Phasic respiratory modulation of pharyngeal collapsibility via neuromuscular mechanisms in rats

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Submitted 3 February 2011; accepted in final form 31 October 2011

Cao Y, McGuire M, Liu C, Malhotra A, Ling L. Phasic respiratory modulation of pharyngeal collapsibility via neuromuscular mechanisms in rats. J Appl Physiol 112: 695–703, 2012. First published November 3, 2011; doi:10.1152/japplphysiol.00136.2011.—Obstructive sleep apnea patients experience recurrent upper airway (UA) collapse due to decreases in the UA dilator muscle activity during sleep. In contrast, activation of UA dilators reduces pharyngeal critical pressure (Pcrit, an index of pharyngeal collapsibility), suggesting an inverse relationship between pharyngeal collapsibility and dilator activity. Since most UA muscles display phasic respiratory activity, we hypothesized that pharyngeal collapsibility is modulated by respiratory drive via neuromuscular mechanisms. Adult male Sprague-Dawley rats were anesthetized, vagotomized, and ventilated (normocapnia). In one group, integrated genioglossal activity, Pcrit, and maximal airflow (Vmax) were measured at three expiration and five inspiration time points within the breathing cycle. Pcrit was closely and inversely related to pharyngeal collapsibility, with the value measured at peak inspiration being the lowest. In other groups, the variables were measured during expiration and peak inspiration, and after and after each of five manipulations. Pcrit was 26% more negative (−15.0 ± 1.0 cmH2O, −18.9 ± 1.2 cmH2O; n = 23), Vmax was 7% larger (31.0 ± 1.0 ml/s, 33.2 ± 1.1 ml/s), nasal resistance was 12% bigger [0.49 ± 0.05 cmH2O/ml/s, 0.59 ± 0.05 cmH2O/ml/s], and latency to induced UA closure was 14% longer (55 ± 4 ms, 63 ± 5 ms) during peak inspiration vs. expiration (all P < 0.005). The expiration-inspiration difference in Pcrit was abolished with neuromuscular blockade, hypocapnic apnea, or death but was not reduced by the superior laryngeal nerve transection or altered by tracheal displacement. Collectively, these results suggest that pharyngeal collapsibility is moment-by-moment modulated by respiratory drive and this phasic modulation requires neuromuscular mechanisms, but not the UA negative pressure reflex or tracheal displacement by phasic lung inflation.

pharyngeal critical pressure; upper airway; neuromuscular activity; respiratory drive; obstructive sleep apnea

OBSTRUCTIVE SLEEP APNEA (OSA), a major sleep disorder affecting at least 2–4% of the adult population (36), leads to many pathological consequences, including daytime sleepiness, neurocognitive deficits, and cardiovascular diseases (4, 7, 11, 13, 18, 19, 33). OSA is characterized by recurrent upper airway (UA) collapse, which blocks gas flow, causing paroxysms of inspiratory effort that trigger partial or complete arousals (2), thereby disrupting normal sleep architecture. As a result, OSA patients suffer from intermittent hypoxemia and sleep fragmentation every night. Thus UA collapse is crucial to OSA syndrome. Pharyngeal critical closing pressure (Pcrit, an index of UA collapsibility) is correlated with susceptibility to UA collapse in normal subjects, snorers, and OSA patients (9), and has thus been considered as an integrated measure of many combined factors (3, 9, 14, 21, 27–29, 32). However, although UA collapsibility has been extensively studied, its phasic respiratory modulation and underlying mechanisms have not been adequately studied.

To date, the majority of previous Pcrit studies have shown no ΔPcrit, an expiration-inspiration difference in Pcrit. In animal studies, UA collapse was artificially induced, but the latency to UA closure was usually longer than peak inspiration itself. In human studies, Pcrit was usually assessed at peak inspiration only. However, the existence of ΔPcrit has been revealed in several special cases. For example, a ΔPcrit was reported in anesthetized dogs when Pcrit was measured at expiration and inspiration in the presence of high levels of CO2 (30). A small ΔPcrit was also reported in several tracheostomized OSA patients when using a reverse airflow regimen (24). These results indicate that Pcrit values can be different when measured during expiration or at peak inspiration.

