Upper airway muscle responsiveness to rising \( P_{\text{CO}_2} \) during NREM sleep

Giora Pillar, Atul Malhotra, Robert B. Fogel, Josee Beauregard, David I. Slamowitz, Steven A. Shea, and David P. White. Upper airway muscle responsiveness to rising \( P_{\text{CO}_2} \) during NREM sleep. J Appl Physiol 89: 1275–1282, 2000.—Although pharyngeal muscles respond robustly to increasing \( P_{\text{CO}_2} \) during wakefulness, the effect of hypercapnia on upper airway muscle activation during sleep has not been carefully assessed. This may be important, because it has been hypothesized that \( \text{CO}_2 \)-driven muscle activation may importantly stabilize the upper airway during stages 3 and 4 sleep. To test this hypothesis, we measured ventilation, airway resistance, genioglossus (GG) and tensor palatini (TP) electromyogram (EMG), plus end-tidal \( P_{\text{CO}_2} \) (\( \text{PETCO}_2 \)) in 18 subjects during wakefulness, stage 2, and slow-wave sleep (SWS). Responses of ventilation and muscle EMG to administered \( \text{CO}_2 \) (\( \text{PETCO}_2 = 6 \text{ Torr} \)) above the eu-pneic level were also assessed during SWS (\( n = 9 \)) or stage 2 sleep (\( n = 7 \)). \( \text{PETCO}_2 \) increased spontaneously by 0.8 ± 0.1 Torr from stage 2 to SWS (from 43.3 ± 0.6 to 44.1 ± 0.5 Torr, \( P < 0.05 \)), with no significant change in GG or TP EMG. Despite a significant increase in minute ventilation with induced hypercapnia (from 8.3 ± 0.1 to 11.9 ± 0.3 l/min in stage 2 and 8.6 ± 0.4 to 12.7 ± 0.4 l/min in SWS, \( P < 0.05 \) for both), there was no significant change in the GG or TP EMG. These data indicate that supraphysiological levels of \( \text{PETCO}_2 \) (50.4 ± 1.6 Torr in stage 2, and 50.4 ± 0.9 Torr in SWS) are not a major independent stimulus to pharyngeal dilator muscle activation during either SWS or stage 2 sleep. Thus hypercapnia-induced pharyngeal dilator muscle activation alone is unlikely to explain the paucity of sleep-disordered breathing events during SWS.

Obstructive sleep apnea syndrome; dilator muscle; genioglossus; hypercapnia; slow-wave sleep; nonrapid eye movement

Obstructive sleep apnea is a common disorder characterized by the repetitive collapse of the pharyngeal airway during sleep. Our laboratory has previously shown, in apnea patients, that pharyngeal dilator muscle activation is high during wakefulness, which probably protects the airway from collapse (17). During sleep, the loss of muscle activation results in airway collapse (18). Considerable effort has been made to determine the stimuli that drive activation of the pharyngeal muscles during both sleep and wakefulness. Although these dilator muscles respond robustly to increasing \( P_{\text{CO}_2} \) during wakefulness (22), the effect of hypercapnia on upper airway (UAW) muscle activation during sleep has only been minimally assessed in humans. This may be clinically relevant because CO2-stimulated muscle activation has been proposed as an important variable in maintaining airway patency during stages 3 and 4 sleep (1, 5, 9, 26, 37).

A number of studies indicate that the majority of sleep-disordered breathing occurs during stages 1 and 2 sleep, generally in the wake-sleep transition, or during rapid-eye-movement (REM) sleep. On the other hand, there are relatively few apneas or hypopneas observed during stages 3 and 4 sleep [slow-wave sleep (SWS)] (8, 14, 15). The reason ventilation appears to be more stable during SWS remains unclear.

