Phasic respiratory modulation of pharyngeal collapsibility via neuromuscular mechanisms in rats

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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 phasic genioglossal activity, with the value measured at peak inspiration being the lowest. In other groups, the variables were measured during expiration and peak inspiration, before 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% O2 (∆N2 balance) to improve the emphysema condition. PETCO2 was monitored in the expired line of the ventilator circuit using 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.

The esophagus was tied near the larynx. A catheter was inserted into the right femoral vein for fluid administration. End-tidal CO2 level. The esophagus was tied near the larynx. A catheter was inserted 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, from which the minute EMGgg or phrenic nerve 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 descending inspiration and expiration, respectively. This timing was achieved by properly setting the delay 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. Pcrit was measured four times (separated by 30-s intervals) and averaged for each time point in all six rats.

Pcrit and Vmax After Different Experimental Manipulations

To explore the underlying mechanisms, both Pcrit 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. Pcrit 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). Pcrit and Vmax were measured ~5 min after the urethane injection.

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.
PHASIC PHARYNGEAL COLLAPSE REQUIRES NEUROMUSCULAR MECHANISMS

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 Perit 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 Perit (also Vmax and ΔPerit) 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 airway 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 Perit and Vmax, respectively, occurred at exactly the same time point, and that the magnitude of Perit (i.e., the absolute value of Perit) was much smaller than 100 cmH2O, 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).

In all five groups of rats, Perit was measured alternatively in the middle of expiration and at peak inspiration (separated by 30-s intervals) for 2–3 times, and averaged for each inspiratory Perit (E-Perit) and inspiratory Perit (I-Perit). The whole procedure was conducted twice, before and after the manipulation described above.

The induced changes in pharyngeal airflow mechanics were numerically different when the negative pressure was delivered at different phases, e.g., expiration and peak inspiration. Perit was more negative (i.e., Perit value was decreased or UA became less collapsible), Vmax was larger, and Rn (upstream to the collapsing site) was bigger during peak inspiration vs. expiration in each rat. Mean Perit was 26% more negative (−15.0 ± 1.0 vs. −18.9 ± 1.2 cmH2O; n = 23; Fig. 4), Vmax was 7% larger (31.0 ± 1.0 vs. 33.2 ± 1.1 ml/s), Rn was 12% bigger [0.49 ± 0.05 vs. 0.59 ± 0.05 cmH2O/ml/s] (Table 1), and the onset latency to UA closure, which was defined by the time from the point when velopharyngeal pressure started to decrease to the point when the pressure reached its nadir (Fig. 2), was 14% longer (55 ± 4 vs. 63 ± 5 ms) during peak inspiration vs. expiration (all P < 0.005). Thus the mean ΔPerit, ΔVmax, and ΔRn was −3.9 ± 0.3 cmH2O, 2.2 ± 0.4 ml/s, and 0.1 ± 0.02 cmH2O/ml/s, respectively. Mean PETCO2 level was ~45 mmHg (ranged from 42 to 49 mmHg).