In OSA patients, UA collapses at the caudal velopharynx and/or oropharynx behind the soft palate and tongue during sleep. However, no such collapse occurs during wakefulness, no matter how severe OSA is. The UA collapse occurs due to decreases in the UA dilator muscle activity during sleep in those who are anatomically predisposed (1, 25, 26) and is associated with increases in UA collapsibility defined by Pcrit (3, 9, 14, 21, 27–29, 32, 34). In contrast, activation of UA dilator muscles (e.g., hypoglossal, genioglossal, or tensor palatini stimulation) reduced Pcrit in humans and animals (5, 6, 8, 15, 30); general activation of respiratory muscles with hypercapnia also decreased Pcrit (30), suggesting an inverse relationship between Pcrit and UA dilator activity. While some OSA patients experience collapse at end-exhalation (due to passive collapse of the vulnerable UA), others have collapse/narrowing during inspiration (due likely to deficient UA muscle responsiveness), making within-breath dynamics clinically important (16). In the present study, we used a modified method with an improved “resolution” to measure Pcrit and the maximum UA airflow (Vmax) directly at different phases of a breathing cycle. Since most, if not all, UA muscles display phasic respiratory activity and are involved in the control of UA patency, we hypothesized that pharyngeal collapsibility is dynamically modulated by respiratory drive via neuromuscular mechanisms under normocapnic conditions.

METHODS

Experiments were conducted on adult male Sprague-Dawley rats (~3 mo old, 250–350 g, CDIGS colony, Charles River, Wilmington, MA). All experimental procedures used herein were in accordance with National Institutes of Health Animal Care and Use Guidelines and were approved by the Harvard Medical Area Standing Committee on Animals.
Experimental Preparation

Rats were anesthetized initially with isoflurane in a closed chamber and then injection of urethane (~1.6 g/kg ip). The depth of anesthesia was adjusted so that the animal failed to show a reflex withdrawal of the hindpaw to a strong pinch. In one group ($n = 6$), rats were also neuromuscularly blocked using pancuronium bromide injection (2.5 mg/kg iv), and the adequacy of anesthesia was assessed by testing phrenic nerve responses to toe pinch. In all rats, atropine sulfate (0.3 mg/kg sc) was given to reduce airway salivation. The trachea was sectioned. The caudal, sectioned trachea was cannulated and the rats were mechanically ventilated (Harvard Apparatus, Holliston, MA). The inspired gas mixture was 50% $O_2$, (N$2$ balance) to improve the tolerance to experimental stresses and prolong the viability of the preparation. Bilateral vagotomy was performed at the midcervical level. The esophagus was tied near the larynx. A catheter was inserted into the right femoral vein for fluid administration. End-tidal CO2 was monitored by a flow-through capnograph (Novametrix, Wallingford, CT) with sufficient response time (~75 ms) to measure PETCO2, in rats. The CO2-apneic threshold was defined as the PETCO2 at which respiratory rhythmic activity resumed from hypocapnic apnea.

PETCO2 was maintained at 3 mmHg above the threshold throughout the hypocapnic apnea. Artificial hyperventilation (the PETCO2 level was below the CO2-apneic threshold) was delivered by instantaneously opening an electrical valve connected in series to the cannula. The negative pressure was quickly delivered to the upper airway via a tracheal cannula to induce a pharyngeal airway collapse. Velopharyngeal pressure was measured by a pressure sensor inserted via a nostril to a position immediately rostral to the pharyngeal collapsing portion near the soft palate rim. Velopharyngeal airflow was measured by a pneumotachometer system (not shown in this figure) connected in series to the cannula.

Recording of Genioglossal Electromyogram (EMGgg) or Phrenic Nerve Activity

Respiratory rhythmic activity was monitored by measuring integrated EMGgg activity in all rats except the group with neuromuscular blockade, in which respiratory rhythm was monitored by recording integrated phrenic nerve activity. The EMGgg activity was measured by two insulated fine silver wires inserted into genioglossus muscle via the floor of the mouth. The right phrenic nerve was dissected via a ventral approach, cut distally, desheathed, and prepared for recording with a bipolar silver wire electrode. The EMGgg or phrenic nerve activity was filtered (300–10,000 Hz) and amplified (2,000–10,000×). BMA-200 AC/DC Bioamplifier CWE, Ardmore, PA). The amplified signals were full-wave rectified and integrated (Paynter Filter, BAK Electronics, Mount Airy, MD; time constant: 100 ms). The integrated signals were digitized and acquired with computer software (LabView 8.0, National Instruments) and analyzed with a customized program. This software determined the amplitude and timing of integrated EMGgg or phrenic nerve activity, from which the minute EMGgg or phrenic activity was calculated.

