Application of negative expiratory pressure during expiration and activity of genioglossus in humans

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The negative expiratory pressure (NEP) method has been recently introduced to detect intrathoracic expiratory flow limitation (EFL) under different conditions in humans (6, 12). It consists in applying a negative pressure (usual range: −3 to −5 cmH2O) during tidal expiration, thus widening the pressure difference between the alveoli and the airway opening. In the absence of EFL, there is an increase in expiratory flow, whereas, in the presence of EFL, the expiratory flow during NEP does not increase throughout the entire or part of the tidal expiration compared with the flow of the preceding control expiration. In spontaneously breathing subjects at rest, however, the application of such levels of NEP at the onset of expiration sometimes results in a drop in flow below the flow rate generated during the preceding tidal expiration. This finding, which is believed to reflect partial or total upper airway narrowing (11), may occur in healthy subjects (9, 15), suggesting some degree of upper airway instability.

It has been shown in awake, normal humans that sudden application of NEP at end expiration was associated with reflex activation of genioglossus (4). This phenomenon may be viewed as a compensatory mechanism to maintain upper airway patency.

We reasoned that airway narrowing or collapse during application of NEP at the beginning of expiration could be due to neurophysiological characteristics of the above-mentioned reflex. For instance, its latency could be too long for it to operate after application of NEP in early expiration, or there could be interindividual variations in the sensitivity of the trigger or in the intensity of response. Such reflex elements might be relevant to certain aspects of the assessment of upper airway function in sleep-disordered breathing.

Therefore, the present study was designed to assess and compare the effect of NEP applied at the mouth at the onset of expiration and during the end-expiratory pause on genioglossus muscle reflex activity in normal subjects.

METHODS

Ten healthy, nonobese subjects without evidence or history of cardiopulmonary disease, obstructive sleep apnea syndrome, or upper airway abnormality were studied (Table 1). Subjects took no medications or alcohol on the day of the study. Informed consent was obtained from each individual.

Flow (V) was measured with a Hans-Rudolph pneumotachograph with a ±2.6 l/s linearity range (model 4700A, Hans-Rudolph, Kansas City, MO) connected to the mouthpiece and a differential pressure transducer (MP45, ±2 cmH2O; Validyne). Pressure was measured at the mouth (Pmouth) via a noncompliant polyethylene tube (1.7 mm ID; Validyne, Northridge, CA). The system used to measure Pmouth had no appreciable shift or alteration in amplitude up to 20 Hz.

