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Effects of hypoxia on genioglossus and scalene reflex responses to brief pulses of negative upper-airway pressure during wakefulness and sleep in healthy men

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Eckert DJ, McEvoy RD, George KE, Thomson KJ, Catcheside PG. Effects of hypoxia on genioglossus and scalene reflex responses to brief pulses of negative upper-airway pressure during wakefulness and sleep in healthy men. *J Appl Physiol* 104: 1426–1435, 2008. First published February 21, 2008; doi:10.1152/japplphysiol.01056.2007.—Hypoxia can depress ventilation, respiratory load sensation, and the cough reflex, and potentially other protective respiratory reflexes such as respiratory muscle responses to increased respiratory load. In sleep-disordered breathing, increased respiratory load and hypoxia frequently coexist. This study aimed to examine the effects of hypoxia on the reflex responses of *1*) the genioglossus (the largest upper airway dilator muscle) and *2*) the scalene muscle (an obligatory inspiratory muscle) to negative-pressure pulse stimuli during wakefulness and sleep. We hypothesized that hypoxia would impair these reflex responses. Fourteen healthy men, 19–42 yr old, were studied on two separate occasions, ~1 wk apart. Bipolar fine-wire electrodes were inserted orally into the genioglossus muscle, and surface electrodes were placed overlying the left scalene muscle to record EMG activity. In random order, participants were exposed to mild overnight hypoxia (arterial oxygen saturation ~85%) or medical air. Respiratory muscle reflex responses were elicited via negative-pressure pulse stimuli (approximately −10 cmH_2O at the mask, 250-ms duration) delivered in early inspiration during wakefulness and sleep. Negative-pressure pulse stimuli resulted in a short-latency activation followed by a suppression of the genioglossus EMG that did not alter with hypoxia. Conversely, the predominant response of the scalene EMG to negative-pressure pulse stimuli was suppression followed by activation with more pronounced suppression during hypoxia compared with normoxia (mean ± SE suppression duration 64 ± 6 vs. 38 ± 6 ms, $P = 0.006$). These results indicate differential sensitivity to the depressive effects of hypoxia in the reflex responsiveness to sudden respiratory loads to breathing between these two respiratory muscles.

respiratory reflexes; suppression; sleep-disordered breathing

IN SLEEP-DISORDERED BREATHING, increased respiratory load and hypoxia frequently coexist. Several recent studies have demonstrated that hypoxia can lead to impairment of a range of vital protective responses, including respiratory load sensation, arousal from sleep to respiratory stimuli, and the cough reflex (12, 13, 17, 30). Depression of respiratory afferent transmission below the level of the cortex appears, at least in part, to mediate hypoxia-induced decrements in respiratory load sensation (11). Together, these findings suggest that other protective respiratory reflexes may be vulnerable to suppression during hypoxia.

Upper-airway (UA) patency is importantly modulated by the balance between downstream respiratory pump muscle activation, creating negative airway pressure, vs. UA dilator muscle activity opposing UA collapsing forces. The genioglossus (gg) is the largest UA dilator muscle and is reflexly activated in response to negative UA pressure in humans during wakefulness (19). Earlier studies in healthy individuals suggested that the response was solely excitatory and was largely attenuated during sleep, thereby potentially contributing to the development of sleep-disordered breathing in individuals with an anatomically narrow airway (18, 38, 42). However, more recent data demonstrate maintenance of gg reflex activity to negative-pressure pulse stimuli in the supine posture during non-rapid eye movement (NREM) sleep (14, 25) and the presence of a state-dependent longer latency reflex suppression, likely inhibitory in origin (14). These findings suggest that the underlying mechanisms of UA reflex activity to negative UA pressure are more complicated than first believed. Notwithstanding, the UA negative-pressure reflex appears to be essential for maintaining UA patency in anatomically compromised airways and for modulating airway size during tidal breathing under normal physiological states (43). Since we have found that hypoxia can depress other protective respiratory reflexes, our primary aim in this study was to determine the effects of hypoxia on the gg negative-pressure reflex.

Unlike the stretch or loading responses in limb muscles, which consist of reflex excitation without suppression (26), the response of human inspiratory muscles (e.g., scalene, parasternal intercostal, and diaphragm) to a sudden increase in respiratory load (or more negative airway pressure) is an initial suppression followed by an increase in EMG activity above baseline levels (3, 7, 32). Inspiratory muscle reflex suppression may play a protective or adaptive role by preventing further increased negative airway pressure (3), thus minimizing the chance of further UA collapse in the presence of any sudden upper obstruction, or by reducing the work of breathing in situations of chronic airflow obstruction (e.g., snoring, asthma) (4, 21). Indeed, measured during wakefulness, recent data show more pronounced reflex suppression of inspiratory muscles to sudden respiratory loading in obstructive sleep apnea

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(OSA) patients and a positive correlation between reflex suppression and the respiratory disturbance index (21). Thus a further aim of this study was to examine the effects of hypoxia on inspiratory muscle responses to negative-pressure stimuli presented in wakefulness and sleep.

We hypothesized that hypoxia would suppress the gg UA negative-pressure reflex and impair inspiratory muscle reflex responses to negative-pressure pulse stimuli. The morphology of the EMGgg negative-pressure reflex from the normoxia experiments in this study has been described in detail previously (14).

MATERIALS AND METHODS

Subject Selection

Twenty-one young healthy nonsmoking men, without a history of respiratory disease, sleep-disordered breathing, or regular medication use and with baseline forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) >80% predicted gave informed written consent to participate in the study. The study was approved by the Daw Park Repatriation General Hospital and Adelaide University Human Research and Ethics Committees.