An Inverse Relationship between ΔPerit and EMGgg Activity

In the group of rats (n = 6) whose phasic changes in Perit were more extensively examined, the three E-Perit values appeared to decrease linearly during the expiration period (P = 0.017), but the only significant difference among the three E-Perit 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-Perit were very different from one another, and were closely and inversely related to the integrated EMGgg 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 (F4,20 = 19.677, P = 0.000001), and significant differences (all P < 0.017) existed between any two of the five I-Perit except two pairs (i.e., the 1st vs. 4th, and the 2nd vs. 3rd Perit). Therefore, the ΔPerit (also ΔVmax and ΔRn) shown in the preceding paragraph was virtually the maximal expiration-inspiration difference in Perit, as I-Perit 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-Perit 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 ($P = 0.11$). $\Delta$Pcrit was eliminated (Fig. 6, Table 1). Additionally, both I- and E-$V_{\text{max}}$ became smaller (both $P < 0.03$), while $\Delta V_{\text{max}}$ was not significantly changed (Table 1). Finally, both E- and I-Rn did not change but $\Delta$Rn 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 $\Delta$Pcrit ($P < 0.02$; Fig. 7). On the other hand, $V_{\text{max}}$ 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-$V_{\text{max}}$ 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, $V_{\text{max}}$, 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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<tbody>
<tr>
<td></td>
<td>E</td>
<td>I</td>
<td>$\Delta$</td>
<td>E</td>
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<tr>
<td>NB (n = 6)</td>
<td></td>
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<tr>
<td>Pcrit</td>
<td>$-15.0 \pm 2.9$</td>
<td>$-17.9 \pm 3.4$</td>
<td>$-3.0 \pm 0.7$</td>
<td>$-11.9 \pm 1.6$</td>
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<tr>
<td>$V_{\text{max}}$</td>
<td>$33.3 \pm 1.5$</td>
<td>$34.5 \pm 1.7$</td>
<td>$1.2 \pm 0.2$</td>
<td>$29.6 \pm 2.5^*$</td>
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<tr>
<td>Rn</td>
<td>$0.47 \pm 0.1$</td>
<td>$0.54 \pm 0.1$</td>
<td>$0.08 \pm 0.02$</td>
<td>$0.42 \pm 0.07$</td>
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<tr>
<td>Apnea (n = 6)</td>
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<tr>
<td>Pcrit</td>
<td>$-14.0 \pm 0.9$</td>
<td>$-18.8 \pm 1.8$</td>
<td>$-4.8 \pm 1.3$</td>
<td>$-13.1 \pm 0.7$</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>$30.6 \pm 1.6$</td>
<td>$33.7 \pm 1.6$</td>
<td>$3.1 \pm 0.5$</td>
<td>$29.5 \pm 1.8$</td>
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<tr>
<td>Rn</td>
<td>$0.66 \pm 0.1$</td>
<td>$0.8 \pm 0.1$</td>
<td>$0.2 \pm 0.05$</td>
<td>$0.62 \pm 0.1$</td>
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<td>SLN cut (n = 8)</td>
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<tr>
<td>Pcrit</td>
<td>$-16.6 \pm 1.9$</td>
<td>$-21.5 \pm 2.2$</td>
<td>$-4.9 \pm 0.5$</td>
<td>$-12.5 \pm 1.0^*$</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>$28.8 \pm 3.0$</td>
<td>$31.5 \pm 3.4$</td>
<td>$2.7 \pm 0.6$</td>
<td>$27.9 \pm 3.0$</td>
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<tr>
<td>Rn</td>
<td>$0.52 \pm 0.1$</td>
<td>$0.63 \pm 0.1$</td>
<td>$0.1 \pm 0.01$</td>
<td>$0.5 \pm 0.1$</td>
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<td>Trachea move (n = 6)</td>
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<tr>
<td>Pcrit</td>
<td>$-16.0 \pm 1.3$</td>
<td>$-20.3 \pm 1.3$</td>
<td>$-4.8 \pm 0.6$</td>
<td>$-18.5 \pm 1.9^*$</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>$27.2 \pm 3.2$</td>
<td>$29.8 \pm 3.5$</td>
<td>$2.7 \pm 0.6$</td>
<td>$28.2 \pm 3.3$</td>
</tr>
<tr>
<td>Rn</td>
<td>$0.64 \pm 0.1$</td>
<td>$0.74 \pm 0.03$</td>
<td>$0.1 \pm 0.01$</td>
<td>$0.7 \pm 0.07$</td>
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<tr>
<td>Death (n = 6)</td>
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<tr>
<td>Pcrit</td>
<td>$-14.0 \pm 1.6$</td>
<td>$-19.3 \pm 2.4$</td>
<td>$-5.3 \pm 1.8$</td>
<td>$-13.8 \pm 1.1$</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>$26.8 \pm 2.3$</td>
<td>$33.8 \pm 1.4$</td>
<td>$5.0 \pm 2.2$</td>
<td>$26.5 \pm 2.7$</td>
</tr>
<tr>
<td>Rn</td>
<td>$0.6 \pm 0.1$</td>
<td>$0.57 \pm 0.1$</td>
<td>$0.07 \pm 0.04$</td>
<td>$0.6 \pm 0.1$</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. Pcrit, pharyngeal critical pressure (cmH$_2$O); $V_{\text{max}}$, upper airway maximal airflow (ml/s); Rn, nasal resistance (cmH$_2$O/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; $\Delta$, E-I difference in Pcrit, $V_{\text{max}}$, 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 \(\Delta\text{Pcrit}\) was not significantly changed \((P = 0.89, \text{Fig. 9})\). \(V_{\text{max}}\) and \(R_n\) 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. \(V_{\text{max}}\), \(R_n\), and latency to UA closure were all increased during peak inspiration vs. expiration. The maximal \(\Delta\text{Pcrit}\) 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 \(~60\, \text{ms} \) \((300–600\, \text{ms} \) in almost all other studies). This speed is actually not too unphysiological, since the duration of the rising phase of inspiratory output is \(60–80\, \text{ms} \) 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 \((-100\, \text{cmH}_2\text{O})\) over a shorter period \((150\, \text{ms})\). 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\, \text{h})