A Venturi device capable of rapidly generating a negative pressure (Aeromech Devices, Almonte, ON, Canada) was connected in series with the pneumotachograph. The Venturi device was attached via an electrically operated solenoid valve to a source of compressed air. A pressure regulator was used to obtain the desired levels of negative pressure at
was placed on the right arm. To compare the individual
terminal 5 mm of the electrodes, which were in the contact parallel and symmetrically on each side of the midline. The electrodes were sewn through the impression material and placed apposed to the upper surface of the genioglossus. Electrodes onto the floor of the mouth so that the material was closely still soft, it was fitted to the subject's lower teeth and pushed connected to a mouthpiece and constructed from dental pack Sigma). In two of the subjects, the intraoral EMGgg was cally integrated by using a time constant of 100 ms (Neuro-
ally between 100 and 5,000 Hz, full-wave rectified, and electroni-
The raw EMGgg signal was amplified, band-pass filtered by the following equation: 
and its pressure (P)-V˙relationship was characterized
E 20 ml, and its pressure (P)-V˙relationship was characterized by the following equation: 
P = 0.85V˙+0.70V², where P is in centimeters of water and V˙is in liters per second. Calibration of the flow and pressure transducers was done before and after each study.
Surface recordings of the genioglossal electromyogram (EMGgg) were obtained by using a pair of disposable unipolar skin-taped electrodes clipped on the chin and connected to a multichannel recording system (Neurorack Sigma, Nihon Kohden, Tokyo, Japan). The electrode-to-skin impedance was checked before each experiment to keep it always below 10 kΩ. The raw EMGgg signal was amplified, band-pass filtered between 100 and 5,000 Hz, full-wave rectified, and electronically integrated by using a time constant of 100 ms (Neurorack Sigma). In two of the subjects, the intraoral EMGgg was also recorded by means of a noninvasive intraoral electrode connected to a mouthpiece and constructed from dental impression material (2). While the impression material was still soft, it was fitted to the subject's lower teeth and pushed onto the floor of the mouth so that the material was closely apposed to the upper surface of the genioglossus. Electrodes were sewn through the impression material and placed parallel and symetrically on each side of the midline. The terminal 5 mm of the electrodes, which were in the contact with the floor of the mouth, were bared. A grounding electrode was placed on the right arm. To compare the individual EMGgg activity obtained at different levels of NEP, we determined the maximal EMGgg activity by performing the following maneuvers: maximal inspiratory effort against occluded airway, sniff, and maximal protrusion of the tongue. In all subjects, the maximal peak integrated EMGgg activity was always achieved during maximal protrusion of the tongue. Once maximum (100%) was defined, the peak of the integrated EMGgg signal could be scaled between 0 (electrical 0) and 100.
The V and Pmouth signals were amplified, low-pass fil-
tered (Demodulator model CD 15, Validyne), and sent as analog signals to Neurorack Sigma. The time course of the raw EMGgg, V, and Pmouth signals were continuously displayed on the Neurorack Sigma screen. When required, the signals were recorded and stored in the hard disk of the computer installed on the Neurorack for subsequent analy-
Data analysis was performed by using the managing waveforms software provided with the Neurorack Sigma system (Fig. 1).
The V and Pmouth signals were also amplified (AC Bridge Amplifier-ABC module, Raytech Instruments), low-pass filtered at 50 Hz, sent to a 16-bit analog-to-digital converter (Direc Physiologic Recording System, Raytech Instruments) installed on a IBM-compatible computer (486DX, 66 MHz), and sampled at 200 Hz. Both digitized signals were displayed in real time on the computer screen together with the volume (V) signal obtained by numerical integration of the V signal. The tracings were continuously monitored both with respect to time and as V-V curves (Fig. 1). The recordings were stored on the computer hard disk in Direc format and used for subsequent analysis when necessary. Data analysis was performed by using the Direc data-analysis software (version 3.1, Direc NEP software, Raytech Instruments).

Procedure and data analysis. During the study, the sub-
jects, wearing a noseclip and breathing through a rigid mouthpiece, were sitting upright in a comfortable dentist's chair with the neck fixed in neutral position. After regular quiet breathing had been achieved, NEP values of −3, −5, −7, and −10 cmH2O were randomly applied 0.2 s after the onset of expiration and maintained for 500 ms. NEP of −10 cmH2O was also applied at the end-expiratory pause. Each level of NEP was repeated six times. Recordings of Pmouth, V, and raw EMGgg (surface or intraoral) were initiated before NEP application and lasted 2 s.
To distinguish voluntary from reflex responses to NEP, the voluntary reaction time taken for protruding the tongue was measured in each subject. The subjects were asked to pro-
trude the tongue as quickly as possible in response to a visual stimulus given while they were relaxed in the experimental sitting position. Six responses were recorded, and the respec-
tive reaction times were averaged for each subject.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Gender</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
</tr>
</thead>
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<tr>
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<td>M</td>
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<td>4</td>
<td>M</td>
<td>51</td>
<td>174</td>
<td>83</td>
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<td>5</td>
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<td>172</td>
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<td>M</td>
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<td>F</td>
<td>28</td>
<td>162</td>
<td>58</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>35</td>
<td>170</td>
<td>70</td>
</tr>
</tbody>
</table>

M, male; F, female.