Assessment of Pharyngeal Airflow Mechanics in an Isolated UA

Pharyngeal mechanical variables (Pcri and Vmax) were measured in all rats with an isolated UA (Fig. 1). All rats were laid naturally in a supine position on a small flat table. Caudal velopharyngeal airflow pressure was measured by a nasally inserted pressure sensor (Millar, Houston, TX), which was positioned (at ~8 mm rostral to the soft palate rim) rostral to the most collapsible portion of the UA. A cannula was inserted into the rostral, sectioned trachea with its tip just passing through the epiglottis to the oral pharyngeal cavity, and then was carefully clamped in the position. Airflow was measured by a pneumotachometer system (Kent Scientific, Torrington, CT) connected in series to the cannula. UA collapse was induced by quickly delivering a negative pressure via the cannula. The negative pressure was delivered by instantaneously opening an electrical valve connected via plastic tubing to the negative pressure source (~100 cmH2O). Both Pcri and Vmax were measured during expiration and peak inspiration, with integrated EMGgg signal (integrated phrenic signal in the rats with neuromuscular blockade) being used to trigger the valve opening. The velopharyngeal pressure and airflow signals were amplified (PCU-2000 Pressure Control Unit; Millar Instruments, Houston, TX), digitized, and acquired with computer software (Lab-view 8.0, National Instruments). Rn (nasal resistance) upstream to the collapsing site was calculated from Pcri and Vmax values $[Rn = (0 - Pcri)/Vmax]$. The expiration-inspiration difference in Pcri ($\Delta$Pcri), $V_{max}$ ($\Delta$Vmax), or $Rn$ ($\DeltaRn$) was defined by the inspiratory value subtracting the corresponding expiratory value.

In one group of rats ($n = 6$), the phasic change in Pcri within a breathing cycle was more extensively examined. The negative pressure was delivered at three expiratory and five inspiratory time points. A custom-made computer software system (CWE, Ardmore, PA), which continuously monitored the integrated EMGgg activity, was used to trigger precisely the negative pressure delivery at target time points. The delivery during expiration was triggered at 1/6, 3/6, and 5/6 of the average expiration period via properly adjusting the delay time after detecting onset of inspiration. But the delivery during inspiration was not based on timing but triggered by designated EMGgg activity levels. Thus the valve opening was automatically triggered when the integrated EMGgg activity reached 1/3 and 2/3 of the average amplitude in ascending inspiratory phase, peak inspiration, and 2/3 and 1/3 of the average amplitude in descending inspiratory phase, respectively. This timing was achieved by properly setting the triggering threshold and/or carefully adjusting the delay time in that software system. Pcri was measured four times (separated by 30-s intervals) and averaged for each time point in all six rats.

Pcri and Vmax After Different Experimental Manipulations

To explore the underlying mechanisms, both Pcri and Vmax were measured during expiration and peak inspiration, before and after each of the following five experimental manipulations. 1) Neuromuscular blockade was achieved by systemic injection of pancuronium bromide (2.5 mg/kg iv). 2) Hypocapnic apnea was achieved by artificial hyperventilation (the PETCO2 level was below the CO2-apneic threshold). 3) Tracheal displacement was achieved by moving the cannula, which was inserted into and tied with the rostral, sectioned trachea, 2 mm caudally and horizontally. 4) The superior laryngeal nerve (SLN) denervation was achieved by bilateral transection of the SLN. Pcri and Vmax were measured ~30 min after the transection to test the possible role of the UA negative pressure reflex. 5) Death was achieved by injecting a lethal dose of urethane (3.2 g/kg ip). Pcri and Vmax were measured ~5 min after the urethane injection.
In all five groups of rats, Pcrit was measured alternatively in the middle of expiration at peak inspiration (separated by 30-s intervals) for 2–3 times, and averaged for each expiratory Pcrit (E-Pcrit) and inspiratory Pcrit (I-Pcrit). The whole procedure was conducted twice, before and after the manipulation described above.

Data Analysis

A one-way ANOVA with repeated measures, followed by the Student-Newman-Keuls post hoc tests (SigmaStat version 3.0, Jandel, San Rafael, CA), was used to analyze statistically the differences among the Pcrit values measured during the three expiratory time points or the five inspiratory time points. A paired t-test was used to analyze statistically the differences between the absolute Pcrit (also V<sub>max</sub> and ΔPcrit) values measured during expiration and peak inspiration, and before and after the manipulations. All data are presented as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Dynamic UA Responses to the Negative Pressure

The negative pressure, which was quickly delivered to the oropharyngeal cavity through the tracheal cannula, induced a dynamic change in pharyngeal airflow mechanics, including an initial, rapid increase and then a sudden decrease in UA airflow (Fig. 2), because of pharyngeal airflow collapse. The pressure sensor also recorded a rapid decrease and then a sudden increase in velopharyngeal pressure (Fig. 2) because the collapse occurred near the soft palate rim caudal to the pressure sensor. Note that the pressure nadir and airflow peak, which define Pcrit and V<sub>max</sub>, respectively, occurred at exactly the same time point, and that the magnitude of Pcrit (i.e., the absolute value of Pcrit) was much smaller than 100 cmH<sub>2</sub>O, further indicating the negative pressure-induced UA collapse. Similar traces of velopharyngeal pressure and airflow were also obtained from other rats under the same experimental condition (Fig. 3).