Three mechanisms are possible to explain the association of SWS with relatively stable respiration. 1) SWS has a protective effect on UAW patency. 2) The instability of sleep state associated with frequent sleep-disordered breathing events does not allow the individual to achieve SWS. 3) The increase in arousal threshold during SWS contributes to respiratory and UAW stability. There are reasonable arguments for all of these mechanisms. However, it seems clear that, if the patient does achieve SWS, ventilation stabilizes. It has been suggested that the gradual increment in \( P_{\text{CO}_2} \) from stage 2 to SWS (5, 26) may adequately stimulate UAW dilator muscles so that pharyngeal patentcy can be maintained (9). Our laboratory has also observed that, with inspiratory resistive loading, there is a delayed (60–90 s) increment in genioglossus (GG) muscle activation compatible with a chemical (\( P_{\text{CO}_2} \)) stimulus (37). Finally, Badr et al. (1) reported variable responses of the GG electromyogram (EMG) to induced hypercapnia among seven subjects during non-rapid-eye-movement (NREM) sleep. However, in both of these studies (1, 37), the muscle responsiveness to rising \( \text{CO}_2 \) may have been confounded by a simultaneous, progressively negative epiglottic pressure because subjects

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Address for reprint requests and other correspondence: D. P. White, RP 485, 221 Longwood Ave., Brigham and Women’s Hospital, Boston, MA 02115 (E-mail: dwhite@grc.bwh.harvard.edu).
slept in the supine posture. The one patient with a substantial increase in GG EMG in the Badr et al. study (1) had a very large negative esophageal pressure (−30 cmH₂O) while inspiratory resistive loading in the Wieck et al. study (37) is known to result in increasingly negative epiglottic pressure (although not directly measured in that study). Thus, to date, the isolated relationship between PCO₂ and pharyngeal dilator muscle activity during sleep has not been fully examined. We hypothesized that dilator muscles are sensitive to chemostimulation (PCO₂) during sleep and that hypercapnia will result in increased dilator muscle activation. We also hypothesized that hypercapnia-induced dilator muscle activation may protect UAW patency during sleep. Thus the present protocol was designed to address, in normal subjects, the following questions.

Does the transition from stage 2 to SWS lead to important increments in end-tidal PCO₂ (PETCO₂)? In order for the hypercapnia of SWS to mediate a protective effect on pharyngeal patency, a measurable change in PCO₂ would seem necessary.

Does SWS provide a protective influence on UAW patency through activation of pharyngeal dilator muscles? By measuring the activity of both a representative phasic dilator muscle (i.e., GG) and a tonic one [i.e., tensor palatini (TP)], we can evaluate whether the protective effect of SWS is mediated through dilator muscle activation.

Do supraphysiologic levels of hypercapnia drive pharyngeal dilator muscle activation during NREM sleep (particularly SWS)? By administering CO₂, we determined the responsiveness of these muscles to rising PCO₂ in both sleep stages.

METHODS

Subjects

Eighteen historically healthy subjects were studied [9 men, 9 women, age 27.7 ± 1.3 (SE) yr, body mass index 22.9 ± 0.5 kg/m²]. Subjects denied any chronic diseases, daytime somnolence, or snoring. None had any pharyngeal anatomic abnormality on physical examination. The study was approved by the Brigham and Women’s Human Subjects Review Committee, and the subjects gave written, informed consent before participation in the study.

Instrumentation and Techniques

Ventilation. Subjects wore a nasal mask (Healthdyne Technologies, Marietta, GA) connected to a two-way valve that partitioned inspiration and expiration. Inspiratory flow was determined with a pneumotachometer (Fleish, Laussanne, Switzerland) and a differential pressure transducer (Validyne, Northridge, CA), calibrated with a rotameter. The subject’s breathing was exclusively nasal, ensured by mouth taping and video camera monitoring to document that the mouth remained closed. The dead space of the mask system was about 50 ml, depending on facial configuration. Tidal volume (VT) was obtained from the integrated flow signal, and minute ventilation (Ve) was calculated as the sum of all VT per minute.

Muscle activation. GG EMG was measured with a pair of unipolar intramuscular electrodes referenced to a single ground, thus producing a bipolar recording. Two stainless steel, Teflon-coated, 30-gauge wire electrodes were inserted 15–20 mm into the body of the GG muscle 3 mm lateral to the frenulum on each side, using a 25-gauge needle that was quickly removed, leaving the wires in place. TP EMG was also measured, with a pair of referenced, unipolar, intramuscular electrodes producing a bipolar recording. On each side of the palate, the tip of the pterygoid hamulus was located at the junction of the hard and soft palates. A 25-gauge needle with a 30-gauge, stainless steel, Teflon-coated wire was then inserted at a 45° angle along the lateral surface of the medial pterygoid plate, to a depth of ~10–15 mm into the palate. The needle was then removed, leaving the electrode in place. These techniques have been used previously in our laboratory (18). To confirm electrode placement, the following respiratory maneuvers, which have previously been shown to activate the TP muscle, were performed: sucking, blowing, and swallowing (35, 36).

