Site of phrenic nerve stimulation-induced upper airway collapse: influence of expiratory time

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Sériès, F., and G. Éthier. Site of phrenic nerve stimulation-induced upper airway collapse: influence of expiratory time. J Appl Physiol 92: 665–671, 2002. First published October 12, 2001; 10.1152/japplphysiol.00582.2001.—Electrical phrenic nerve stimulation (EPNS) applied at end expiration during exclusive nasal breathing can be used to characterize upper airway (UA) dynamics during wakefulness by dissociating phasic activation of UA and respiratory muscles. The UA level responsible for the EPNS-induced increase in UA resistance is unknown. The influence of the twitch expiratory timing (200 ms and 2 s) on UA resistance was studied in nine normal awake subjects by looking at instantaneous flow, esophageal and pharyngeal pressures, and genioglossal electromyogram (EMG) activity during EPNS at baseline and at −10 cm H2O. The majority of twitches had a flow-limited pattern. Twitches realized at 200 ms and 2 s did not differ in their maximum inspiratory flows, but esophageal pressure measured at maximum inspiratory flow was significantly less negative with late twitches (−6.6 ± 2.7 and −5.0 ± 3.0 cm H2O respectively, P = 0.04). Pharyngeal resistance was higher when twitches were realized at 2 s than at 200 ms (6.4 ± 2.4 and 2.7 ± 1.1 cm H2O · l−1 · s−1 respectively). EMG activity significant rose at peak esophageal pressure with a greater increase for late twitches. We conclude that twitch-induced UA collapse predominantly occurs at the pharyngeal level and that UA stability assessed by EPNS depends on the expiratory time at which twitches are performed.

maximum inspiratory flow; electrical phrenic nerve stimulation; flow limitation

The stability of upper airway (UA) structures throughout respiration contributes to determine ventilatory characteristics, particularly during sleep. This is convincingly illustrated by the development of obstructive breathing disorders with increasing UA resistance in normal subjects (15, 28) and by the increase in UA resistance and in UA collapsibility in obstructive sleep apnea (OSA) patients (5). The pharyngeal level of UA structures has a key role in determining UA patency because it is not supported by rigid cartilaginous or bony structures as are nasal and laryngeal airways. This accounts for the fact that pharyngeal tissues represent the most collapsible UA region (9) and that they represent 75% of the primary site of UA closure in OSA (14). For these reasons, the pharyngeal airway patency mainly relies on the adapted contraction of UA dilator muscles (17).

Two different factors independently contribute to determine the stabilizing effect of UA dilator muscle contraction, namely the timing of UA activation and the effective force that is developed during their contraction. This last factor is of first importance in UA physiology because it directly counterbalances the collapsing forces of the transmural pharyngeal negative pressure gradient and tissue weight. However, only in vitro measurements of the dilating forces developed by UA contraction have been completed in the investigation of OSA pathophysiology. These studies suggest that UA dilators behave as highly trained muscles without loss in their capacity to generate tension (22). The timing of UA activation plays an important role in the stabilization of UA patency. Physiologically, UA dilator muscles are activated before inspiratory muscles, their peak electromyographic (EMG) activity being reached before that of the diaphragm (25). A loss of this preactivation pattern has been described in sleep disorders, phasic activation, and peak EMG activity of UA dilators being reached after the inspiratory chest wall muscle maximal EMG activity, with a consecutive increase in UA resistance (8). Such absence of UA dilator preactivation can be provoked by stimulating the phrenic nerve during the expiratory phase, resulting in the occurrence of flow-limited twitches and a dramatic increase in UA resistance in animals (7) and awake humans (23). In these circumstances, phrenic nerve stimulation (PNS) is a unique method that allows the evaluation of UA mechanical properties of passive UA awake subjects. It must be clear that we qualify UA as passive to take into account that twitch-induced flow has not been preceded by any phasic EMG activity of UA muscles. In this context, PNS can be used to measure passive UA mechanical properties resulting from UA shape and dimension, as well as from intrinsic tissue properties, and to characterize the effect of treatment in awaked OSA with this method (24).