Fig. 1. Experimental setup used to produce sud-
ne negative expiratory pressure (NEP) in a sub-
ject breathing through a mouthpiece. Flow (V), pressure at the mouth (Pmouth), and raw surface EMG of genioglossus (EMGgg) were si-
multaneously recorded before and during applica-
tion of NEP pulses (500 ms), both at onset of tidal expiration and during end-expiratory pause. V, volume; P, pressure.
The latency of the genioglossus muscle activation, if present, was determined by eye as that time, after the onset of the pressure change, when the poststimulus raw EMG activity appreciably increased above the prestimulus activity, according to the judgment of three independent observers. Increased raw EMG activity was considered as expression of a true reflex if it was detected within the first 150 ms after application of NEP and lasted at least 30 ms. The magnitude of the EMG response was expressed as percent ratio between the peak integral of the rectified EMG activity over a 150-ms poststimulus period and the peak integral of the corresponding maximal EMG activity obtained during maximal tongue protrusion. At each level of NEP the record with the worst quality in EMG signal was always discarded, and the average of five NEP responses was used for subsequent analysis. On four occasions (scattered among subjects) at end expiration a further test was rejected because of technical problems, and then four NEP responses were averaged for analysis.

One-way analysis of variance for repeated measurements was used for statistical comparisons of EMGg response after NEP applied at the onset of expiration, with the NEP level as within-group factor; paired Student's t-test was performed to compare NEP at the onset of expiration with NEP at the end-expiratory pause. P values < 0.05 were considered as significant. Data are expressed as means ± SE unless otherwise specified.

RESULTS

NEP at onset of expiration. In seven subjects, no detectable reflex surface EMGg activity was observed after application of all NEP levels, as shown for a representative subject (subject 3) with NEP of −10 cmH2O (Fig. 2). A consistent reflex response to all levels of NEP was found in only one subject (subject 6), with a mean latency of 57 ± 6 ms with NEP of −10 cmH2O (Fig. 3). Comparable mean latencies were found with NEP values of −3 (56 ± 9 ms), −5 (56 ± 8 ms) and −7 cmH2O (59 ± 8 ms) for this subject.

In two subjects (subjects 2 and 9), reflex responses to NEP were observed intermittently and only with NEP of −7 and −10 cmH2O. During five NEP tests for each level of NEP, a reflex activity of genioglossus was detected four times in subject 2 (twice with NEP of −10 cmH2O) and two times in subject 9 (once with NEP of −10 cmH2O).

The magnitude of the peak integrated surface EMGg activity with the different levels of NEP applied at onset of expiration is shown in subjects 2, 6, and 9 (Fig. 4).

NEP at end-expiratory pause. When NEP of −10 cmH2O was applied during the end-expiratory pause,
that is, at functional residual capacity before the ensuing inspiration, consistent reflex responses in surface EMGg activity were found in seven subjects, as exemplified for a representative subject (subject 3) in Fig. 5, and occasionally in three subjects (once in 5 NEP tests). The latency of the reflex of the seven responders amounted to 68 ± 5 ms and was significantly quicker than the reaction time for protruding the tongue (203 ± 5 ms; range 168–228 ms).

In the seven subjects who did not respond and in two subjects (subjects 2 and 9) who responded intermittently to the application of NEP at the onset of expiration, the mean peak integrated surface EMGg activity amounted to 10.3 ± 3.5% maximum when NEP was applied at end expiration. The corresponding mean value with NEP of −10 cmH2O applied in early expiration was much lower (5.3 ± 1.5% maximum; P = 0.07). Conversely, in subject 6 the peak integrated surface EMGg activity was similar when NEP of −10 cmH2O was applied both at end expiration and in early expiration, amounting to 62.9 and 67.1% maximum, respectively.

In the group of nine subjects, except subject 6, a significant increase of peak integrated EMGg activity compared with baseline (NEP = 0 cmH2O) occurred only when NEP of −10 cmH2O was applied during the end-expiratory pause (P < 0.01).