For both muscles, the raw EMG was amplified, band-pass filtered (between 30 and 1,000 Hz), rectified, and electronically integrated on a moving-time-average basis, with a time constant of 100 ms (CWE, Ardmore, PA). The EMG was quantified as percentage of maximal activation. To define maximal muscle EMG activity, subjects performed four maneuvers. They were instructed to 1) maximally inspire against an occluded tube, 2) maximally protrude their tongue against the maxillary alveolar ridge, 3) swallow, and 4) suck and blow. Each maneuver was performed several times, and the maximal EMG recording for each muscle during this calibration was assigned a level of 100%. Electrical zero was then determined, and, thereafter, each EMG was quantified as a percentage of maximal activation for that individual.

Because GG is an inspiratory phasic muscle, its level of activation was assessed at two points in the respiratory cycle. The tonic activation was defined as the lowest EMG level during expiration (the minimal activation in each breath), and peak phasic EMG was defined as the maximal activation during inspiration. As TP is a tonic muscle, without phasic activation, the EMG is reported as the average activation across each breath.

To ensure that recording time or duration did not affect EMG responsiveness, two actions were taken. First, the EMG activation in response to naturally occurring swallows was assessed for TP and GG in each subject during the first and last 15-min period of each recording. In each condition, electrical zero was also recorded to ensure no drift in EMG signal. Second, we studied three additional subjects in a modified protocol. This included GG EMG measurements in six conditions: basal breathing and hypercapnia while awake, basal breathing and hypercapnia during stable NREM sleep, and basal breathing and hypercapnia awake again, at the end of the study (after 2–5 h of recordings).

Polysomnography. Wakefulness and/or sleep was documented with two channels of electroencephalography (C3-A2, C4-O1), two channels of electrooculography, and submental EMG. Sleep stages were scored using standard criteria (24). Subjects maintained the lateral decubitus posture throughout the study, as verified by video camera. We chose to study all subjects in the lateral posture to minimize changes in pharyngeal resistance and epiglottic pressure during sleep. This was done to assess the relatively isolated effects of hypercapnia on muscle activation.

Pressure and resistance. Pressures were monitored in the nasal mask (Validyne) and in the airway at the level of the choanae and the epiglottis. One nostril was decongested with oxymetazoline HCl and anesthetized using lidocaine HCl. Two pressure-tipped catheters (MPC-500, Millar, Houston,
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TX) were inserted through this nostril and localized to measure choanal and epiglottic pressures. Before insertion, all three pressure signals were calibrated simultaneously in a rigid cylinder using a standard water manometer. These three signals, plus flow, were demonstrated to be without amplitude or phase lags at up to 2 Hz. Pharyngeal resistance (the pressure difference between choanae and epiglottis divided by flow), nasal resistance (the pressure difference between mask and choanae divided by flow) and supraglottic resistance (nasal resistance + pharyngeal resistance) were determined at both peak flow and 0.2 l/s inspiratory flow.

CO₂ administration and PETCO₂ measurement. PETCO₂ was measured from expired air sampled within the mask using a calibrated infrared CO₂ analyzer (Capnograph Monitor, BCI, Waukesha, WI). To assess the hypercapnic response, the inspired fraction of CO₂ was increased using a calibrated gas source (25% CO₂-21% O₂-balance N₂) fed into the inspiratory line, to achieve an PETCO₂ of 5–6 Torr above eucapnic basal levels. This level was reached and remained stable for 3 min, data were recorded for 3 min.

Study Protocol

Subjects reported to the laboratory in the evening, having abstained from food for at least 4 h. After informed consent was obtained, all instrumentation was performed, and the equipment was calibrated. Data were then recorded during basal wakefulness (see Fig. 1) for a period of 5 min. Subjects were then allowed to fall asleep. Once stable stage 2 sleep was observed, 5 min of nasal breathing were recorded. If subjects awakened during the recordings, these data were excluded and another 5-min period was recorded after stable stage 2 sleep was again achieved. After subjects entered SWS, an additional 5 min of recording took place. Two subjects did not reach SWS. Finally, CO₂ was administered to elevate PETCO₂ to 5–6 Torr above baseline levels during sleep (see Fig. 1). In 9 of the 18 subjects, CO₂ administration was performed during SWS, whereas CO₂ was delivered to 7 subjects during stage 2 sleep. Recordings of supraphysiologic hypercapnia were performed after a steady-state level of PETCO₂ with no arousals was reached. The time interval between recording of baseline and CO₂-stimulated muscle activation was, on average, 31.7 min. In two subjects, CO₂ administration could not be completed due to repetitive awakenings.