Several factors may contribute to determine the flow dynamic response obtained during PNS. Apart from

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the role of nasal resistance, other important determinants are the location of UA collapse and the UA caliber immediately preceding the twitch. A previous short report identified that PNS used as a ventilation procedure during wakefulness induces a laryngeal collapse (18), which is in disagreement with the fact that the velopharynx is the most compliant UA level during sleep (9). If PNS-induced UA collapse effectively occurs at the laryngeal level, it could make comparing flow dynamic properties obtained with PNS during wakefulness and during spontaneous breathing when asleep difficult. Because we did not obtain oropharyngeal pressure recordings in our previous reports using this technique (23, 24), it is therefore of first importance to identify the site(s) responsible for the highest increase in resistance with PNS. We hypothesized that localization of the collapsing site could be influenced by the differences in breathing route between the different studies and that the UA collapse should occur above the larynx in our stimulation protocol. Thus the first aim was to evaluate the contribution of oropharyngeal and laryngeal airways to phrenic nerve-induced UA collapse. Furthermore, we were interested in documenting the influence of UA caliber on the characteristics of the twitch-induced flow response. It is known that UA cross-sectional area largely varies during expiration, the largest area being reached early during this respiratory phase with a progressive decrease in UA area as expiration is completed (19). We reasoned that these expiratory changes in UA caliber could be used to illustrate the influence of UA caliber on UA mechanical properties and hypothesized that the propensity for oropharyngeal airway to collapse should increase when the twitch is applied during late (2 s) instead of early (200 ms) expiration.

MATERIALS AND METHODS

Subjects. Nine nonsnoring subjects (5 men and 4 women) were recruited for this study. A screening history in each subject disclosed no medical illnesses or anatomic abnormalities that could cause UA occlusion. No subject complained of any symptoms suggestive of obstructive sleep disorder, nor was any subject on any 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 anterior chest wall in the six to eight right and left intercostal spaces and connected to an EMG (Biopac, Santa Barbara, CA). An esophageal balloon was inserted through one nares after local anesthesia (1 ml of viscous 2% Xylocaine) and located into the lower third of the esophagus as assessed by the occlusion technique (1). A pressure-tipped catheter (model CT/S X1058, Gaeltec, Hackensack, NJ) was introduced through the other nostril in the pharynx at 14 cm from the nares to record oropharyngeal 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. Occluding its opening during maximal inspiratory efforts assessed its airtightness. Another catheter was passed through another opening of the mask to measure pressure inside the mask (Pmask). Esophageal and mask catheters were connected to differential pressure transducers (Validyne MP 45 ± 100 cmH2O), esophageal pressure being referenced to Pmask (driving pressure). The mask was connected to a pneumotachograph (model 112467-3850A, Hans Rudolph, Kansas City, MO), and, when measurements were made with continuous negative airway pressure (see Study design), the breathing circuit was completed with a non-rebreathing valve connected to a 180-liter capacitance and to vacuum source through a variable orifice used to maintain a predetermined level of subatmospheric pressure throughout the respiratory cycle (5). The subjects were installed in a standardized semirecumbent position in a dentist chair with the head lying in a premolded pillow. The inclination of the back and head were preset, and a headrest supported the head so that the neck was in the neutral position. This ascertained that head and neck position did not change during the experiment.

The genioglossus (GG) EMG activity was recorded by using intraoral electrodes mounted on a mouthpiece made from dental impression, as described by Stable et al. (4). EMG signals were amplified (Grass CP122, Quincy, MA), filtered (10–3,000 Hz), rectified, and integrated with a moving time averager with a time constant of 100 ms (MA 1000, CWE, Ardmore, PA).

Study design. Three consecutive GG recruitment maneuvers were recorded (swallowing, pushing the tongue on the maxillary edge, Mueller maneuver) at baseline to determine the maximal voluntary GG activity. Electrical PNS (EPNS) was realized by using conventional techniques (3) with all measurements made with subjects breathing exclusively by the nose. Twitch electrical pulses were delivered from a Grass stimulator (model S88) through a stimulus isolation unit (Grass SIU 5A). The phrenic nerve was stimulated at the neck by using a square-wave pulse of 0.1-ms duration delivered by two bipolar electrodes. After phrenic nerve location, a recruitment procedure was realized to determine the 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.

A baseline spontaneous-breathing 1-min file was recorded before EPNS was completed. Twitches were applied during expiration 200 ms or 2 s after expiration onset. This was completed by a timer that triggered the Grass stimulator output once the chosen expiratory delay (200 ms or 2 s) had been reached. Ten twitches were applied at each expiratory time in random order. Subjects were then connected to a negative pressure source (−10 cmH2O) with a 2- to 5-min stabilization period before initiating recording. The same procedure was realized with a spontaneous-breathing recording followed by 10 twitches obtained at each expiratory time (200 ms or 2 s). Twitches were applied every consecutive four to five breaths.