**Fig. 5.** Inverse relationship between \(\Delta\text{Pcrit}\) and integrated genioglossal electromyogram (EMGgg) activity. **Top:** integrated (curve line) and raw (vertical lines) EMGgg activity in arbitrary units, both of which are the average of the same 60 phase-aligned breathing cycles recorded continuously from one rat. The 5 black circles on the curve denote 1/3, 2/3, and 3/3 of the integrated EMGgg activity amplitude, respectively. **Bottom:** mean \(\Delta\text{Pcrit}\) measured at 3 expiratory time points and 5 inspiratory time points in one group of rats \((n = 6)\). Each E-\(\Delta\text{Pcrit}\) was calculated by subtracting the grand mean of all E-Pcrit from the E-Pcrit at that specific time point. Each I-\(\Delta\text{Pcrit}\) was calculated by subtracting the E-Pcrit from I-Pcrit at that specific time point. The dotted curve line is a combination of a parabolic regression curve (calculated from the 5 mean I-\(\Delta\text{Pcrit}\) values, whose x-axis values were taken from the 5 black circles on the solid curve line) and a linear regression line (calculated from the 3 mean E-\(\Delta\text{Pcrit}\) values using their own x-axis values), which has been split into two parts. Data are expressed as means \(\pm\) SE.

**Fig. 6.** Effect of neuromuscular blockade (NB) on Pcrit and \(\Delta\text{Pcrit}\). **Top:** both E- and I-Pcrit was measured before (as baseline) and after the NB with systemic injection of pancuronium bromide \((2.5\, \text{mg/kg iv})\). **Bottom:** \(\Delta\text{Pcrit}\) was calculated by I-Pcrit subtracting the corresponding E-Pcrit. Data are expressed as means \(\pm\) SE. *Significant difference between E- and I-Pcrit. #Significant difference from the corresponding baseline value \((P < 0.05)\).
only limited variation, suggesting that damage to UA, if any, is
minimal in terms of its effect on Pcrit.

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 Pcrit at each
position. Major findings include:

1) the sensor could detect −100 cmH2O 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) Pcrit magnitude tended to decrease (Vmax kept the same) but overall Pcrit changes were
very limited when the sensor was moved gradually from one
position (−2 mm rostral to the collapsing site) to the other
(−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 extrapo-
late Pcrit from a pressure-flow relationship. Pcrit 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
Pcrit, because UA must have collapsed and the sensor is
positioned rostral to the collapsing portion (it can record −100
cmH2O if positioned caudal to the collapsing portion). Thus

![Fig. 7. Effect of bilateral transection of the superior laryngeal nerve (SLN cut) on Pcrit and ΔPcrit. Top: both E- and I-Pcrit were measured before (as baseline) and ~30 min after the SLN cut. Bottom: ΔPcrit was calculated by I-Pcrit subtracting the corresponding E-Pcrit. Data are expressed as means ± SE. *Significant difference between E- and I-Pcrit. #Significant difference from the corresponding baseline value (P < 0.05).]