Data Recording and Analysis

All signals (electroencephalogram, electrooculogram, submental EMG, inspiratory flow, PETCO₂, GG EMG and TP EMG) were recorded on a 16-channel Grass model 78 polygraph (Grass Instruments, Quincy, MA). Certain signals (VT, Ve, PETCO₂, muscle EMG, and inspiratory flow) were also recorded onto computer using signal-processing software (Spike 2, Cambridge Electronic Design, Cambridge, UK). Sampling frequency was 125 Hz.

For each recording period (awake, stage 2, SWS, CO₂ administration) all breaths from each 5-min recording (3 min in the administered CO₂ portion) were signal averaged. Thus, for each state, VT, PETCO₂, GG EMG (tonic and peak phasic) and TP EMG (tonic only) were determined from this averaged breath. Ve, as stated, was determined by summing all VT values per minute.

All statistical analyses were performed with commercially available software (Excel 97, Microsoft; and SigmaStat + Sigmaplot, SPSS, Chicago, IL). All data are presented as means ± SE unless otherwise stated. Repeated-measures ANOVA with post hoc Student-Newman-Keuls testing was used to assess state-dependent changes. Whenever data were not normally distributed, Friedman repeated-measures ANOVA on ranks was used. P < 0.05 was taken to indicate significance.

RESULTS

Ventilation, PETCO₂, UAW resistances, and activation levels of both dilator muscles in the three states are shown in Table 1. Ve decreased significantly from wakefulness to stage 2 sleep, and further to SWS, although this further decline was not statistically significant. Although PETCO₂ increased significantly from wakefulness to stage 2 sleep (P < 0.05), and further

<p>| Table 1. Ventilation, airway mechanics, and muscle activation in different sleep stages |
|---------------------------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Awake</th>
<th>Stage 2</th>
<th>SWS</th>
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<tbody>
<tr>
<td>Ve, l/min</td>
<td>9.4 ± 0.4</td>
<td>8.6 ± 0.3</td>
<td>8.1 ± 0.3</td>
</tr>
<tr>
<td>Tidal volume, ml</td>
<td>595 ± 22</td>
<td>563 ± 25</td>
<td>543 ± 31</td>
</tr>
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<td>Respiratory rate, breaths/min</td>
<td>15.7 ± 0.7</td>
<td>15.3 ± 0.5</td>
<td>14.8 ± 0.6</td>
</tr>
<tr>
<td>VT/Ttot, %</td>
<td>42.5 ± 1.8</td>
<td>43.8 ± 1.6</td>
<td>43.3 ± 1.5</td>
</tr>
<tr>
<td>PETCO₂, Torr</td>
<td>39.3 ± 0.7</td>
<td>43.3 ± 0.6</td>
<td>44.1 ± 0.5</td>
</tr>
<tr>
<td>GG tonic, % of maximum</td>
<td>5.9 ± 1.3</td>
<td>5.9 ± 1.2</td>
<td>5.6 ± 1.2</td>
</tr>
<tr>
<td>GG peak, % of maximum</td>
<td>7.4 ± 1.3</td>
<td>7.4 ± 1.3</td>
<td>7.7 ± 1.5</td>
</tr>
<tr>
<td>TP, % of maximum</td>
<td>8.1 ± 1.9</td>
<td>4.8 ± 1.0</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>P choanal, cmH₂O</td>
<td>−1.5 ± 0.1</td>
<td>−2.0 ± 0.4</td>
<td>−1.9 ± 0.2</td>
</tr>
<tr>
<td>P epiglottic, cmH₂O</td>
<td>−1.9 ± 0.1</td>
<td>−3.1 ± 0.5</td>
<td>−4.3 ± 0.8</td>
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<tr>
<td>Peak flow, l/s</td>
<td>0.48 ± 0.02</td>
<td>0.50 ± 0.03</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>Resistance</td>
<td>1.2 ± 0.2</td>
<td>2.3 ± 0.8</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Pharyngeal</td>
<td>0.7 ± 0.2</td>
<td>2.5 ± 0.8</td>
<td>4.8 ± 1.0</td>
</tr>
<tr>
<td>Supraglottic</td>
<td>1.9 ± 0.2</td>
<td>4.8 ± 1.2</td>
<td>6.2 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. SWS, slow-wave sleep; Ve, minute ventilation; Ti, inspiratory time; Ttot, total time; PETCO₂ end-tidal PCO₂; GG, genioglossus muscle; TP, tensor palatini muscle; P, pressure. *P < 0.05 vs. awake; †P < 0.05, stage 2 vs. SWS.
from stage 2 to SWS ($P < 0.05$), no significant change in GG EMG was observed. TP EMG decreased significantly from wake to stage 2 sleep but did not change from stage 2 to SWS. Pharyngeal resistance significantly increased from wakefulness to sleep and tended to increase further from stage 2 to SWS, although this change did not reach statistical significance. There was no correlation between the change in pharyngeal resistance and the change in ventilation from wakefulness to stage 2 sleep, but there was a significant correlation between the change in these variables from stage 2 sleep to SWS ($r = 0.55$, $P < 0.05$). Nasal resistance did not change significantly between conditions.