Measurements and Equipment

Electroencephalograms (C3 and C4), left and right electrooculograms, and submental EMG were applied for sleep staging and arousal scoring. Both nostrils were decongested with xylometazoline hydrochloride nasal spray (Otrivin, Novartis Australasia, Rowville, Victoria, Australia) and anesthetized (2% lignocaine, 2 sprays <1 ml total dosage). The half-life of this agent is ~10 min. Two custom-made air-perfused catheters were inserted via the most patent nostril and attached to pressure transducers (MP45, Validyne Engineering, Northridge, CA). One catheter was advanced to the epiglottis 1–2 cm below the base of the tongue under direct visualization (Pepi), the other to the level of the choanae (Pcho). After surface anesthesia (4% lignocaine), two fine-wire Teflon-coated intramuscular electrodes (316SS3T wire, Medwire, Mt. Vernon, NY) were inserted ~4 mm either side of the frenulum to a depth of approximately 1–1.5 cm to measure genioglossus EMG activity (EMGgg). Surface electrodes were also placed overlying scalene (sc), parasternal intercostal, and diaphragm muscles as described previously (3). Each subject was fitted with a nasal mask (Gel mask, Respironics, Murrysville, PA) with a two-way nonrebreathing valve attached (series 2600, Hans Rudolph, Kansas City, MO), and his mouth was taped throughout the sleep period. An additional pressure transducer was fitted to the mask (Pmask). Ear pulse oximetry and continuous sampling of the expirate were used to determine arterial oxygen saturation (Sa_{O_2}) and end-tidal partial pressure of CO_2 ($P_{ET}{}_{CO_2}$, POET II model 602-3 Criticare Systems, Waukesha, WI), respectively. ECG was measured continuously. A pneumotachograph (PT36, Erich Jaeger) on the inspiratory side of the breathing valve was used to monitor inspiratory flow and calculate ventilatory parameters. UA negative-pressure pulses (Pmask approximately −10 cmH_2O, 250-ms duration) were delivered during early inspiration via a computer-controlled rapid actuating solenoid valve system (Iso star, SXE9575-A70-00, Norgren, Switzerland). A schematic of the breathing circuit is displayed in Fig. 1. Negative-pressure pulse delivery was controlled via custom-written software that continuously monitored the inspiratory flow signal and triggered solenoid valve switching during early inspiration when flow reached 2 l/min (e.g., Fig. 2). In addition, the software continuously monitored the ECG signal and suppressed pulse delivery during QRS activation to avoid ECG artifact contamination of surface EMG reflex recordings. Pulses were delivered at random during stable breathing every 2–10 breaths.

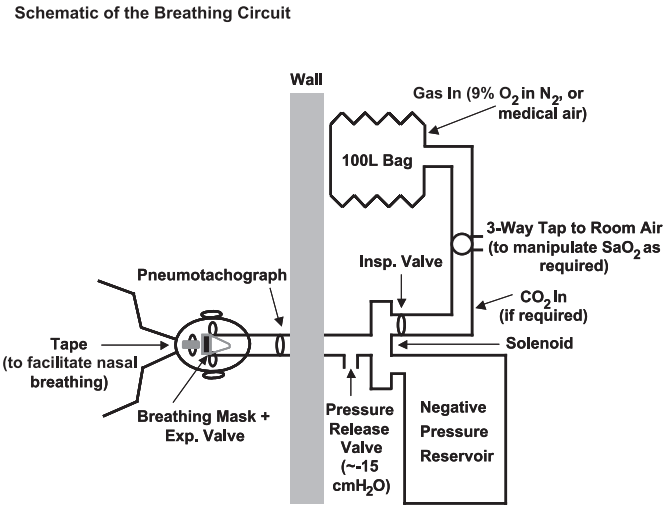

Fig. 1. Schematic of the breathing circuit used to deliver negative-pressure pulse stimuli and experimental gas conditions. Refer to text for further detail. Insp, inspiratory; Exp, expiratory; Sa_{O_2}, arterial oxygen saturation.

Data were acquired simultaneously on two separate recording systems. A Compumedics system (E series, Abbotsford, Victoria, Australia) was used to determine sleep stage and to score arousals. All other data were acquired using a Windaq data-acquisition system (DI-720 DATAQ Instruments). To capture fast-frequency reflex components and synchronize key stimulus magnitude parameters for event-related analysis, inspiratory flow, ECG, EMG, and pressure channels were band-pass filtered (30–1,000 Hz) and sampled at 2 kHz. The remaining channels not directly used for reflex and event-related timing purposes were sampled at 200 Hz. An event mark was simultaneously placed on both recording systems coincident with solenoid activation of each pulse allowing both data-acquisition systems to be synchronized.

Protocol

Preliminary visit. Initially, subjects attended a preliminary visit during the day for familiarization with the testing environment, recording equipment, and staff and to obtain informed consent. Spirometry was performed to ensure normal lung function (JLab software version 4.53; Compactlab, Jaeger, Wuerzburg, Germany).

Main experimental visits. On two separate occasions, ~1 wk apart, subjects arrived at the laboratory 2.5 h before their usual bedtime. Subjects abstained from alcohol and caffeine for at least 12 h before each visit. Once all the sensors and equipment were fitted, several negative-pressure pulses were delivered for familiarization purposes. The lights were then switched off, and subjects were given the opportunity to sleep. Subjects were asked to lie on their backs throughout the study. In the event that subjects became uncomfortable maintaining the supine posture during the night, they were given the opportunity to stretch before returning to sleep on their backs.

After at least 5 min of stable stage 2 sleep, subjects were randomly allocated to breathe either a normoxic or an isocapnic hypoxic gas mixture throughout the night. During normoxia trials, subjects breathed via a circuit attached to a 100-liter reservoir bag filled from compressed dry medical air. During hypoxia trials, the bag was filled from compressed dry ~9% O_2 in N_2, and the inspired O_2 fraction was adjusted as necessary by adding room air to the breathing circuit via a three-way tap to maintain Sa_{O_2} at ~85%. A manual inspiratory bleed of CO_2 was employed as necessary to ensure isocapnia (Fig. 1). Subjects remained blinded to the test gas condition. Following 15 min of sleep under each gas condition, UA negative-pressure pulses were delivered every 2–10 breaths during stable sleep. Thus reflex data were not collected until after the topical anesthetic agents should have