NEP at onset of expiration and at end-expiratory pause with intraoral EMGg electrode. In two of the above subjects (subjects 1 and 3) who did not respond to NEP applied at the onset of expiration, we also repeated the measurements by using intraoral EMGg electrodes and again found no response to NEP applied at the onset of expiration, as shown in subject 3 (Fig. 6). In contrast, when NEP was applied during the end-expiratory pause, in both subjects there was an increase in EMGg activity similar to that found with the surface electrodes.

Relationship between expiratory flow and reflex activation of genioglossus during NEP at onset of expiration. The individual measurements of expiratory flow obtained during application of the different levels of NEP were reported in relation to the genioglossus reflex activation in Table 2. When the values of the mean expiratory flow during the first 150 and 500 ms of NEP were expressed as percent ratio (i.e., 150/500%), two distinct patterns emerged. The former, characterized by a higher 150/500% ratio, was displayed by subjects 2, 3, 7, and 9 and by subject 6 and documented a stable increase in expiratory flow; the latter, which had a lower 150/500% ratio, was observed in subjects 1, 4, 5, 8, and 10 and was determined by a substantial, early decrease in expiratory flow. Thus, in the absence of a reflex response of genioglossus to NEP applied at the beginning of expiration, both patterns in the expiratory flow could be observed, likely reflecting a sustained patency or a transient narrowing of the upper airways in response to NEP, respectively. In Fig. 7, examples were given of V-V curves obtained in three representative subjects when NEP was applied at the onset of expiration. Despite the absence of a reflex-mediated activation of genioglossus, subject 3 (left panels) exhibited a steady increase in expiratory flow during NEP in each test, as did subjects 2, 7, and 9. In contrast, subject 4 (middle panels) showed almost at all times an abrupt fall in expiratory flow soon after the application of NEP, which was generally more marked at higher levels of NEP, as did subjects 8 and 10. Subjects 1 and 5 behaved similarly to subject 4, although in a less regular fashion. Finally, subject 6 (right panels), who had an invariable EMGg reflex activity in response to NEP, usually showed a steady increase in expiratory flow (right); once or twice at each level of NEP, however, she exhibited a sudden drop in expiratory flow (left).

**DISCUSSION**

The main finding of this study is the modulation of the reflex activity of genioglossus muscle in response to NEP according to the timing of application of NEP during expiration. In fact, whereas a reflex response of
Table 2. Individual flow measurements and reflex activation of genioglossus during application of different levels of NEP at the onset of expiration