Despite significant increases in $V_E$ with induced hypercapnia ($8.3 \pm 0.1$ to $11.9 \pm 0.3$ l/min in stage 2, and $8.6 \pm 0.4$ to $12.7 \pm 0.4$ in SWS, $P < 0.05$ for both), there was no change in the GG EMG or the TP EMG (Table 2). One example, 30 s of raw data from stage 2 sleep, SWS, and during hypercapnia in SWS, is shown in Fig. 2. As can be seen, hypercapnia was associated with substantial increases in ventilation but no important change in muscle activation. Figure 2 also demonstrates the phasic nature of the GG and the tonic nature of the TP.

As stated in METHODS, to ensure EMG signal stability, CO$_2$ responsiveness was assessed in three subjects, awake at the beginning of the study, during stable NREM sleep, and during wakefulness thereafter. Adequate data were obtained in two subjects. As shown in Fig. 3 (data from one representative subject), both ventilation and GG EMG increased in response to CO$_2$ during wakefulness on both occasions (before and after NREM sleep), but little to no GG EMG response was observed during NREM sleep. This suggests stable signals throughout the recordings. In addition, no consistent changes were observed in the response of either the GG or TP to spontaneous swallows over the course of the study. Average GG EMG during a swallow was $59.1 \pm 13\%$ of maximum during the first 15 min vs. $57.5 \pm 12.2\%$ during the last 15-min period (not significant). Average TP EMG was $56.8 \pm 17.7\%$ during the first 15 min vs. $55.2 \pm 20.3\%$ of maximum during the last 15-min period. Therefore, EMG responsiveness to spontaneous swallows was as robust at the end of the study as at the beginning. Thus we believe we had a stable EMG signal. Finally, two subjects occasionally snored, but no evidence of inspiratory flow limitation was observed in the buffered (signal-averaged) breath.

**DISCUSSION**

This study suggests that, in normal subjects, pharyngeal dilator muscle activation is not importantly modulated by CO$_2$ during either SWS or stage 2 sleep. Although PETCO$_2$ did increase significantly from stage 2 sleep to SWS, it was not associated with an increase in activation of either tonic or phasic pharyngeal dilator muscles. Even with supraphysiological levels of CO$_2$ that were clearly effective in increasing ventilation, dilator muscle activation did not change significantly. These data strongly suggest that hypercapnia alone is not a strong stimulus to pharyngeal dilator muscle activation during NREM sleep.

