Functional Genomics of Sleep and Circadian Rhythm
Selected Contribution: Influence of genioglossus tonic activity on upper airway dynamics assessed by phrenic nerve stimulation

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Sériès, F., and I. Marc. Selected Contribution: Influence of genioglossus tonic activity on upper airway dynamics assessed by phrenic nerve stimulation. J Appl Physiol 92: 418–423, 2002.—Upper airway (UA) dynamics can be evaluated during wakefulness by using electrical phrenic nerve stimulation (EPNS) applied at end-expiration during exclusive nasal breathing by dissociating twitch flow and phasic activation of UA muscles. This technique can be used to quantify the influence of nonphasic electromyographic (EMG) activity on UA dynamics. UA dynamics was characterized by using EPNS when increasing tonic EMG activity with CO₂ stimulation in six normal awake subjects. Instantaneous flow, esophageal and nasopharyngeal pressures, and genioglossal EMG activity were recorded during EPNS at baseline and during CO₂ ventilatory stimulation. The proportion of twitches presenting an inspiratory-flow limitation pattern decreased from 100% at baseline to 78.7 ± 21.4% (P < 10⁻⁴) during CO₂ rebreathing. During CO₂ stimuli, maximal inspiratory twitch flow (V₁max) of flow-limited twitches significantly rose, with the driving pressure at which flow limitation occurred being more negative. For the group as a whole, the increase in V₁max and the decrease in pressure were significantly correlated with the rise in end-expiratory EMG activity. UA stability assessed by EPNS is dramatically modified during CO₂ ventilatory stimulation. Changes in tonic genioglossus EMG activity significantly contribute to the improvement in UA stability.

electromyographic tonic activity; diaphragm twitch; electrical phrenic nerve stimulation

UPPER AIRWAY (UA) STRUCTURES are largely involved in determining ventilatory characteristics and breathing stability. Whereas nasal and laryngeal levels are supported by rigid cartilaginous or bony structures, pharyngeal airway patency relies mainly on the adapted contraction of UA dilator muscles. The force developed by these muscles is of first importance in UA physiology because it counterbalances the collapsing forces of the transpharyngeal negative-pressure gradient and tissue weight. An imbalance between these collapsing forces and output of UA dilator muscle contraction will result in partial or complete UA closure (15).

Tonic and phasic activities of UA dilator muscles contribute to determine UA patency, as illustrated by the dramatic influence of the sleep-induced decrease in these respective electromyographic (EMG) activities on end-expiratory and inspiratory UA caliber (6). Furthermore, the influence of UA muscle activity on UA stability on the improvement of sleep-related obstructive breathing disorders is clearly illustrated by the reduction in UA collapsibility and/or resistance and by the relief of obstructive events associated with the pharmacologically (11, 13, 14, 28), electrically (8, 20), or metabolically induced (10, 12) increase in UA activity. However, even if these procedures may influence tonic and/or phasic UA activities (27, 29), the literature has mainly focused on the mechanical effects of modulating phasic UA EMG activity. This may be accounted for by the fact that it is difficult to independently manipulate tonic and phasic EMG activities.

Physiologically, UA dilator muscles are activated before inspiratory muscles, with peak EMG activity being reached before that of the diaphragm (25). This preactivation pattern stabilizes UA structures during inspiratory flow and decreases work of breathing by decreasing UA resistance. A sleep-related loss of coordination between UA and inspiratory muscle activities...
may play an important role in the pathophysiology of obstructive sleep apnea; the loss of this pattern in apneics during sleep and the delay in phasic activation of UA dilators compared with inspiratory chest wall muscles are associated with inspiratory flow limitation and a dramatic increase in UA resistance (9). Studies completed in dogs (7) and more recently in humans (22, 23) using phrenic nerve stimulation (PNS) techniques support this concept. By primarily stimulating the diaphragm without previous UA dilator activation, PNS mimics the dissociation between UA and respiratory muscle activation. Flow elicited by the twitch almost always corresponds to a flow-limited regimen that is not physiologically seen in normal awake subjects. Furthermore, we have observed in previous reports that PNS is not accompanied by a rise in genioglossus (GG) EMG activity until the maximal driving pressure has been reached (22). Therefore, PNS allows the evaluation of UA mechanical properties independently of phasic UA activity in awake subjects. For convenience in this paper, UA studied in these conditions with electrical PNS (EPNS) will be called “passive” to illustrate the fact that no phasic EMG activity preceded twitch-induced flow.

Based on the above-mentioned knowledge of the influence of UA dilator activities on UA mechanical properties, we reasoned that PNS could be a useful tool to evaluate the effect of changes in tonic UA dilator activity on UA dynamics. The present study was designed to characterize UA dynamics by using EPNS when increasing tonic EMG activity with CO₂ stimulation in normal awake subjects.