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>GG Reflex Activation (Yes/No)</th>
<th>VT/TE NEP 150 ms, l/s</th>
<th>VT/TE NEP 500 ms, l/s</th>
<th>Ratio 150/500, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/5</td>
<td>0.38 ± 0.05</td>
<td>0.43 ± 0.02</td>
<td>88 ± 8</td>
</tr>
<tr>
<td>2</td>
<td>0/5</td>
<td>0.53 ± 0.10</td>
<td>0.58 ± 0.04</td>
<td>91 ± 12</td>
</tr>
<tr>
<td>3</td>
<td>0/5</td>
<td>0.38 ± 0.10</td>
<td>0.92 ± 0.06</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>4</td>
<td>0/5</td>
<td>0.40 ± 0.12</td>
<td>0.45 ± 0.08</td>
<td>88 ± 13</td>
</tr>
<tr>
<td>5</td>
<td>0/5</td>
<td>0.57 ± 0.11</td>
<td>0.76 ± 0.20</td>
<td>79 ± 18</td>
</tr>
<tr>
<td>6</td>
<td>5/0</td>
<td>0.61 ± 0.11</td>
<td>0.66 ± 0.08</td>
<td>94 ± 14</td>
</tr>
<tr>
<td>7</td>
<td>0/5</td>
<td>0.26 ± 0.05</td>
<td>0.25 ± 0.03</td>
<td>105 ± 13</td>
</tr>
<tr>
<td>8</td>
<td>0/5</td>
<td>0.75 ± 0.10</td>
<td>0.87 ± 0.04</td>
<td>87 ± 9</td>
</tr>
<tr>
<td>9</td>
<td>0/5</td>
<td>0.51 ± 0.04</td>
<td>0.47 ± 0.02</td>
<td>109 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>0/5</td>
<td>0.50 ± 0.20</td>
<td>0.76 ± 0.17</td>
<td>65 ± 22</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>GG Reflex Activation (Yes/No)</th>
<th>VT/TE NEP 150 ms, l/s</th>
<th>VT/TE NEP 500 ms, l/s</th>
<th>Ratio 150/500, %</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0/5</td>
<td>0.31 ± 0.11</td>
<td>0.45 ± 0.06</td>
<td>68 ± 19</td>
</tr>
<tr>
<td>2</td>
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<td>0.62 ± 0.09</td>
<td>0.68 ± 0.06</td>
<td>91 ± 9</td>
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<tr>
<td>3</td>
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<td>1.00 ± 0.05</td>
<td>0.94 ± 0.02</td>
<td>106 ± 4</td>
</tr>
<tr>
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<tr>
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<td>0.80 ± 0.10</td>
<td>92 ± 14</td>
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<tr>
<td>7</td>
<td>0/5</td>
<td>0.38 ± 0.05</td>
<td>0.35 ± 0.05</td>
<td>110 ± 3</td>
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<tr>
<td>8</td>
<td>0/5</td>
<td>0.63 ± 0.09</td>
<td>0.90 ± 0.05</td>
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<td>9</td>
<td>0/5</td>
<td>0.73 ± 0.04</td>
<td>0.65 ± 0.02</td>
<td>112 ± 3</td>
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<td>10</td>
<td>0/5</td>
<td>0.63 ± 0.07</td>
<td>1.04 ± 0.08</td>
<td>60 ± 7</td>
</tr>
</tbody>
</table>

Flow data are means [over 5 negative expiratory pressure (NEP) responses] ± SD. GG, genioglossus; VT/TE NEP 150 ms and VT/TE NEP 500 ms, mean expiratory flow during the first 150 and 500 ms of NEP, respectively; Ratio 150/500, (VT/TE NEP 150 ms)/(VT/TE NEP 500 ms).

Genioglossus was elicited in most subjects by the application of NEP at end expiration as previously described (4), definitely this was not the case at the onset of expiration. In addition, no relationship was found between steady increase or abrupt fall in inspiratory flow and the presence or the absence of a reflex activity of genioglossus during sudden application of NEP in early expiration. It follows that, under these dynamic conditions, the maintenance of upper airway patency does not seem to imply the occurrence of a reflex-mediated activation of genioglossus and vice versa.

Studies in awake normal subjects have shown that continuous subatmospheric pressure applied selectively to upper airways during active inspiration increases the phasic activity of the upper airway dilator muscles by reflex mechanisms in a proportional way to stabilize and maintain pharyngeal patency (1, 7). Furthermore, by application of pulses of negative pressure in the upper airway at the onset of inspiration, a reflex activation of genioglossus with a mean latency of 54 ± 11 ms was consistently found in six normal subjects during wakefulness (13).

Recently, by using intraoral bipolar surface electrodes to record EMGgg activity, in 10 awake supine normal subjects application of pulses of 500 ms of negative airway pressure at end expiration has been shown to induce a significant reflex response of genioglossus at levels equal to and higher than −10 cmH2O with glottis closed and −15 cmH2O with glottis open (4). The results of our study showing a reflex activity of surface and intraoral EMGgg during the end-expiratory pause in the majority of awake, seated normal subjects in response to NEP of −10 cmH2O add evidence for reflex pharangeal dilator muscle activation by negative airway pressure at end expiration in normal humans.