GG EMG did not change from wake to sleep; however, TP activation fell significantly. These observations are generally in agreement with previous studies in normal humans, which have demonstrated a substantial fall in TP EMG with the change from wake to sleep but highly variable changes in GG EMG with state changes (18, 31, 32). Hypercapnia has previously been shown to be a potent stimulator of ventilation (7) and leads to increases in GG EMG during wakefulness (22). However, GG responsiveness to CO$_2$ has not been tested during sleep in humans. As stated, we observed that induced hypercapnia during sleep increased ventilation but failed to increase pharyngeal dilator activation. When this observation is added to the previous reports that document negative pressure stimuli activating UAW muscles during wakefulness but not during sleep (11, 35), we have to conclude that pharyngeal dilator muscles are generally unresponsive to either mechanoreceptive or chemoreceptive stimuli during sleep. In apnea patients, although increments in pha-
Ryngeal dilator muscle activation have been observed over the course of an apnea (as intrapharyngeal pressure becomes progressively negative and hypoxia plus hypercapnia develop), the majority of such muscle activation occurs with arousal at apnea termination (3, 19). This may explain the necessity for sleep apneics to arouse to regain pharyngeal patency. In other words, because pharyngeal dilators fail to adequately respond to respiratory stimuli during sleep, arousal from sleep is required to terminate sleep-disordered breathing events.

Although individuals with sleep apnea were not studied, the lack of response of pharyngeal dilator muscles to hypercapnia (physiological and supraphysiological levels) does not support the hypothesis that SWS-induced hypercapnia drives muscle activation and thereby protects UAW patency. Although the study of Basner et al. (2) previously reported increased GG activation in five subjects during SWS compared with stage 2 sleep (without significant change in PETCO₂ or ventilation), we could not replicate the results of that study. In fact, in our group of 16 subjects, PETCO₂ significantly increased from stage 2 to SWS without activation of either GG or TP. However, the subjects of Basner et al. (2) were studied in the supine posture, whereas ours were in the lateral decubitus posture, which may have influenced airflow resistance, epiglottic negative pressure, and muscle activation. Henke et al. (9) also reported an increase in PETCO₂ from stage 2 sleep to SWS, in association with an increase in the EMG of ventilatory muscles (diaphragm and scalene) in five snorers (measured with surface electrodes). Flow limitation was noted in both stage 2 and SWS, and the change from stage 2 to SWS was associated with a significant increase in pharyngeal resistance. When patients were unloaded by con-

![Figure 2. Representative example in a single subject: 30 s of raw data collected during stage 2 sleep (left), SWS (middle), and CO₂ administration (hypercapnia; right) during SWS are shown. As can be seen in this case, end-tidal PETCO₂ increased from during stage 2 sleep, SWS, and with hypercapnia. This was associated with a substantial increase in ventilation (only inspiratory tidal volume is shown) but no change in genoglossus (GG) or tensor palatini (TP) muscle activation. The data also demonstrate the phasic nature of GG and the tonic nature of TP. max, Maximum.](http://jap.physiology.org/Downloaded from 12.220.33.6 on July 10, 2017)
continuous positive airway pressure application, both PETCO$_2$ and ventilatory muscle activation declined. When CO$_2$ was added to restore eucapnia (with continuous positive airway pressure in place), EMG increased toward baseline levels, suggesting some effect of CO$_2$ on scalene and diaphragm activity in snorers. Pharyngeal dilator muscles, however, were not monitored in that study.

Interestingly, in animal models, induced hypercapnia resulted in decreased pharyngeal airflow resistance and increased EMG of the GG and ala nasi (27). This decrease in resistance was also observed in cats, independent of GG or strap muscle activation (25). Other studies in animals have also found a reduction in airway resistance with induced hypercapnia (20). However, these studies were not conducted in humans and not during sleep, making it difficult to compare with our observations. The one study that did measure GG EMG in humans with induced hypercapnia found highly variable responses. In the single subject from that study for whom raw data were presented, esophageal pressure became extremely subatmospheric (~30 cmH$_2$O, in the supine posture), and there was a robust response of GG EMG (1). Thus hypercapnia and airway negative pressure could potentially work in combination to activate pharyngeal dilators.