MATERIALS AND METHODS

Subjects. Six nonsnoring subjects (4 men, 2 women) were recruited for this study. A screening history in each subject disclosed no medical illness, clinical history, or anatomic abnormalities that could cause UA occlusion. No subject complained of any symptoms suggestive of obstructive sleep disorder nor was any on medication. The internal review board of our institution approved this protocol, and informed consent was obtained from each subject.

Protocol. Surface recording of the right and left costal diaphragmatic EMG activities were obtained by silver cup electrodes placed on the axillary line in the six to eight right and left intercostal spaces and connected to an EMG (Biopac system/Biopac, Santa Barbara, CA). Changes in end-expiratory lung volumes (EELV) were obtained from the sum tracing of an inductance plethysmographic recording operated in the DC mode and calibrated with the isovolume calibration technique (3). An esophageal balloon was inserted through one nares after local anaesthesia (1 ml of viscous xylocaine 2%) and located in the lower third of the esophagus as assessed by the occlusion technique (1). A pressure-tipped catheter (model CTF X1058, Gaeltec, Hackensack, NJ) passed through the other nostril in the nasopharynx at 8 cm from the nares recorded pharyngeal pressure. A plastic nasal stent was placed in the anterior nares to prevent nasal collapse, and the two catheters were secured on the nose. A tight-fitting nasal continuous-positive-pressure mask was then placed over the nose. Its airtightness was assessed by occluding its opening during maximal inspiratory efforts. Another catheter was passed through another opening of the mask to measure pressure inside the mask, and a fourth catheter was connected to a CO₂ analyzer (LB2, Beckman, Fullerton, CA). Esophageal pressure was referenced to mask pressure. The breathing circuit connected to the mask consisted of a pneumotachograph (Hans Rudolph, model 112467–3850A, Kansas City, MO) fixed to a unidirectional three-way valve (Hans Rudolph) whose inspiratory side could be switched from atmosphere to a rebreathing bag. This one was filled with a 7% CO₂-93% O₂ mixture. Subjects were studied supine with their heads supported by a premolded firm pillow to make certain that head and neck position did not change during the experiment.

GG EMG activity was recorded by using intra-oral electrodes mounted on a mouthpiece made from dental impression as described by Dohle et al. (5). EMG signals were amplified (Grass CP122, Quincy, MA), filtered (10 Hz to 10 kHz), rectified, and integrated with a moving time averager with a time constant of 100 ms (MA 1000, CWE, Ardmore, PA).

Study design. All measurements were made with subjects breathing exclusively by the nose. EPNS were realized by using conventional techniques (4). Twitch electrical pulses were delivered from a Grass stimulator (S88, Quincy, MA) through a stimulus isolation unit (Grass SIU 5A). The phrenic nerve was stimulated at the neck using a square wave pulse of 0.1-ms duration delivered by two bipolar electrodes. Following phrenic nerve location, a recruitment procedure was realized to determine supramaximal level of stimulus intensity, which was associated with a plateau in the amplitude of the diaphragmatic M-waves (motor evoked potential). EPNS was then further increased by 10–20% to ascertain supramaximality.

All twitches were applied at end-expiration as assessed by direct monitoring of instantaneous flow tracing. Four twitches were applied at baseline while subjects were breathing room air, and then they were switched to the rebreathing bag. Twitches were obtained every four to five breaths until subjects were unable to breathe exclusively by the nose. For each subject, this sequence was repeated three times.

Data and statistical analysis. Flows, integrated GG EMG, and all pressure tracings were recorded on a microcomputer. Twitch stimuli were retained for analysis when EPNS was performed at expiratory flow values <150 ml/s, with a pre-stimulation esophageal pressure between +1 and 0 cmH₂O and in the absence of unstable GG EMG (swallow or any rise in EMG that could correspond to phasic preinspiratory activation). Breathing cycles were identified as inspiratory flow limited (IFL) when inspiratory flow plateaued or decreased while twitch inspiratory efforts (esophageal pressure) increased. For each stimulus, we measured 1) maximal inspiratory twitch flow (V₁max) of flow-limited twitches, 2) esophageal pressure (Pes) at V₁max (Peslim), 3) peak esophageal pressure (driving pressure), 4) nasopharyngeal resistance at peak flow by the ratio pharyngeal pressure at V₁max/V₁max, 5) changes in EELV compared with baseline value, 6) tonic GG EMG activity estimated by GG end-expiratory EMG activity, and 7) the difference between GG EMG activity measured at V₁max and the preceding end-expiratory value. Regression analysis was completed with least-square correlation between the studied variables on the pooled data collected during the three trials. Differences in UA dynamic variables between baseline and the last twitch of the ventilatory stimulation period were analyzed with unpaired t-test. Statistical significance was set at P < 0.05.
RESULTS

Subjects’ mean age was 27.1 \pm 3.2 yr (SD), body mass index was 22.8 \pm 1.6 kg/m², and neck circumference was 36.3 \pm 1.9 cm.