An Inverse Relationship between ΔPcrit and EMG<sub>ggg</sub> Activity

In the group of rats (n = 6) whose phasic changes in Pcrit were more extensively examined, the three E-Pcrit values appeared to decrease linearly during the expiration period (P = 0.017), but the only significant difference among the three E-Pcrit values was between the 1/6 and 5/6 points of the expiration period (P = 0.014), suggesting that UA is slightly less collapsible immediately before vs. after an inspiration in anesthetized, vagotomized rats (Fig. 5). In contrast, the five I-Pcrit were very different from one another, and were closely and inversely related to the integrated EMG<sub>ggg</sub> activity, with the value measured at peak inspiration being the lowest (Fig. 5). The one-way ANOVA with repeated measures revealed a significant time point effect (F<sub>4,20</sub> = 19.677, P = 0.00001), and significant differences (all P < 0.017) existed between any two of the five I-Pcrit except two pairs (i.e., the 1st vs. 4th, and the 2nd vs. 3rd Pcrit). Therefore, the ΔPcrit (also ΔV<sub>max</sub> and ΔRn) shown in the preceding paragraph was virtually the maximal expiration-inspiration difference in Pcrit, as I-Pcrit was measured at peak inspiration and Perit changes during expiration period were much smaller.

Pharyngeal Mechanical Responses after the Manipulations

Neuromuscular blockade (n = 6). After neuromuscular blockade, I-Pcrit was increased compared with baseline (P <
0.03, paired t-test; Fig. 6, Table 1). E-Pcrit also appeared to increase (Fig. 6, Table 1), but the increase was not significant (P = 0.11). ΔPcrit was eliminated (Fig. 6, Table 1). Additionally, both I- and E-Vmax became smaller (both P < 0.03), while ΔVmax was not significantly changed (Table 1). Finally, both E- and I-Rn did not change but ΔRn was decreased after neuromuscular blockade (Table 1).

Bilateral transection of the SLN (n = 8). About 30 min after the SLN transection, I-Pcrit was not significantly changed (P = 0.47), but E-Pcrit was increased (P < 0.05; Fig. 7), leading to an increased magnitude of ΔPcrit (P < 0.02; Fig. 7). On the other hand, Vmax and Rn were not significantly changed after the transection (both P > 0.42; Table 1).

Hypocapnic apnea (n = 6) or death (n = 6). There was no rhythmic respiratory activity during hypocapnic apnea or after death, so only E-Pcrit was measured after these two manipulations. During hypocapnic apnea, E-Pcrit was not significantly changed (P = 0.16; Fig. 8). About 5 min after death, E-Pcrit was also not significantly changed (P = 0.35; Fig. 8). In addition, E-Vmax and E-Rn were also not significantly changed during hypocapnic apnea or after death (both P > 0.35; Table 1).