This finding seems consistent with phasic activation of upper airway dilator muscles before inspiration in humans acting to stiffen the compliant structures of upper airway against the collapsing forces generated by inspiratory efforts of diaphragm and chest wall muscles (10).

We focused, however, on the genioglossus reflex response after application of NEP in early expiration because sometimes during resting breathing in normal subjects transient reduction and even cessation of flow can occur by application of NEP at the onset of expiration, as used to detect EFL.

In this respect, the substantial lack of reflex EMGgg activity we observed in awake normal subjects suggests that sensory feedback from upper airways in response to subatmospheric pressure might be differently modulated to mediate the reflex activation of upper airway dilator muscles during inspiration in humans.

In 24 anesthetized spontaneously breathing rabbits, the effect of upper airway negative pressure pulses (between −5 and −20 cmH2O) on EMGgg activity has been shown to depend on the time of application within the breathing cycle (14). Indeed, after application of negative pressure pulses, the activation of genioglossus was much lower in the middle expiration than at the onset of inspiration and decreased when negative pressure was applied in late rather than in early inspiration. Thus the time of the application of negative pressure during the respiratory cycle seems to play an important role in determining a reflex response of
pharyngeal dilator muscles to upper airway negative pressure.

In one subject (subject 6), a reflex surface EMG activity has been invariably found at all levels of NEP applied at onset of expiration. We do not have a clear explanation for this response, which is opposite to that exhibited by the other subjects. However, in this subject after surface anesthesia of the mouth floor sprayed with 60 mg of 10% lidocaine solution, the reflex surface EMG activity after application of NEP of −5 cmH₂O at the onset of expiration was markedly reduced (peak integrated surface EMG activity was almost 50% less than before anesthesia). This fact suggests that in some individuals the reflex activation of genioglossus muscle may be mediated, at least in part, by inputs coming from superficial receptors stimulated by NEP and probably traveling by nonvagal afferents (lingual and glossopharyngeal nerves) (5, 8). A similar observation was reported by Horner and colleagues (3), who found an EMG reflex activity decreased by 21% in response to negative airway pressure of −25 cmH₂O applied at end expiration in four normal subjects after selective anesthesia of the oropharyngeal mucosa.

Surface records of EMG activity are less sensitive and specific than those obtained by invasive technique or by using a special intraoral electrode, which has been recently validated for this purpose (2). In our study, however, the reflex EMG activity was always detected by surface electrodes in all subjects during different maneuvers such as inspiratory effort, sniff, and protrusion of the tongue and was consistently observed when NEP was applied at the end expiration and in early expiration in subject 6. In addition, the EMG tracings obtained via intraoral electrode in two subjects under the same experimental conditions re-
NEP AND REFLEX ACTIVITY OF GENIOGLOSSUS DURING EXPIRATION

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The activity of pharyngeal dilator muscles.

upper airways cannot be ascribed to an impaired reflex when NEP is applied in early expiration the maintenance of upper airway patency does not imply a reflex-mediated activation of genioglossus, suggesting that in these circumstances a partial or total narrowing of upper airways cannot be ascribed to an impaired reflex activity of pharyngeal dilator muscles.

Thus, although we are well aware of these technical limitations, we are very confident that our surface EMG records were sufficiently reliable to exclude a reflex activity of genioglossus when they were unchanged and to detect an activation pertaining mainly to genioglossus when they displayed a markedly increased activity with NEP.

In conclusion, our results confirm that a reflex response of genioglossus may occur in humans when negative airway pressure is applied during the expiratory phase, showing, however, that this happens much more commonly at the end-expiratory pause than at the onset of expiration. These findings also indicate that when NEP is applied in early expiration the maintenance of upper airway patency does not imply a reflex-mediated activation of genioglossus, suggesting that in these circumstances a partial or total narrowing of upper airways cannot be ascribed to an impaired reflex activity of pharyngeal dilator muscles.

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