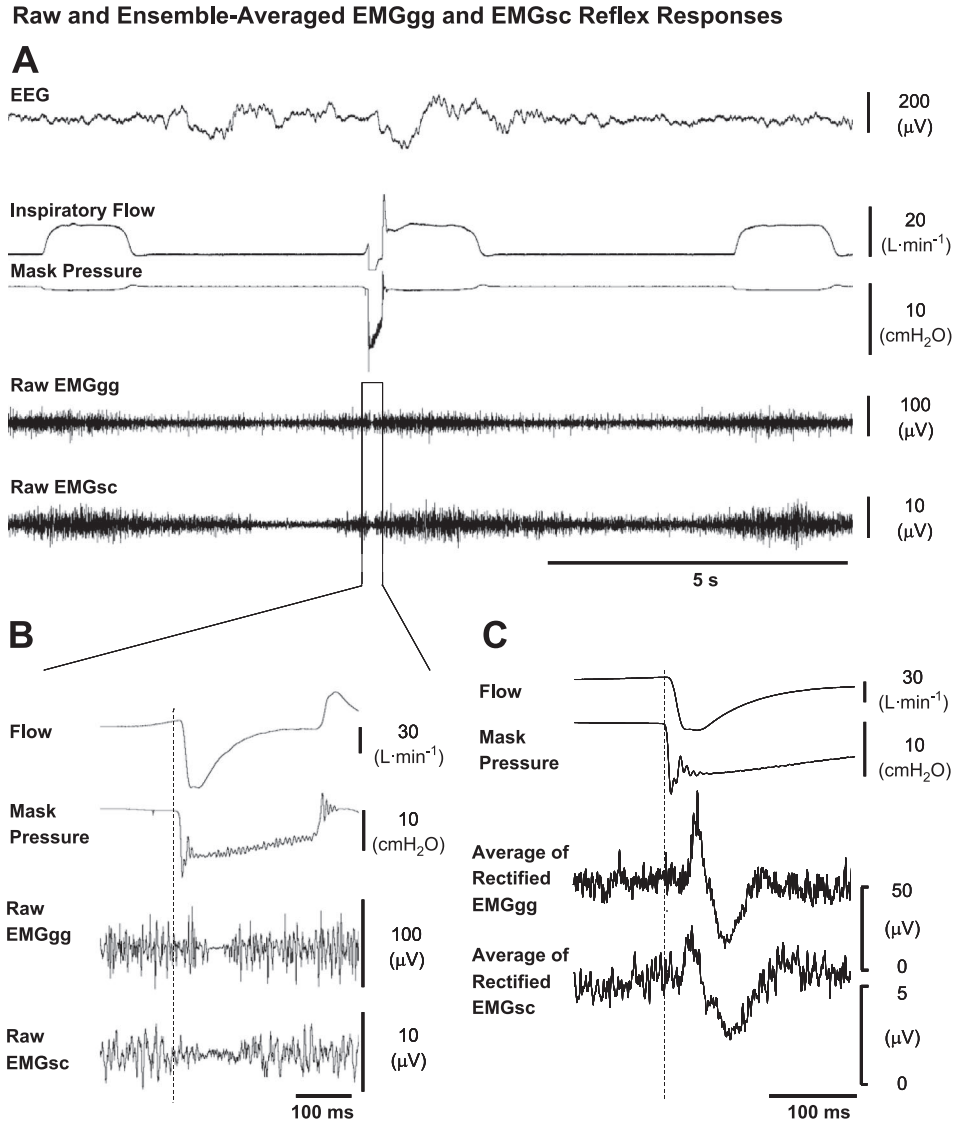

Fig. 2. *A*: example tracings of electroencephalogram (EEG), inspiratory flow, mask pressure, raw genioglossus EMG (EMGgg), and raw scalene EMG (EMGsc) during a negative-pressure pulse and the breaths immediately before and following pulse application. *B*: an expanded view of flow, mask pressure, and the raw EMGgg and raw EMGsc responses during the negative-pressure pulse. *C*: ensemble-averaged (n = 69 replicate trials) flow, mask pressure, and rectified EMG responses in a representative subject. Vertical dashed lines represent stimulus onset. Example tracings are from a hypoxia experiment during non-rapid eye movement (NREM) sleep.

worn off, at least 90 min after their administration. In the event of an arousal, pulses were ceased until there was at least 1 min of arousal-free sleep. In the event that the subject woke during the night, the subject was given a 5-min opportunity to return to sleep while the experimental gas remained on. However, if the subject was unable to return to sleep within 5 min, the subject was switched back to room air. Once stable sleep was achieved the subject was returned to the experimental gas condition and pulses recommenced following at least 10 min of stable sleep after returning to breathing the experimental gas. Upon awakening the following morning, the test gas remained on and approximately 50–60 pulses were delivered every 2–10 breaths during wakefulness to elicit EMG reflex responses during wakefulness.

Data Analysis

A single trained sleep technician, blinded to the gas condition, defined the presence of arousals and performed sleep staging according to standard criteria (1, 35). Custom-designed software to detect the most rapid change in Pmask during pulse presentation was employed to align each individual pulse to an accurately identifiable and highly reproducible reference point for EMGgg event-related analyses. Briefly, on breaths preidentified as having a negative-pressure pulse presented, the software identified the point in Pmask at which the rate of change in pressure was most negative. This point was then used to time-align all replicate pulses for ensemble averaging. Stimulus onset (*time 0*) was defined in the conventional manner as the last point preceding the sudden decrement in the ensemble-averaged Pmask following solenoid activation. Negative-pressure pulse stimulus magnitude was calculated as the minimum pressure after the initial "ringing" observed in the pressure channels as described previously (14). Stimulus rise-time was quantified as the time from the first sudden deflection in Pmask to the nadir of Pmask.

For each subject, all EMG trials free from swallows or movement artifact and, during sleep, from arousal (no arousal in the 1 min before application of the negative-pressure pulse and within the 700 ms immediately after the application of the pulse) were grouped and ensemble-averaged according to sleep stage and gas condition. The three states examined were *1*) wakefulness, *2*) non-rapid eye movement (NREM; stages 2–4 combined), and *3*) rapid eye movement (REM). Raw EMG recordings were full-wave rectified for each subject. Using custom-designed semiautomated software, individual subject's ensemble-averaged, rectified EMG reflex responses were visually inspected to measure the presence, timing, and amplitude of each positive and negative component of the EMG response. Examples of representative EMGgg and EMGsc reflex responses and the

criteria used to define the various reflex characteristics are displayed in Fig. 3. EMG reflex amplitude data were expressed as a percentage of the baseline average EMG activity for the 100-ms preceding pulse onset (14). This approach is similar to that described previously in which reflex amplitude data were expressed as the percent change from the 100-ms preceding period (5, 21). Excitation onset was defined as the point at which the rectified EMG signal crossed baseline before the first sustained (lasting >10 ms) positive EMG peak. Suppression onset was defined as the first point at which the rectified EMG recording crossed the baseline level for a sustained period of >10 ms following the peak of the excitation response if present. The first point at which the rectified EMG returned to baseline levels after the suppression nadir was used to define the cessation of suppression and the onset of the secondary excitation for EMGsc responses.