### Table 1. Pharyngeal mechanical variables (Pcrit, Vmax, and Rn) after each of the 5 manipulations

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
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<th>After</th>
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<tr>
<td></td>
<td>E</td>
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<tr>
<td>NB (n = 6)</td>
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<tr>
<td>Pcrit</td>
<td>-15.0 ± 2.9</td>
<td>-17.9 ± 3.4</td>
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<td>-11.9 ± 1.6</td>
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<td>Vmax</td>
<td>33.3 ± 1.5</td>
<td>34.5 ± 1.7</td>
<td>1.2 ± 0.2</td>
<td>29.6 ± 2.5*</td>
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<td>Rn</td>
<td>0.47 ± 0.1</td>
<td>0.54 ± 0.1</td>
<td>0.08 ± 0.02</td>
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<td>Apnea (n = 6)</td>
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<tr>
<td>Pcrit</td>
<td>-14.0 ± 0.9</td>
<td>-18.8 ± 1.8</td>
<td>-4.8 ± 1.3</td>
<td>-13.1 ± 0.7</td>
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<td>Vmax</td>
<td>30.6 ± 1.6</td>
<td>33.7 ± 1.6</td>
<td>3.1 ± 0.5</td>
<td>29.5 ± 1.8</td>
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<td>Rn</td>
<td>0.66 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.2 ± 0.05</td>
<td>0.62 ± 0.1</td>
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<td>SLN cut (n = 8)</td>
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<tr>
<td>Pcrit</td>
<td>-16.6 ± 1.9</td>
<td>-21.5 ± 2.2</td>
<td>-4.9 ± 0.5</td>
<td>-12.5 ± 1.0*</td>
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<tr>
<td>Vmax</td>
<td>28.8 ± 3.0</td>
<td>31.5 ± 3.4</td>
<td>2.7 ± 0.6</td>
<td>27.9 ± 3.0</td>
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<td>Rn</td>
<td>0.52 ± 0.1</td>
<td>0.63 ± 0.1</td>
<td>0.1 ± 0.01</td>
<td>0.5 ± 0.1</td>
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<td>Trachea move (n = 6)</td>
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<tr>
<td>Pcrit</td>
<td>-16.0 ± 1.3</td>
<td>-20.3 ± 1.3</td>
<td>-4.8 ± 0.6</td>
<td>-18.5 ± 1.9*</td>
</tr>
<tr>
<td>Vmax</td>
<td>27.2 ± 3.2</td>
<td>29.8 ± 3.5</td>
<td>2.7 ± 0.6</td>
<td>28.2 ± 3.3</td>
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<tr>
<td>Rn</td>
<td>0.64 ± 0.1</td>
<td>0.74 ± 0.03</td>
<td>0.1 ± 0.01</td>
<td>0.7 ± 0.07</td>
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<tr>
<td>Death (n = 6)</td>
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<tr>
<td>Pcrit</td>
<td>-14.0 ± 1.6</td>
<td>-19.3 ± 2.4</td>
<td>-5.3 ± 1.8</td>
<td>-13.8 ± 1.1</td>
</tr>
<tr>
<td>Vmax</td>
<td>26.8 ± 2.3</td>
<td>33.8 ± 1.4</td>
<td>5.0 ± 2.2</td>
<td>26.5 ± 2.7</td>
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<tr>
<td>Rn</td>
<td>0.6 ± 0.1</td>
<td>0.57 ± 0.1</td>
<td>0.07 ± 0.04</td>
<td>0.6 ± 0.1</td>
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Data are expressed as means ± SE. Pcrit, pharyngeal critical pressure (cmH2O); Vmax, upper airway maximal airflow (ml/s); Rn, nasal resistance (cmH2O/ml/s); NB, neuromuscular blockade with pancuronium; SLN cut, transection of the superior laryngeal nerve; trachea move, 2-mm trachea caudal displacement; E, expiration; I, inspiration; Δ, E-I difference in Pcrit, Vmax, or Rn. *Significant difference from the corresponding baseline value (P < 0.05).
Trachea caudal displacement (n = 6). After the 2-mm trachea displacement, both E- and I-Pcrit were decreased (both \( P < 0.02 \)), while \( \Delta \)Pcrit was not significantly changed (\( P = 0.89 \), Fig. 9). \( V_{\text{max}} \) and Rn were also not significantly changed after the tracheal displacement (both \( P > 0.1 \); Table 1).

**DISCUSSION**

The present study demonstrated that Pcrit was closely and inversely related to phasic EMGgg activity within a breathing cycle in anesthetized rats, with the one measured at peak inspiration being the lowest. \( V_{\text{max}} \), Rn, and latency to UA closure were all increased during peak inspiration vs. expiration. The maximal \( \Delta \)Pcrit was not reduced after the superior laryngeal nerve transection or changed by trachea caudal displacement (despite that both E- and I-Pcrit became more negative), but was completely eliminated by neuromuscular blockade, or after hypocapnic apnea or death (but E-Pcrit was not significantly altered). These results suggest that pharyngeal collapsibility was moment-by-moment modulated by respiratory drive via neuromuscular mechanisms, but the UA negative pressure reflex and respiration-induced tracheal displacement are not crucial for this phasic modulation.

Methodology Consideration

The method, which measures Pcrit by delivering negative pressure to induce collapse in an isolated UA, has been used for many years and has been successfully applied to rats, cats, dogs, and mice (8, 12, 17, 20, 30, 31). Our method is a modified one with three new features. 1) We induced collapse more quickly with mean onset latency being \(~60\) ms (300–600 ms in almost all other studies). This speed is actually not too unphysiological, since the duration of the rising phase of inspiratory output is 60–80 ms in most awake rats. 2) Pcrit was measured during expiration and peak inspiration (at more time points in one group), while respiratory phase is usually not discriminated by others except in a few special cases. 3) We induced collapse by delivering a relatively more negative pressure (\(~100\) cmH\(_2\)O) over a shorter period (150 ms). This negative pressure is clearly supraphysiological and might cause changes in Pcrit. However, the reproducibility we achieved suggests that the UA functioned well through the experiments, which might be partially attributed to our shorter exposures to the negative pressure. For example, Pcrit was measured 4 times in each point in all six rats in Fig. 5, and the mean coefficient of variation is 4.4% for three E-Pcrit and 3.6% for five I-Pcrit values. The between-rat variations, reflected in our standard error bars (Fig. 5), are also reasonable. Moreover, studies for other purposes in our lab also demonstrated that Pcrit could be measured repeatedly in experiments lasting \(~2\) h, showing...
only limited variation, suggesting that damage to UA, if any, is minimal in terms of its effect on $P_{crit}$.