Data and statistical analysis. Flows, integrated GG EMG, and all pressure tracings were recorded on a microcomputer. Twitch stimuli were retained for analysis 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 the inspiratory flow plateaued or decreased while the twitch inspiratory efforts (esophageal and pharyngeal pressures) increased. For each stimulus, we measured 1) maximal twitch flow (Vimax), 2) driving pressure at Vimax (Pdlim), 3) peak driving pressure, 4) pharyngeal resistance at peak flow and peak inspiratory efforts by the pharyngeal pressure-to-flow ratio obtained at the corresponding flow and pressure values, and 5) end-inspiratory EMG activity as well as the
difference between peak inspiratory efforts and end-expiratory GG EMG activities. EMG activity was expressed in percentage of maximal values obtained during voluntary maneuvers. An ANOVA with randomized split-block design was first performed. When significant interaction factors (expiratory timing, Pmask) were identified, they were followed by a randomized block design for each condition. Normality and variance assumptions were always fulfilled. Data were analyzed by using the SAS statistical package program (SAS, Cary, NC). Statistical significance was set at $P < 0.05$.

RESULTS

Five men and four women participated in the study. The anthropomorphic characteristics of the subjects are reported in Table 1. Expiratory duration was $2.9 \pm 0.6$ s (mean $\pm$ SD) in breathing room air and $2.9 \pm 1.0$ s at $-10$ cmH$_2$O.

**Characteristics of twitch-induced flow pattern.** There was no evidence of IFL during spontaneous breathing in any of our subjects. EPNS induced IFL in the majority of the trials as illustrated in Fig. 1 with an initial parallel increase in instantaneous flow and inspiratory efforts up to a $V_{imax}$ value that is reached once a threshold driving pressure value is developed ($P_{dlim}$); this is followed by a decrease of twitch flow despite a progressive increase in driving pressure (Fig. 2). $V_{imax}$ and peak driving pressure values after EPNS were significantly higher than the respective values obtained during tidal breathing. Peak flow and peak pressure pharyngeal resistance were significantly higher during twitches than during spontaneous breaths (Fig. 3).

**Effects of twitch timing and Pmask.** The percentage of flow-limited twitches was significantly higher when twitches were realized at 2 s than at 200 ms (Table 2). No difference was found in the percentage of flow-limited twitches between atmospheric and $-10$ cmH$_2$O pressures when expiratory timing was not taken into account. There was no difference in $V_{imax}$, flow at peak driving pressure, nor in peak driving pressure between twitches realized at 200 ms and 2 s at atmosphere. However, at $-10$ cmH$_2$O, Pmask, $V_{imax}$, and flow at peak driving pressure were significantly lower, and peak driving pressure was significantly more negative when twitches were realized at 2 s than at 200 ms expiratory time (Table 2). $P_{dlim}$ was significantly less negative when twitches were realized at 2 s than at 200 ms at both pressure levels (Table 2). Pharyngeal pressure tracing paralleled that of esophageal pressure (Figs. 1 and 2). Pharyngeal resistance measured at peak flow was significantly higher when twitches were realized at 2 s than at 200 ms (Fig. 3). Differences in peak pressure pharyngeal resistance between 200 ms and 2 s were also significant ($6.8 \pm 4.6$ and $11.3 \pm 9.9$ cmH$_2$O$^{-1}$s at room air, and $14.6 \pm 8.4$ and $26.9 \pm 21.4$ cmH$_2$O$^{-1}$s at $-10$ cmH$_2$O, both $P < 0.01$). The contribution of pharyngeal resistance to total resistance of the respiratory system measured at peak flow in the different study conditions is represented in Fig. 4. The contribution of pharyngeal resistance did not differ significantly between tidal breathing and 200-ms twitch applied at atmospheric pressure but signifi-

Table 1. Individual values of the anthropomorphic characteristics of the participating subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Neck Circumference, cm</th>
<th>Body Mass Index, kg/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>43</td>
<td>36.5</td>
<td>24.6</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>32</td>
<td>35.5</td>
<td>23.7</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>27</td>
<td>36.7</td>
<td>26.4</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>25</td>
<td>29.9</td>
<td>19.2</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>F</td>
<td>23</td>
<td>32.5</td>
<td>23.5</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>28</td>
<td>41.5</td>
<td>28.9</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>40</td>
<td>36.3</td>
<td>22.1</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>22</td>
<td>32.4</td>
<td>21.7</td>
</tr>
</tbody>
</table>

Mean $\pm$ SD 29 $\pm$ 7 34.8 $\pm$ 3.5 23.5 $\pm$ 2.9

M, male; F, female.
sificantly increased when twitches were performed at 2 s and at -10 cmH₂O (Fig. 4).