Ventilatory parameters were calculated on a per-breath basis using custom-designed software only for the breaths immediately preceding each pulse presentation. Ventilatory parameters for these selected breaths were then separated according to condition (hypoxia vs. normoxia) and state (wakefulness vs. NREM sleep) and ensemble-averaged for each subject.

Statistical Procedures

ANOVA for repeated measures was used to examine gas (hypoxia vs. normoxia), state (wakefulness vs. sleep state), and interaction effects for EMG reflex peak amplitudes and timing characteristics (SPSS version 12.1, SPSS, Chicago, IL). Similarly, ventilatory parameters across study periods (wakefulness vs. sleep and between gas conditions) were explored using ANOVA for repeated measures. Where significant ANOVA main effects were observed, post hoc comparisons were performed using Dunn-Sidak adjusted Student's paired *t*-tests (24). Given variable signal dropouts between subjects and conditions, complete data were not obtained for all variables. In these instances, the reasons for data loss and the sample size used for analysis are reported for each variable. Statistical significance was inferred when $P < 0.05$. All group data are reported as means ± SE.

RESULTS

A total of seven subjects did not complete the full study. Four subjects had insufficient sleep on the first experimental visit (3 during hypoxia, 1 during normoxia) and were excluded

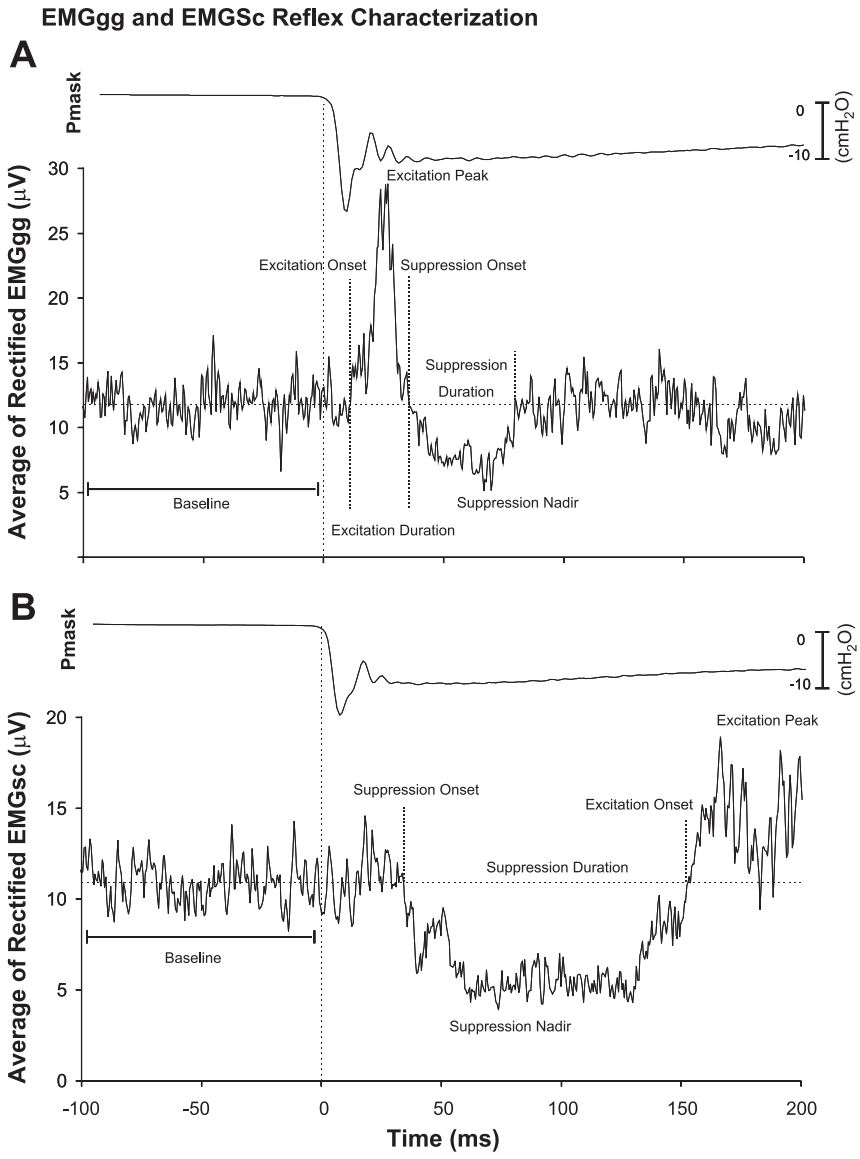

Fig. 3. Characterization criteria used to define EMGgg and EMGsc reflex components and timing properties for each subject under each experimental condition. Tracings represent the ensemble-average of the rectified EMGgg ($n = 75$ replicate trials; *A*) and EMGsc ($n = 57$ replicate trials; *B*) and corresponding mask pressure (Pmask) profiles in a representative subject. Example tracings are from a hypoxia visit during NREM sleep for the EMGgg and during wakefulness for the EMGsc example. Vertical dashed line at *time 0* corresponding to the first rapid dip in Pmask was used to define stimulus onset. Refer to the text for further detail.

from further participation. One subject slept poorly on his second visit (hypoxia) and was unable to return for a repeat visit. One subject was excluded because he demonstrated significant sleep-disordered breathing on the first visit (normoxia). One other subject successfully completed the first visit (normoxia) but was unable to return for his final visit. Thus 14 subjects completed the study protocol.

Anthropometric Characteristics and Sleep Architecture

The mean age and the body mass index for the 14 subjects studied were 24 ± 2 yr and 24 ± 1 kg/m^2, respectively. Subjects had normal lung function (mean FEV_1 102 ± 4 and FVC 107 ± 4% of predicted). All subjects were able to successfully sleep in the supine posture for the entire data collection period. The background resistance of the breathing circuit was 2.50 ± 0.02 $cmH_2O \cdot l^{-1} \cdot s$. Epiglottic pressure catheters were prone to blockage and did not provide reliable recordings in most subjects. Of the limited data available under both gas conditions, stimulus intensity at the level of the epiglottis was similar during normoxia and hypoxia during wakefulness (−6.7 ± 1.1 vs. −5.7 ± 0.9 cmH_2O, $P = 0.969$; $n = 4$ subjects) and NREM sleep (−8.2 ± 0.6 vs. −8.3 ± 1.9 cmH_2O; $n = 2$ subjects). There were no differences in sleep architecture variables between gas conditions (Table 1).