No IFL was observed during spontaneous breathing in any subjects. In each trial, all EPNS induced partial UA closure as demonstrated by the presence of twitch flow-limited breaths. A representative example of twitch-flow limitation induced by EPNS is shown in Fig. 1: a clear flow-limitation pattern is observed as illustrated by the dissociation between Pes and twitch flow once Peslim has been reached. Then, instantaneous flow decreases while driving pressure becomes more negative. This flow-pressure pattern is consistent with our previous observations made in another population (22). Twitches were accompanied by a dramatic increase in UA resistance, with nasopharyngeal resistance measured at peak flow averaging 2.3 \pm 1.0 cmH₂O·l⁻¹·s during spontaneous breathing and 40.9 \pm 13.3 cmH₂O·l⁻¹·s following twitches (P = 10⁻⁴). It is important to note that, in all of our subjects and as previously reported in other subjects, no phasic GG activity was observed before EPNS. Furthermore, the rise in GG EMG activity only followed the increase in driving pressure, as supported by similar EMG activity recorded before PNS and at Vmax (Table 1).

Values of the parameters characterizing UA dynamics and GG EMG activity during EPNS at baseline and with CO₂ are reported in Table 1. Their values were significantly higher at the end of the CO₂ trials than during baseline (Table 1). Peslim significantly decreased (i.e., became more negative) with a significant drop during CO₂ trial compared with baseline (Table 1). Nasopharyngeal resistance measured at Vmax was significantly less during CO₂ trial than during baseline twitches (Table 1). Interestingly, the maximal driving pressure that was developed during twitches was significantly less during CO₂ trial (Table 1). EELV preceding twitches significantly increased during CO₂ trial (315 \pm 197 ml). GG EMG activity measured at end-expiration and the difference between GG EMG activity measured at Vmax and the preceding end-expiratory value significantly rose with CO₂ (Table 1).

To analyze the possible influence of the simultaneous changes in GG EMG activity and in EELV on UA dynamics, we looked at the correlation between closing
pressure and these variables when analyzing together the results obtained during the three different trials in each individual. The increase in $V_{\text{Imax}}$ was significantly correlated with the rise in end-expiratory EMG activity in every subject ($R > 0.32, P < 0.01$; Fig. 3) and the decrease in $P_{\text{eslim}}$ was significantly correlated with end-expiratory GG EMG activity in four of six subjects ($R > 0.34, P < 0.01$; Fig. 3). In three of six subjects, $V_{\text{Imax}}$ was correlated with GG EMG activity measured at $V_{\text{Imax}}$. For the whole group, changes in $V_{\text{Imax}}$ or in $P_{\text{eslim}}$ from air to $CO_2$ were positively correlated with changes in GG activity measured at end-expiration ($R = 0.65, P = 0.002$ and $R = 0.36, P = 0.06$, respectively), but no significant relationship was found with changes in GG EMG measured at $V_{\text{Imax}}$. A positive relationship was found between changes in $V_{\text{Imax}}$ and in EELV in only two subjects. The decrease in $P_{\text{eslim}}$ was never correlated with changes in EELV.

DISCUSSION

Our results demonstrate that passive UA mechanical properties are dramatically modified during $CO_2$ ventilatory stimulation; congruent changes in $V_{\text{Imax}}$, $P_{\text{eslim}}$, and instantaneous flow at maximal $\text{Pes}$ demonstrate an improvement in UA stability compared with its baseline assessment. Furthermore, changes in tonic GG EMG activity significantly contribute to the improvement in UA stability.

The originality of EPNS in assessing UA dynamics is that it bypasses the effect of physiological preinspiratory UA muscles activation, then allowing the study of passive UA during wakefulness. This represents an ideal model to quantify the effects of the changes in end-expiratory EMG activity on UA dynamics.

GG EMG was assessed to quantify UA dilator muscle activity. Even if we did not measure other EMG activities than that of the GG, we are confident that changes in GG EMG activity can be extended to other UA dilator muscles because an identical increase in tonic and phasic activities has been observed in tensor pala- tini, levator palatini, and palatopharyngeus during hypoxic hypercapnia (27). It can be asked to what extent some methodological issues could flaw the linkage that we observed between UA mechanics and tonic UA muscle activity, for example as a consequence of the effects of hypercapnic stimulation on preactivation of UA-dilator EMG up to several hundred milliseconds.
before onset of inspiration (25). However, PNS trials were retained for analysis only if the GG EMG activity remained stable before stimulation, thereby discarding any phasic preinspiratory activation. Furthermore, the GG EMG value measured at end expiration just before PNS always corresponded to the tonic EMG level (na-dir EMG value) and EMG activity began to rise before $V_{\text{max}}$ had been reached. This last result strongly supports the fact that no preinspiratory phasic activity had occurred before PNS because one should expect maximal phasic EMG activity to be reached in the 50 ms following inspiratory onset (this delay corresponds to the average time required for $V_{\text{max}}$ to be reached). For these reasons we are convinced that the observed changes in tonic EMG activity can account for the modification in UA mechanics.