![Fig. 8. Effect of hypocapnic apnea or death on expiratory Pcrit. There was no rhythmic respiratory activity (thus no I-Pcrit) during hypocapnic apnea or after death. E-Pcrit was measured before (as baseline) and after hypocapnic apnea or death. Data are expressed as means ± SE. There was no significant difference in E-Pcrit before and after either experimental manipulation (both P > 0.16).]

![Fig. 9. Effect of 2-mm trachea caudal displacement (trachea move) on Pcrit and ΔPcrit. Top: both E- and I-Pcrit were measured before (as baseline) and after the trachea move. Bottom: ΔPcrit was calculated by I-Pcrit subtracting the corresponding E-Pcrit. Data are expressed as means ± SE. *Significant difference between E- and I-Pcrit. #Significant difference from the corresponding baseline value (P < 0.05).]
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: Vmax 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 Vmax) 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 Vmax appeared to be minimal. In several pilot experiments, as in our experimental setting, mouth from nostrils via velopharynx.

creased if the nostrils were loosely closed and almost stopped the experiments. More importantly, we noticed that respiratory since rats’ respiratory rhythm always fluctuated slightly during the experiments. Learn that it was impossible to keep an exact 1:1 (or 1:2), thereby causing periodic, irregular respiratory rhythms and unstable EMGgg 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 EMGgg signals, 2) the capnograph could show the same digital PETCO2 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), PdCO2 values were always very close to PETCO2 ones.

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 ΔPcrit. In one vagi-intact rat, E- and I-Pcrit were −13.2 and −17.9 cmH2O, respectively. In our other studies, we also found similar ΔPcrit 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 ΔPcrit, 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 ΔPcrit (~3 cmH2O) was reported in anesthetized dogs in the presence of 11.7% CO2 (30). ΔPcrit (~1 cmH2O) 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 cmH2O lower during asphyxia (vs. apnea) when EMGgg activity was greatly increased (12). About 6.4 cmH2O ΔPcrit 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 ΔPcrit and EMGgg activity. However, the ΔPcrit 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 EMGgg 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 ~60 ms). We also noticed that there was a slight time delay in the integrated vs. raw EMGg activity (Fig. 5), which might also somehow contribute to this asymmetrical distribution. The EMGg 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 EMGg 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 Pcrit and ΔPcrit. After neuromuscular blockade, ΔPcrit 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. ΔPcrit also disappeared when there was no respiratory drive during hypocapnic apnea or after death. E-Pcrit 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-Pcrit and I-Pcrit were significantly decreased, whereas ΔPcrit 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-Pcrit was not significantly changed, while E-Pcrit was increased, which may reflect the delicate balance of SLN tonic influences on both dilators and constrictors (23). The magnitude of ΔPcrit 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 ΔPcrit in the present study may be somewhat underestimated. On the other hand, vagotomy might slightly increase the magnitude of ΔPcrit, 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-Pcrit and Vmax looked similar to those observed in real UA (Fig. 3). They also responded to the “trachea displacement,” e.g., “Pcrit” became more negative and “Vmax” 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 Pcrit 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-Pcrit approximately describes a passive UA’s collapsibility, and the maximal ΔPcrit 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.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Y.C., M.M., and L.L. conception and design of research; Y.C., M.M., and C.L. performed experiments; Y.C. and C.L. analyzed data; Y.C. interpreted results of experiments; Y.C. prepared figures; Y.C. drafted manuscript; Y.C., A.M., and L.L. edited and revised manuscript; Y.C. and L.L. approved final version of manuscript.

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