It is well known that UA collapses at sites near the soft palate rim, i.e., at caudal velopharynx and/or oropharynx close to the soft palate. Because we used a modified method, we were worried about the possible change it might cause and thus did our own “mapping” throughout the pharyngeal passage in several rats. In these pilot experiments, we first inserted the pressure sensor to a position near the epiglottis and then pulled it bit by bit toward nostrils while measuring $P_{crit}$ at each position. Major findings include: 1) the sensor could detect $-100$ cmH$_2$O when sensor was near the epiglottis (i.e., caudal to the collapsing site); 2) UA did collapse at caudal velopharynx or oropharynx near the soft palate rim (actually most rats collapsed at both sites); and 3) $P_{crit}$ magnitude tended to decrease ($V_{max}$ kept the same) but overall $P_{crit}$ changes were very limited when the sensor was moved gradually from one position ($\sim 2$ mm rostral to the collapsing site) to the other ($\sim 12$ mm rostral to the collapsing site), provided that the sensor insertion did not cause bleeding inside that segment. Thus the bulk of the Rn came from more rostral segments of the passage. We also did anatomic examination at the end of each experiment. If the sensor or cannula was not in the proper position, the data collected would not be included. Compared with those in human studies, the method with induced collapse is more direct and does not need to extrapolate $P_{crit}$ from a pressure-flow relationship. $P_{crit}$ is the critical pressure below which UA collapses. Thus no matter how negative the negative pressure is, the nasal pressure sensor (theoretically) cannot record any value more negative than $P_{crit}$, because UA must have collapsed and the sensor is positioned rostral to the collapsing portion (it can record $-100$ cmH$_2$O if positioned caudal to the collapsing portion).
the lowest value recorded by the sensor (immediately before collapse) is Pcrit (Fig. 2). The UA collapse is also reflected by an abrupt drop in a rapidly rising airflow (note: V\text{max} always matches Pcrit in time; Fig. 2).

There has been no perfect way to measure Pcrit, as all known methods change Pcrit in one way or another. For example, CPAP suppresses UA muscle activity, thus increasing physiological Pcrit. Our method, which measures Pcrit momentarily, has minimal confounding effects from CPAP and/or the UA negative pressure reflex. However, it is not perfect either. Theoretically, Pcrit value should be independent of applied negative pressures as long as they are more negative than Pcrit, but we noticed that Pcrit obtained was slightly decreased when a more negative pressure was applied, due likely to the fact that UA collapse is not instantaneous. When we measured the variables after closing both nostrils with two fingers, the overall airflow (also V\text{max}) was substantially decreased if the nostrils were loosely closed and almost stopped if tightly closed, suggesting that the airflow is indeed coming from nostrils via velopharynx.

We were aware of the effect of mouth leak on pharyngeal airflow mechanics, but we did not seal the rats’ mouth in these experiments, as in our experimental setting, the effect of mouth leak on Pcrit and V\text{max} appeared to be minimal. In several pilot experiments, as in our experimental setting, the effect of mouth airflow mechanics, but we did not seal the rats’ mouth in these experiments, we found that Pcrit and V\text{max} values were not significantly changed after mouth sealing. We did find that mouth sealing reduced the airflow but this flow reduction only occurred after UA had collapsed (i.e., the ascending limb and the peak of the airflow traces remained almost the same after mouth sealing, but the descending limb was indeed greatly changed). We believe that two factors contribute to this phenomenon. 1) Rats are nose-breathing animals. Mouth leak is usually negligible as long as velopharyngeal flow via nose is not severely interrupted. 2) Our latency to UA closure (∼60 ms) is much shorter than others (300–600 ms). Thus before mouth passage starts to leak, the UA probably has already collapsed (the collapse did not prevent or stop but facilitated mouth leak). All events that we are interested in happened within 100 ms after the negative pressure initiation. The UA would collapse in ∼60 ms and stay collapsed as long as the negative pressure is applied. After pharyngeal collapse, the velopharyngeal pressure and airflow did not quickly return to zero in most rats (Fig. 2), indicating that the velopharyngeal airway was not completely sealed. So far we have observed complete closure in only a few rats, in which the velopharyngeal pressure did return to zero rapidly after the pharyngeal collapse. Thus we used a reproducible metric of pharyngeal airflow mechanics, recognizing that no one metric is ideal for this assessment.

