amplitude at baseline during eupnea compared to sighs (n = 6; sighs must be greater than 2 times the