**GG EMG response to twitches.** No difference was found in the GG activity that preceded EPNS between the twitches realized at 200 ms and 2 s (Fig. 5). A rise in EMG activity was observed at peak driving pressure, the difference between 2-s and 200-ms twitches being borderline significant when twitches were realized at atmosphere (P = 0.07), and not significant at -10 cmH₂O (Fig. 5). No relationship was found between Vᵢₘₐₓ, Pd₁₉₃ₐ₃ₙ, or UA resistance values and the rise in GG EMG activity.

**DISCUSSION**

Our results demonstrate that the increase in UA resistance induced by this method mainly occurs at the pharyngeal level and that the expiratory timing of EPNS influences passive UA mechanical properties.

Our study was designed to compare twitch-induced flow dynamics at two different expiratory durations. The influence of expiratory characteristics could also have been explored by applying twitches at fixed expiratory flows during initial and late expiration. However, this could have been accompanied by important intra- and interindividual differences in twitch application timing, then adding important confounding variables and making elaborating on possible explanations of the results difficult. To account for the importance of body and neck position in the characteristics of UA dynamics, special attention was paid to keep neck position unchanged throughout the trial. The use of a premolded pillow prevented anteroposterior movements of the head and also limited the possibility of lateral movements.

Twitch-induced UA closure was found to mainly occur at the pharyngeal level; the contribution of pharyngeal resistance to the resistance of the respiratory system was higher at 2 s than at 200 ms. It has been previously reported that closure of the vocal cords mainly accounts for the increase in UA resistance observed during phrenic nerve pacing in a quadriplegic patient (18). However, in this case report, measurements were completed during mouth breathing that bypasses the velopharyngeal airway, which is known to be the most compliant part of UA in humans (9, 14). In the present study, as in our recently published ones, measurements were obtained during exclusive nasal breathing, thereby respecting the physiological breathing route. The recording of oropharyngeal pressure in combination with esophageal pressure was used to document the changes in upstream (pharyngeal) and downstream (larynx and intrathoracic airways) resistance. In this situation, the occurrence of UA closure above the laryngeal level is in accordance with the respective compliance of the different UA levels. It can be speculated that the higher percentage of pharyng-
Flow and driving pressure values of flow-limited twitches were higher at 2 s than at 200 ms at each pressure level. *P < 0.05 between 200 ms and 2 s (P < 0.05).

Values are means ± SE. V˙max, maximal inspiratory flow; V˙, flow; Pdlim, driving pressure at V˙max. *Significant difference between 200 ms and 2 s (P < 0.05).

Figure 4. Means ± SD values of the contribution of peak flow pharyngeal resistance to total respiratory resistance measured during tidal breathing and following 200-ms and 2-s twitches. This contribution was higher at 2 s than at 200 ms at each pressure level. *P < 0.05 with spontaneous breathing. †P < 0.05 between 200-ms and 2-s twitches.

Figure 5. Means ± SD values of genioglossus (GG) electromyographic (EMG) activity measured just before twitches (pretwitch) and at peak Pd at each expiratory time and pressure level. max, Maximum; atm, atmosphere.
ues. However, the conjunction of the absence of difference in $V_{\text{max}}$ between 200-ms and 2-s twitches, with the decrease in downstream resistance (see previous paragraph) and the decrease in pharyngeal pressure at $V_{\text{max}}$, can only occur if $P_{\text{crit}}$ decreases to more negative values from early to late expiration. It could be argued that such a decrease in UA collapsibility (less negative $P_{\text{crit}}$ value) may be in discrepancy with the above-mentioned simultaneous changes in UA pharyngeal caliber throughout expiration (19). However, changes in UA collapsibility also have to take into account the effects of laryngeal airway dilation on the stability of pharyngeal tissues. Laryngeal and pharyngeal structures are anatomically and functionally dependent. The changes in laryngeal airway loading have been shown to interact with the stability of pharyngeal airway: UA pressure drops when lowering the transmural pressure of the extrathoracic and laryngeal airway (30). On the other hand, $P_{\text{crit}}$ values are less negative when external loading is applied on the larynx (10). In this instance, our results corroborate with those previously published by Gottfried et al. (7) in anesthetized dogs in which elevation of the hyoid bone led to a significant decrease in UA pressure with no changes in $V_{\text{max}}$ compared with PNS alone. These data demonstrate the importance of laryngeal patency in the PNS flow-induced response.