All experiments were conducted in anesthetized, ventilated, vagotomized, and unparalyzed rats (except one group), in which PET\text{CO}_2 and body temperature could be controlled. On the other hand, these animals would likely “fight the ventilator” and the unpredictable afferent feedbacks could cause some unknown influences on the respiratory control system, which would complicate the experiments. In the beginning, we tried to synchronize the pump rate with respiratory rhythm and tried very hard to keep 1:1 (or 1:2) rhythmic ratio, but we later learned that it was impossible to keep an exact 1:1 (or 1:2) ratio since rats’ respiratory rhythm always fluctuated slightly during the experiments. More importantly, we noticed that respiratory entrainment to mechanical ventilation somewhat remained even after vagotomy, especially when the rhythmic ratio was very close to 1:1 (or 1:2), thereby causing periodic, irregular respiratory rhythms and unstable EMG\text{gg} activities. We thus intentionally set the ratio for the rat’s breathing rhythm and the pump rate to approximately 2:3 (e.g., if rat’s respiratory rhythm was 40 bursts/min, pump rate would be set at ∼60 cycles/min) by carefully adjusting the pump’s rate and/or tidal volume. This approach turned out to be a successful model because 1) rats had a very stable respiratory rhythm and EMG\text{gg} signals, 2) the capnograph could show the same digital PET\text{CO}_2 value (e.g., 44 mmHg) for many minutes without any fluctuations, and 3) whenever we measured blood gases (in our other studies using this model), P\text{ACO}_2 values were always very close to PET\text{CO}_2.

In some pilot experiments, we noticed that respiratory activity and rhythm were not very stable in vagi-intact, unparalyzed, and ventilated rats. In addition, relatively quicker rhythm and shorter inspiratory time in those rats would also somewhat increase the difficulty of precisely triggering negative pressure delivery at target respiratory time points. Therefore, vagotomy was performed in all rats (except one; see below) for more stable recordings. However, It is unequivocal that vagotomy is not necessary for the existence of ∆Pcrit. In one vagi-intact rat, E- and I-Pcrit were −13.2 and −17.9 cmH\text{O}, respectively. In our other studies, we also found similar ∆Pcrit in several vagi-intact rats. These data were not included in this study because these rats were anesthetized differently (about half of the urethane dose was replaced by other anesthetics) and thus might obscure/complicate our message.

Respiratory Phasic Pharyngeal Collapsibility

Although the concept of phasic Pcrit is straightforward, it is challenging to illustrate. Most previous studies have not shown ∆Pcrit, as latency to UA closure in their methods (300–600 ms) was longer than peak inspiration (∼200 ms) in the animal studies and Pcrit has been primarily measured at peak inspiration in the human studies. However, respiratory-modulated UA collapsibility is not a new concept, as some pioneers in the field have already proposed and demonstrated it. The ∆Pcrit (−3 cmH\text{O}) was reported in anesthetized dogs in the presence of 11.7% CO\text{2} (30). ∆Pcrit (−1 cmH\text{O}) was also reported in one group of tracheostomized OSA patients when a reverse airflow regimen was used (24). In addition, Pcrit was reported to be −8.2 cmH\text{O} lower during asphyxia (vs. apnea) when EMG\text{gg} activity was greatly increased (12). About 6.4 cmH\text{O} ∆Pcrit was also reported between closure and reopening phases of the respiratory cycle (10). In the present study, we modified the method and thus could measure Pcrit directly at several different time points even within the inspiratory phase. To our knowledge, the present study is the first to show that Pcrit changes continuously within a breathing cycle, and is almost precisely in opposite phase of the central respiratory drive under normocapnic conditions.

Figure 5 shows a close and inverse relationship between ∆Pcrit and EMG\text{gg} activity. However, the ∆Pcrit distribution was unexpectedly asymmetrical in the inspiratory phase, i.e., although triggered at the same 1/3 and 2/3 of the amplitude of integrated EMG\text{gg} activity, Pcrit measured during the ascend-
ing phase was more negative than those of descending phases. We believe that the main reason is because we triggered the negative pressure delivery not the collapse itself (i.e., the induced UA collapse was not instantaneous; the latency to collapse is \( \sim 60 \) ms). We also noticed that there was a slight time delay in the integrated vs. raw EMGgg activity (Fig. 5), which might also somehow contribute to this asymmetrical distribution. The EMGgg activity data were collected (and averaged) from one rat and exhibited a fairly good symmetry in the inspiratory phase. However, we have also observed rather asymmetrical EMGgg activity distributions (always skewed to the right) in many rats.