The changes in UA mechanical properties that we observed could be attributed to a complex combination of several concordant physiological changes. According to the dynamics of the pressure-flow relationship during flow-limited breaths (18), changes in $V_{\text{max}}$ and $P_{\text{eslim}}$ can be accounted for by changes in UA collapsibility and/or downstream resistance with CO2. The relationship that is known to exist between $V_{\text{max}}$, downstream pressure, and critical pressure ($P_{\text{crit}}$) (18) can be used to determine the factors that contributed to the changes in the studied variables. From this relationship, $P_{\text{eslim}}$ can be determined as a function of other variables [$P_{\text{eslim}} = P_{\text{crit}} - (V_{\text{max}} \times \text{downstream resistance})$]. We are not able to precisely calculate downstream resistance in the present study because pressure measurements were obtained above (nasopharyngeal level) and far below (esophageal pressure) the collapsing site. The change in airway resistance downstream to the nasopharynx can be used to estimate changes in downstream resistance, but it obviously overestimates this resistance value because it includes the resistance of the collapsing site. With this assumption, the decrease in $P_{\text{eslim}}$ resulting from changes in $V_{\text{max}}$, downstream pressure, and critical pressure ($P_{\text{crit}}$) (18) can be used to determine the factors that contributed to the changes in the studied variables. From this relationship, $P_{\text{eslim}}$ can be determined as a function of other variables [$P_{\text{eslim}} = P_{\text{crit}} - (V_{\text{max}} \times \text{downstream resistance})$].

Another interesting finding concerns the significant decrease in flow drop (difference in flow values between $P_{\text{eslim}}$ and peak Pes) and in peak esophageal pressure during CO2 trial. We have previously observed that PNS frequently leads to a rapid decrease in twitch flow while driving pressure continues to decrease, with the flow nadir usually being reached at peak Pes (22). This effect was not related to an increase in nasal resistance during this last part of the twitch, and we previously hypothesized that this could result from the paradoxical movement of the upper portion of the thoracic cage, thus interfering with the effect of tracheal traction. This was supported by the fact that this drop is more pronounced during EPNS than during magnetic stimulation, which is accompanied by a stabilization of the thoracic cage because of simultaneous activation of accessory respiratory muscles (23). The same hypothesis can be drawn during CO2 breathing that is known to increase the tonic activity of accessory inspiratory muscles (intercostals, sternocleidomastoid) (16) with a subsequent stiffening of the thoracic cage, thus reducing paradoxical thoracic movement. It is also possible that increasing EELV could reduce the effect of such a paradoxical movement on UA dynamics. Concerning the decrease in peak esophageal pressure, for the above-mentioned reasons dealing with the consequence of the small increase in EELV on diaphragmatic contractile properties, we don’t believe that this
coefficient at $V_{\text{Imax}}$ did increase, further discrediting Grant MT 13 768 events. The occurrence of nocturnal obstructive-breathing activity associated with sleep may play a major role in can be speculated that the decrease in tonic muscle and particularly UA stability. Based on these results, it drop in peak esophageal pressure level when twitches are realized at the effective continuous positive airway pressure level (23).

We conclude that tonic activity of UA dilator muscles plays an important role in determining UA dynamics and particularly UA stability. Based on these results, it can be speculated that the decrease in tonic muscle activity associated with sleep may play a major role in the occurrence of nocturnal obstructive-breathing events.

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REFERENCES


2. Begle RL, Badr S, Skatrud JB, and Dempsey JA. Effects of phrenic nerve stimulation on upper airway and chest inspiratory muscle activity in obstructive sleep apnea: a significant change of the diaphragm. Furthermore, it must be kept in mind that, even if the twitch-induced peak driving pressure decreased with CO$_2$, the inspiratory pressure measured at $V_{\text{Imax}}$ did increase, further discrediting such a mechanistic hypothesis. We believe that this could be simply because this pressure was developed in a nearly closed circuit at baseline but in an open circuit with CO$_2$ trial. This would result in the same effect that continuous positive airway pressure had on UA dynamic characteristics studied with magnetic PNS in patients with obstructive sleep apnea: a significant drop in peak esophageal pressure level when twitches are realized at the effective continuous positive airway pressure level (23).