Ventilatory Characteristics

The ventilatory characteristics immediately before pulse presentation during wakefulness and NREM sleep are displayed in Table 2. By design, Sa_{O_2} was significantly lower during hypoxia experiments. There were no other significant gas or gas-by-state interaction effects in any other ventilatory parameter. During NREM sleep, minute ventilation and tidal volume were significantly reduced compared with wakefulness. $P_{ET}CO_2$ levels increased, and there was a small increase in breathing frequency from the waking level (Table 2).

Reflex Responses to Brief Pulses of Negative Pressure

Genioglossus negative-pressure reflex. EMGgg reflex data during wakefulness under both gas conditions were not available in three subjects. Post hoc sleep staging revealed that one subject spent the majority of the wakefulness data collection period drifting in and out of stage 1 sleep such that there were insufficient replicate trials to generate rectified EMG reflex responses during wakefulness. In two subjects one of the EMGgg intramuscular electrodes was dislodged before wakefulness measures (1 during a cough on waking in the morning, the other on removal of the mouth tape in the morning). Consequently, data for analysis of EMGgg reflex activity were available in 11 subjects. The number of artifact-free stimuli, EMGgg peak reflex amplitudes, timing, and stimulus properties during wakefulness and NREM sleep are summarized in Table 3. For this analysis, similar numbers of pulses were presented during normoxia and hypoxia in wakefulness (62 ± 3 vs. 59 ± 2, $P = 0.360$) and in NREM sleep (79 ± 8 vs. 73 ± 7, $P = 0.447$). Negative-pressure pulse stimuli resulted in a short-latency peak followed by prolonged suppression of the rectified EMGgg in the normoxia and hypoxia experiments during wakefulness and NREM sleep. Phasic EMGgg activity was observed in all of these subjects (e.g., Fig. 2). The baseline average EMGgg activity in the 100 ms before pulse onset was

Table 1. *Sleep architecture data*

	Normoxia	Hypoxia	*P* Value
SOL, min	19±6	19±2	0.906
TST, min	241±12	247±8	0.633
Sleep efficiency, %	71±4	68±3	0.402
Stage 1, %TST	12±5	15±3	0.319
Stage 2, %TST	53±3	56±3	0.319
SWS, %TST	30±4	25±3	0.096
REM, %TST	5±1	4±1	0.241
AI, arousals/h	22±3	23±4	0.634

Data are means ± SE; $n = 14$. SOL, sleep onset latency; TST, total sleep time; SWS, slow wave sleep; REM, rapid eye movement sleep; AI, arousal index.

Table 2. *Group mean ventilatory characteristics immediately before stimulus presentation during wakefulness and NREM sleep*

	Normoxia		Hypoxia	
	Awake	NREM	Awake	NREM
$\dot{V}_I$, l/min	9.3±0.3	7.1±0.3†	9.6±0.5	7.8±0.4†
V_T, liters	0.76±0.06	0.51±0.03†	0.78±0.04	0.53±0.02†
f_B, min^{-1}	13.4±0.5	14.2±0.5†	13.1±0.4	14.5±0.6†
PIF, l/min	30.6±1.5	28.9±3.1	32.4±2.1	28.9±2.1
$P_{ET}CO_2$, Torr	41.7±0.8	45.2±0.7†	39.7±0.9	44.1±0.7†
Sa_{O_2}, %	97.9±0.2	97.6±0.1	86.2±0.5*	85.9±0.2*

Data are means ± SE for the breath immediately before pulse onset; $n = 14$. Minute ventilation ($\dot{V}_I$), inspiratory tidal volume (V_T), breathing frequency (f_B), peak inspiratory flow (PIF), end-tidal CO_2 ($P_{ET}CO_2$), and arterial blood oxygen saturation (Sa_{O_2}) are shown during wakefulness and non-REM (NREM) sleep. *Significant difference compared with normoxia. †Significant difference compared with wakefulness.

Table 3. *Effect of hypoxia on EMGgg reflex characteristics to negative-pressure pulse stimuli during wakefulness and NREM sleep*

	Normoxia		Hypoxia	
	Awake	NREM	Awake	NREM
Excitation phase				
Onset latency, ms	25±2	22±1	27±1	23±2
Peak amplitude, %baseline	236±36	206±14	226±35	193±9
Peak latency, ms	37±2	32±2*	38±1	34±2*
Duration, ms	24±3	19±1	22±2	21±2
Suppression phase				
Onset latency, ms	50±2	41±2	50±2	45±2
Nadir amplitude, %baseline	67±6	47±5*	63±6	42±4*
Nadir latency, ms	70±5	64±1	72±9	67±2
Duration, ms	41±7	41±2	49±10	40±4
Stimulus properties				
Pmask, cmH_2O	−9.4±0.3	−10.5±0.4*	−9.1±0.2	−10.3±0.3*
Pmask rise time, ms	11±0.3	12±0.1	11±0.7	11±0.3
Pcho, cmH_2O	−8.5±0.5	−9.3±0.7*	−8.1±0.4	−9.3±0.6*
No. of artifact-free pulse presentations	56±3	73±8	53±2	67±8

Values are means ± SE; $n = 11$. Data are presented for the subjects in whom values for all the measured variables were available under all conditions. EMGgg, genioglossus electromyogram; Pmask, mask pressure; Pcho, choanal pressure. *Significant difference compared with wakefulness.

not different between normoxia and hypoxia (16 ± 5 vs. 36 ± 12 μV, $P = 0.174$).

The initial peak occurred earlier during NREM sleep compared with wakefulness under both gas conditions. After the initial peak phase there was a suppression of EMGgg amplitude below baseline that was significantly greater during NREM sleep compared with wakefulness. Stimulus magnitude was greater during NREM sleep compared with wakefulness as measured by mask and choanal pressures (Table 3). However, stimulus rise-times did not differ between NREM sleep and wakefulness (Table 3). There were no differences in EMGgg reflex component amplitudes or latencies between gas conditions.