Mechanisms of Phasic Pharyngeal Collapsibility

To explore the mechanisms underlying the phasic respiratory modulation of pharyngeal collapsibility, we examined the effects of several manipulations on \( P_{\text{crit}} \) and \( \Delta P_{\text{crit}} \). After neuromuscular blockade, \( \Delta P_{\text{crit}} \) was completely eliminated while the central respiratory drive remained intact as shown in rhythmic phrenic nerve activity, suggesting that the phasic modulation of pharyngeal collapsibility depends entirely on neuromuscular mechanisms. \( \Delta P_{\text{crit}} \) also disappeared when there was no respiratory drive during hypocapnic apnea or after death. E-P\(_{\text{crit}}\) appeared to be increased but did not reach a significant level after neuromuscular blockade, hypocapnic apnea, or death, suggesting that in anesthetized rats, basal tonic contribution of UA muscles to UA collapsibility is limited, and a pharyngeal airway without respiratory activity is similar to a dead one in terms of its collapsibility. After trachea caudal displacement (2 mm), both E-P\(_{\text{crit}}\) and I-P\(_{\text{crit}}\) were significantly decreased, whereas \( \Delta P_{\text{crit}} \) was not significantly changed, suggesting that the stiffness caused by the trachea displacement affects UA collapsibility similarly throughout the breathing cycle. It also suggests that trachea displacement induced by phasic lung inflation in respiration is unlikely to be the main reason for the phasic pharyngeal collapsibility. After the SLN denervation, I-P\(_{\text{crit}}\) was not significantly changed, while E-P\(_{\text{crit}}\) was increased, which may reflect the delicate balance of SLN tonic influences on both dilators and constrictors (23). The magnitude of \( \Delta P_{\text{crit}} \) was even increased, thus suggesting that the phasic pharyngeal collapsibility does not depend on the upper airway negative pressure reflex, which is primarily mediated via the SLN in rats (22).

The experimental setting itself can also help rule out several other factors as major contributors to this phasic pharyngeal collapsibility, such as respiratory phasic changes in UA negative pressure (almost not existing in an isolated UA), surface tension (no spontaneous UA closure in rats), and tracheal traction by phasic lung inflation (the trachea cannula was clamped and rats were vagotomized). These factors, however, do exist more or less in intact animals or humans. Thus the magnitude of \( \Delta P_{\text{crit}} \) in the present study may be somewhat underestimated. On the other hand, vagotomy might slightly increase the magnitude of \( \Delta P_{\text{crit}} \), yielding an overestimate, as it increases phasic activity of UA dilator muscles (35).

We also constructed a nonbiological setting with the same pressure sensor and pneumotachograph but using a handmade collapsible tube [made of thin waxy film (parafilm)] instead of a real isolated UA. With a microscope, we directly observed collapses of that tube (but not totally sealed), when the same negative pressure was delivered. The pseudo-P\(_{\text{crit}}\) and \( V_{\text{max}} \) looked similar to those observed in real UA (Fig. 3). They also responded to the “trachea displacement,” e.g., “P\(_{\text{crit}}\)” became more negative and “\( V_{\text{max}} \)” became larger when the tube was prolonged 2 mm horizontally, and returned to their baseline values when the tube returned to its original length (data not shown). This setting does not have any influences of muscle spindles or tendon organs, let alone the stretch reflex, suggesting that the phasic change in P\(_{\text{crit}}\) can largely be explained by simple physical changes (e.g., pharyngeal size and/or stiffness changes).

Physiological Significance

These data have directly demonstrated that pharyngeal collapsibility is not static but changes dynamically throughout a breathing cycle under normocapnic conditions and is lowest at peak inspiration. Pharyngeal collapsibility does not change much during expiration (even after death or during hypocapnic apnea) and its dynamic changes occur mainly during inspiration. Therefore, E-P\(_{\text{crit}}\) approximately describes a passive UA’s collapsibility, and the maximal \( \Delta P_{\text{crit}} \) can be used as an index for assessing the influence of UA neuromuscular activity over the control of UA patency. Finally, the phasic respiratory modulation of UA collapsibility can be viewed as an efficient (energy saving) mechanism that resists suction collapse of the UA during inspiration.

GRANTS

This work was supported by National Institutes of Health (NIH) Grant HL-64912. A. Malhotra reports funding from NIH R01-HL-085188, American Heart Association (AHA)-0840159N, NIH R01-HL090897, NIH K24-HL-093218, and NIH P01-HL-095491.

DISCLOSURES

A. Malhotra reports research and/or consulting income from Philips, Pfizer, Merck, SFG, SGS, Apnex, Apnicure, Ethicon, Medtronic, Cephalon, and Sepracor.

AUTHOR CONTRIBUTIONS


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