It can be asked to what extent some methodological issues could flaw data interpretation, particularly the fact that UA was free of preinspiratory phasic activation. However, GG EMG activity had to remain stable before stimulation trials for each PNS to be retained for analysis; therefore, the presence of any phasic preinspiratory activation was discarded. Furthermore, the GG EMG value measured at end expiration just before PNS always corresponded to the tonic EMG level (nadir EMG value), and EMG activity did not consistently rise before the peak oesophageal pressure had been reached. This last result strongly supports the fact that no preinspiratory phasic activity had occurred before PNS since one should expect that the maximal phasic EMG activity was reached within the 50 ms required to reach $V_{\text{max}}$. These arguments as well as the absence of a relationship between UA activity and posttwitch UA resistance strongly supports the passive nature of UA tissues in these circumstances.

The principle of using EPNS to characterize UA dynamics during wakefulness is that it allows the study of passive UA without previous activation of UA muscles. This is a unique situation because negative UA pressure is a powerful stimulus of EMG activity (12), which is known to dramatically interfere with the end-expiratory and inspiratory UA caliber (8, 6, 27, 21). In this and previous studies, we demonstrated that the GG EMG response occurs lately following the twitch, when the driving pressure is to reach its peak value. Despite the absence of an observed relationship between the EMG rise and UA resistance, $V_{\text{max}}$ or esophageal pressure at $V_{\text{max}}$ clearly illustrates that the increase in EMG activity is passive and does not contribute to the flow dynamic characteristics that are observed by using EPNS. This does not contradict the fact that UA muscles play an important role in UA stability, because, during twitches, UA activation is always delayed compared with twitch-induced inspiratory flow onset, which supports the importance of the physiological preinspiratory activation of UA dilators in reducing UA resistance and increasing UA stiffness at inspiratory onset.

It is interesting to note that even if no difference was found in the peak esophageal pressure reached after 200-ms and 2-s twitches, the GG EMG rise tended to be greater when twitches were applied during late expiration. This is consistent with previous results, which showed negative pressure-induced UA EMG rise, demonstrating an alteration in this reflex response during early compared with late expiration (26). The rise in GG EMG activity that we observed in response to decreasing intrathoracic pressure was lower than previous ones that reported the effects of negative pressure applied to a nasal mask (15). However, conventional methods dedicated to evaluate UA muscle EMG response to negative pressure differ from EPNS in several ways. First, they use sudden drops in negative pressure applied at the nose, and the EMG response is measured after a fixed delay in the application of this square pressure drop. With the use of EPNS, negative pressure is generated by diaphragmatic contraction and, therefore, does not have square negative-pressure shape. This may interact with the amount of EMG response because, with EPNS, negative airway pressure does not reach a minimal duration plateau. Second, the application of negative pressure at the nose induces expiratory-like thoracic and tracheal displacements, whereas EPNS mimics the inspiratory-related changes in lung volume and tracheal movements. In these circumstances, it could be hypothesized that EPNS may represent a more physiological means of quantifying UA reflex responsiveness. This should be prospectively compared to further emphasize the ability of EPNS to quantify UA muscle response to negative pressure.

We conclude that EPNS-induced UA collapse mostly occurs at the pharyngeal level and that the timing at which EPNS is performed during expiration is an important determinant of the characteristics of UA dynamics by influencing UA stiffness as well as the localization of UA collapse. It should be recommended that, to assess the dynamics of the UA pharyngeal component, future studies using EPNS should be realized during late expiration. The results of this study also emphasize that EPNS allows the characterization of UA dynamics independently of the twitch-induced rise in UA muscle activity, further illustrating that this technique evaluates passive UA properties during wakefulness.

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