Sufficient REM sleep to present repeated negative-pressure pulse stimuli was achieved in five subjects under both gas conditions. While replicate trials were limited during normoxia and hypoxia experiments ($n = 7 \pm 2$ vs. $n = 9 \pm 2$, $P = 0.913$), the predominant reflex response was a prolonged period of suppression with (40%) or without (60%) any preceding excitation. As described previously, EMGgg suppression was most pronounced during REM sleep (14). However, in the present study there were no gas or gas-by-state interaction effects in EMGgg reflex peak amplitudes or latencies during REM.

Inspiratory muscle reflex responses. Similar to other reports (5, 21), the signal-to-noise ratio for surface electrode EMG recordings overlying the diaphragm and intercostal muscles proved to be poor and insufficient to discern reflex responses. EMGsc reflex responses were reliably observed in 10 of the 14 subjects during wakefulness and NREM sleep under both gas conditions. The number of artifact-free stimuli, EMGsc peak reflex amplitudes, timing and stimulus properties during wakefulness and NREM sleep are summarized in Table 4. Similar numbers of pulses to allow for EMGsc analysis were presented during normoxia and hypoxia in wakefulness (63 ± 4 vs. 61 ± 3, $P = 0.734$) and in NREM sleep (75 ± 9 vs. 70 ± 9, $P = 0.516$). Two EMGsc reflex patterns were observed to negative-pressure pulse stimuli: *1*) suppression followed by activation, and *2*) an initial short-latency increase in EMGsc activity followed by the pattern described above. Phasic EMGsc activity was observed in all of these subjects (e.g., Fig. 2). The baseline average EMGsc activity in the 100 ms before pulse onset was not different between normoxia and hypoxia (3.4 ± 0.5 vs. 4.3 ± 0.7 μV, $P = 0.246$).

Table 4. *Effect of hypoxia on EMGsc reflex characteristics to negative-pressure pulse stimuli during wakefulness and NREM sleep*

	Normoxia		Hypoxia	
	Awake	NREM	Awake	NREM
Suppression phase				
Onset latency, ms	35±3	36±2	33±3	39±3
Nadir amplitude, %baseline	62±3	63±4	50±3†‡	61±3
Nadir latency, ms	64±8	60±4	87±5†	78±4†
Duration, ms	41±9	36±6	76±9†	51±5*
Excitation phase				
Onset latency, ms	76±9	72±5	109±9†	91±3†
Peak amplitude, %baseline	157±8	145±7	174±10	154±13
Peak latency, ms	121±12	108±9	152±12	162±27
Stimulus properties				
Pmask, cmH_2O	−9.7±0.3	−10.8±0.4*	−9.1±0.3	−10.5±0.3*
Pmask rise time, ms	11±0.3	12±0.2	12±0.6	11±0.2
Pcho, cmH_2O	−8.0±0.6	−10.3±0.5*	−8.1±0.5	−10.1±0.4*
No. of artifact-free pulse presentations	55±4	70±9	52±2	60±8

Values are means ± SE; $n = 10$. Data are presented for the subjects in whom values for all the measured variables were available under all experimental conditions. EMGsc, scalene electromyogram. *Significant difference compared with wakefulness within a gas condition. †Significant difference compared with corresponding state during normoxia. ‡Significant gas-by-state interaction effect.

ANOVA revealed that the latency to the nadir of the suppression response, the latency to the onset of the subsequent excitatory response, and the latency to the peak of the excitatory response were all significantly delayed during hypoxia compared with normoxia. Accordingly, the duration of EMGsc reflex suppression was also significantly prolonged during hypoxia compared with normoxia. However, post hoc tests showed no significant differences between gas conditions for EMGsc reflex suppression duration during NREM sleep ($P = 0.076$) or for the latency to the peak of the subsequent excitatory response during wakefulness ($P = 0.073$) and NREM sleep ($P = 0.119$, Table 4).

There was a trend toward the amplitude of the EMGsc suppression nadir being more pronounced during hypoxia compared with normoxia (56 ± 2 vs. 63 ± 3% of baseline, $P = 0.06$). There was a significant state effect ($P = 0.049$) whereby the suppression nadir amplitude was most pronounced during wakefulness. There was also a significant state-by-gas interaction effect for the amplitude of the suppression nadir such that suppression nadir amplitude was most pronounced during hypoxia in wakefulness (Table 4). Suppression duration was greater during wakefulness compared with NREM sleep (59 ± 6 vs. 43 ± 43, $P = 0.008$). However, this difference was only statistically significant post hoc within the hypoxia condition (Table 4). There were no other state, gas, or interaction effects for EMGsc reflex characteristics. There were too few replicate trials during REM sleep such that the signal-to-noise ratio of the ensemble-averaged surface EMGsc recordings was poor and inadequate to discern any reflex responses from background EMG activity.

In addition to the suppression and subsequent excitation EMGsc reflex morphology, an initial short-latency increase in EMGsc activity before the suppression phase was present in some but not all subjects and to varying degrees between wakefulness and sleep and the two gas conditions. Examples of EMGsc reflex responses in two subjects during wakefulness and NREM sleep are displayed in Fig. 4. Where present, the amplitude and timing characteristics were quantified using the same criteria used for the initial EMGgg peak (Fig. 3*A*) with the exception of a shorter minimum duration (>5 ms) given the short duration of this peak. The reflex characteristics and variability of the presence of an initial short-latency increase in EMGsc activity before the suppression phase are reported in Table 5. In 2 of the 10 subjects there was no initial increase in EMGsc activity before the suppression phase. In two separate subjects an initial increase in EMGsc activity before the suppression phase was present in all four experimental conditions. In the six remaining subjects the presence of an initial increase in EMGsc activity was more variable, occurring in some but not all of the experimental conditions (Table 5).