The changes in ventilation and in UAW resistance observed in the present study are generally in agreement with previous findings. We observed that the change from wakefulness to sleep was associated with an increase in UAW resistance, a decrease in ventilation, and an increase in PETCO$_2$ (4, 5, 7, 9, 12, 13, 23, 26). Increased pharyngeal resistance is likely a substantial contributor to the fall in ventilation from wake to sleep (9, 30). It is not surprising, however, that the correlation between the change in pharyngeal resistance and ventilation in this transition was weak, as many other changes in respiratory control likely occurred as well, thus making the isolated effect of pharyngeal resistance on $V_e$ difficult to detect. We observed a further increment in PETCO$_2$ with the change from stage 2 to SWS, although the trend toward decreases in ventilation and increases in UAW resistance did not reach statistical significance. Several previous studies have reported similar observations (5, 7, 9, 26, 33). Unlike the transition from wakefulness to stage 2 sleep, the decrement in $V_e$ from stage 2 to SWS was significantly correlated with the increment in pharyn-
geal resistance. This suggests that, in the absence of the behavioral influences present during wakefulness, changes in pharyngeal resistance with state change play a substantial role in determining the associated change in ventilation (9, 30, 33).

Our observation that induced hypercapnia leads to an increment in ventilation with no significant change in pharyngeal resistance (Table 2) is in contrast to previous studies that have reported hypercapnia to reduce pharyngeal resistance (16, 28). However, Badr et al. (1), using total pulmonary resistance as an index of UAW patency, found no significant change in this measure with +2, +4, and +6 Torr increments in $P_{CO_2}$ during NREM sleep in nine subjects, although there was a trend toward a decrease in total pulmonary resistance with $P_{CO_2}$ 6 Torr above baseline (1). In anesthetized animals, however, airway resistance decreased with induced hypercapnia (20, 25, 27). The most plausible explanation for this observation is that elevated $P_{CO_2}$ levels (in combination with negative pharyngeal pressure) lead to increased pharyngeal dilator muscle activation or tracheal caudal displacement (1, 20, 34). Our finding that pharyngeal resistance did not decrease with induced hypercapnia may be a result of our subjects’ sleeping in the lateral decubitus posture. In the lateral position, airway resistance tends to be lower, and thus the negative pressure generated by inspiratory muscles is reduced. If a combination of negative airway pressure and elevated $P_{CO_2}$ is required to activate the pharyngeal dilator muscle during NREM sleep, one would expect more muscle activity during hypercapnia in the supine posture. However, we wanted to assess the isolated effect of hypercapnia and observed little such effect in our subjects sleeping in the lateral posture.

There are several potential limitations to our study. First, although our intention was to provide insights into the pathogenesis of obstructive sleep apnea by studying only normals, any conclusions regarding patients with obstructive sleep apnea are speculative. However, because of the fragmented sleep seen in individuals with apnea and their minimal SWS, assessment of muscle activation and chemosensitivity during stable sleep states would have been exceedingly difficult to accomplish. Second, because of the long-time constants of central chemoreceptors, hypercapnic stimulation cannot be meaningfully assessed during wake-sleep transitions, which is why stable sleep was selected for this study. However, as stated, such stable sleep is not commonly encountered in individuals with apnea. Third, we did not directly measure lung volume in this study, and it could be argued that changes in lung volume may change the mechanics of the UAW and pharyngeal dilator muscle activation. Fourth, there is the possibility that, after 2–3 h of recording, our electrode sensitivity was reduced and actual increments in muscle activity with incremental $P_{CO_2}$ were not observed. However, the data in Fig. 3 suggest that robust increments in GG EMG can be observed with rising $P_{CO_2}$ hours after the electrodes were placed. Our laboratory has similarly reported (using the same equipment in the same laboratory as the present study) the muscle responsiveness to negative pressure pulses to be easily demonstrable 3–5 h after electrode placement (29), suggesting no deterioration in our ability to measure muscle responsiveness. Thus we do not believe this represents a problem. Finally, although we did not observe a substantial dilator muscle activation in response to hypercapnia during SWS, we cannot rule out the possibility that hypercapnia could protect UAW patency during SWS via different mechanisms, such as changes in parapharyngeal blood flow (21) or lung volume (10).

In conclusion, we believe that our results demonstrate pharyngeal dilator muscles to be largely unresponsive to hypercapnia during NREM sleep (stage 2 and SWS). The lack of responsiveness of these muscles to physiological $CO_2$ levels, supraphysiological $CO_2$ levels, and negative pharyngeal pressure during NREM sleep may explain the necessity of apnea patients to arouse from sleep to terminate a sleep-disordered breathing event.

We thank Yvonne J. Gilreath for assistance.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-48531 and HL-60292 and National Center for Research Resources Grant RR-02635. In addition, G. Pillar received a Fulbright grant to conduct this research.

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