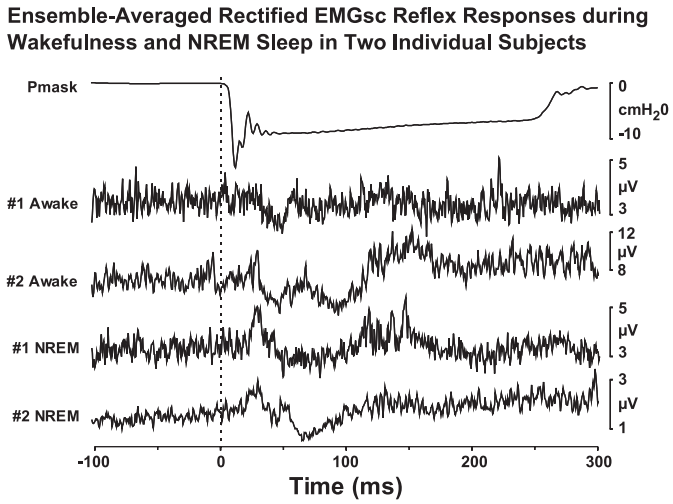

Fig. 4. Ensemble-averaged rectified EMGsc reflex responses and ensemble-averaged Pmask profile to brief negative-pressure pulse stimuli in 2 subjects during wakefulness and NREM sleep. For *subject 1*, $n = 61$ and $n = 69$ replicate trials during wakefulness and NREM sleep, respectively; and for *subject 2*, $n = 61$ and $n = 44$, respectively. Vertical dashed line at *time 0* represents stimulus onset.

DISCUSSION

In this study, EMGgg and EMGsc reflex responses to brief pulses of negative UA pressure during wakefulness and sleep were compared between conditions of mild isocapnic hypoxia (Sa_{O_2} ~85%) and normoxia. The EMGgg negative-pressure reflex was unaffected by hypoxia. However, the latency of several components of the EMGsc reflex response to negative pressure was increased and EMGsc reflex suppression duration was prolonged during hypoxia compared with normoxia.

Respiratory Muscle Reflex Response Patterns to Negative-Pressure Pulse Stimuli

As described in detail in a recent report (14) the response of the genioglossus muscle to brief pulses of negative UA pressure consisted of an initial excitatory phase followed by prolonged suppression below baseline. The morphology of this response was similar during normoxia and hypoxia in wakefulness and NREM sleep.

The response of several human inspiratory muscles to a sudden increase in respiratory load delivered during midinspiration in wakefulness consists of an initial suppression (onset ~35–40 ms) followed by excitation (onset ~80–100 ms) (3, 7, 32). In this study, the morphology and timing of the suppression and subsequent excitatory components of the EMGsc reflex response to a rapid-onset negative-pressure pulse delivered during early inspiration was comparable to previous reports (3–5, 21). The finding that the latencies for suppression onset for EMGsc and EMGgg were similar suggests that similar mechanisms may be involved in the genesis of these reflex components.

In addition to the suppression phase, an initial brief increase in EMGsc activity was also observed in 50% of trials during wakefulness and in 70–80% of trials during NREM sleep. Given its variable nature and short duration, this may reflect an artifactual peak associated with more synchronous motoneuron firing without necessarily a subsequent increase in firing frequency and/or motoneuron recruitment (i.e., excitation) (28, 33, 44). Previous EMGsc reflex studies employed midinspiratory occlusive stimuli delivered in the seated upright position and did not report the presence of an initial short-latency peak (3–5, 21). Thus methodological differences in the timing of the stimulus (early inspiration), stimulus properties (rapid negative-pressure pulse), and posture (supine) may have also contributed to the presence of this initial peak in the present study.

Effects of Hypoxia on Respiratory Reflex Responses to Negative-Pressure Pulse Stimuli

EMGgg. Sustained overnight hypoxia did not alter the EMGgg reflex responses to negative pressure. This finding is consistent with previous wakefulness reflex data (37) and a report demonstrating no change in baseline EMGgg activity during brief periods of isocapnic hypoxia alone (3 min, Sa_{O_2} ~80–85%) or when combined with inspiratory resistive loading (~5–15 $cmH_2O \cdot l^{-1} \cdot s$) during NREM sleep (39). Together these data suggest that the hypoglossal motor nucleus and the various components involved in the EMGgg negative-pressure reflex arc [i.e., the nucleus tractus solitarius (NTS) and UA mechanoreceptors] are relatively insensitive to mild sustained isocapnic hypoxia. This is in contrast to data obtained in adult cats demonstrating low tolerance of hypoglossal motoneurons to mild hypoxia (31) and recent observations in humans of impaired sensory processing of respiratory load (11, 12, 17, 30) and suppression of the cough reflex during sustained hypoxia (13). The respiratory afferent pathways activated during respiratory loading, airway occlusion, cough provocation, and negative airway-pressure pulses all relay through the NTS. Previous studies have suggested that the NTS may be an important site of hypoxia-induced neural inhibition (16, 40). The inhibitory effects of hypoxia on cough provocation sensitivity and respiratory load sensation but not the EMGgg negative-pressure reflex, suggest either that hypoxia does not exert inhibitory effects on these responses at the NTS (i.e., cortical pathways and/or peripheral receptor impairment may be involved) or that they relay through different second-order afferents that are differentially sensitive to hypoxia.

EMGsc. Unlike the EMGgg reflex response, several latency components of the EMGsc reflex response were delayed and the duration of suppression was greater during hypoxia compared with normoxia. Even in the absence of hypoxia, patients with asthma and OSA also demonstrate similar changes in EMGsc reflex responses to sudden respiratory loading (4, 21).

Table 5. *Reflex characteristics and variability of the presence of an initial increase in EMGsc activity before the suppression phase*

	Normoxia		Hypoxia	
	Awake	NREM	Awake	NREM
No. of subjects in whom initial peak occurred	5/10	8/10	5/10	7/10
Onset latency, ms	30±4	25±2	25±4	26±4
Peak amplitude, %baseline	158±6	168±14	149±7	179±14
Peak latency, ms	35±4	30±1	34±4	32±3
Duration, ms	11±3	14±2	13±3	15±2

Values are means ± SE from the subjects in whom an initial peak was present as indicated in first row.

This altered reflex has been proposed to be an adaptive response to repetitive exposure to increased respiratory load (4, 21). In support of this hypothesis, the duration and nadir of suppression measured during wakefulness positively correlate with the respiratory disturbance index in OSA patients (21).

Intramuscular sensory receptors (muscle spindles and tendon organs) rather than intrathoracic or airway receptors appear to be particularly important in mediating suppression of inspiratory muscle activity during transient loading (3, 5, 7). The precise central nervous system sites and synapses to the EMGsc reflex arc are not known although pontomedullary inspiratory neurons may be involved (3, 21, 32). More pronounced EMGsc reflex suppression during hypoxia may be mediated at one or multiple levels within the reflex arc. Hypoxia-induced changes in the sensitivity of intramuscular sensory receptors may be important. Indeed, animal and human data show that hypoxia across a range of magnitudes, including Sa_{O_2} values comparable to the present study, can lead to marked changes in the sensitivity of muscle spindles and Golgi tendon organs in a variety of skeletal muscles (8, 23, 46). Hypoxia may also lead to depressed central drive (i.e., alterations in interneuron and pontomedullary neuron excitability) as has been shown to occur in other reflex pathways (10). Thus a net inhibitory effect at the level of the brain stem to the neurons involved in this reflex response may contribute to more pronounced EMGsc reflex suppression. This altered reflex response may be one of many that occur during hypoxia as part of a central chemosensitive inhibitory network (29).

Methodological Considerations

Given the within-subjects repeated-measures study design (1-wk interval between experiments), we elected to only study men due to the known influence of changes in respiratory stimulant hormones that occur throughout the menstrual cycle and their associated effects on ventilation and genioglossus muscle activation (34). Further, the prevalence of sleep-disordered breathing is greater in men than women (45). However, the absence of data on female subjects remains a relative weakness of the present study, and future carefully designed studies are required to address this important issue.

The applicability of these results to sleep-disordered breathing remains uncertain given that increased respiratory load is normally of much more gradual onset than the rapid onset stimulus required to elicit discernible reflex responses. Thus reflex responses may differ under these two circumstances. Nevertheless, our findings indicate that inspiratory muscle reflex responses may well be importantly modulated by hypoxia when hypoxia accompanies increased inspiratory load. Greater EMG reflex suppression to increased breathing load may help explain overnight apnea prolongation (6) and delayed arousal to respiratory load under conditions of hypoxia (17).

We studied young healthy individuals rather than patients with disease because of the many potential confounding factors associated with sleep-disordered breathing. While this design allowed us to examine the effects of hypoxia per se on respiratory muscle reflexes, several potentially clinically relevant questions arise. For example, EMGgg responses may be more vulnerable during hypoxia in elderly subjects (22). The use of sustained overnight hypoxia in the present may be more akin to disorders such as obesity hypoventilation syndrome rather than OSA, which is characterized by intermittent hypoxia. Indeed, animal data have shown that intermittent hypoxia reduces excitatory hypoglossal nerve output and may be deleterious to UA muscle function (2, 41). Intermittent hypoxia also markedly attenuates baseline EMGgg activity during wakefulness in humans (27). Thus the EMGgg negative-pressure reflex may be impaired during intermittent hypoxia but not sustained hypoxia. Therefore, these variables are worthy of future investigation given that intermittent hypoxia is a predominant feature of OSA and that aging is a risk factor for this disorder.

While care was taken to ensure electrode placement was comparable between gas conditions and surface EMG recordings were reapplied as necessary until impedance values were below 5 kΩ, slight differences in electrode placement and signal-to-noise ratio may have occurred between gas conditions. To minimize these effects, amplitude data were expressed as a percentage of the prepulse baseline level. Prepulse baseline EMGgg and EMGsc activity did not differ between gas conditions. Given these findings and the repeated-measures design, subtle differences in electrode placement, signal-to-noise characteristics, and other between-night effects are unlikely to have systematically influenced the main study findings.

While reflex responses were reliably observed in the majority of subjects for the scalene muscle, the signal-to-noise ratio for surface electrode recordings overlying the diaphragm and intercostal muscles was insufficient to discern reflex responses. Previous studies that have simultaneously recorded reflex responses to brief respiratory loading in several inspiratory muscles suggest that the scalene responds in a similar fashion to other inspiratory muscles such as the diaphragm (3, 4). However, to more fully characterize inspiratory muscle reflex responses during hypoxia and sleep, further studies with more sensitive diaphragm and intercostal recording techniques are required.

In this study we elected to standardize pulse delivery to early inspiration to enable comparison with the majority of the existing EMGgg negative-pressure reflex data in humans. However, the activation patterns of inspiratory motoneurons differ between the genioglossus and other respiratory muscles (20, 36). Thus reflex responses to negative-pressure pulse stimuli, and potentially the vulnerability to the inhibitory effects of hypoxia, may vary between the scalene and genioglossus muscles throughout the respiratory cycle and contribute to the differential effects that we observed.

Finally, epiglottic pressure measurements were prone to drift, most likely because of buildup of airway secretions on the catheter. Thus we cannot be certain that negative-pressure pulse stimuli at the pharyngeal airway were matched between gas conditions. However, most studies suggest that in the absence of changes in ventilatory drive, respiratory muscle tone, respiratory mechanics, and pharyngeal resistance are unchanged during hypoxia in humans (9, 15, 37). Further, the choanal and mask pressures during negative-pressure pulse stimuli were not different between gas conditions, nor were ventilatory parameters on the breath before stimulus application. These data strongly support that negative-pressure pulse stimuli were indeed similar between gas conditions.

Summary and Possible Relevance to Sleep-Disordered Breathing

This study has demonstrated that EMGsc reflex suppression to brief pulses of negative pressure is prolonged during mild sustained hypoxia during wakefulness and NREM sleep. Reflex suppression of inspiratory muscles to airway occlusion has been postulated by Butler and colleagues (3) to be protective by way of preventing greater downstream negative pressures during times of UA narrowing or obstruction. Hypoxia did not change any of the measured EMGgg negative-pressure reflex characteristics, and the initial increase in EMGgg activity was preserved from wakefulness to NREM sleep. These results indicate differential sensitivity to the depressive effects of hypoxia in the reflex responsiveness to sudden respiratory loads to breathing between the scalene and genioglossus muscle in these healthy young men. The onset latency of the EMGgg suppression reflex component was similar to EMGsc reflex suppression, suggesting these reflex responses may share common neural pathways. Future studies that measure these reflex responses in a range of respiratory muscles (i.e., diaphragm, intercostals) during intermittent hypoxia and in patients with OSA are required to determine clinical significance of these findings.

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