Effect of episodic hypoxia on the susceptibility to hypocapnic central apnea during NREM sleep

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Chowdhuri S, Shanidze I, Pierchala L, Belen D, Mateika JH, Badr MS. Effect of episodic hypoxia on the susceptibility to hypocapnic central apnea during NREM sleep. J Appl Physiol 108: 369–377, 2010. First published November 25, 2009; doi:10.1152/japplphysiol.00308.2009.—We hypothesized that episodic hypoxia (EH) leads to alterations in chemoreflex characteristics that might promote the development of central apnea in sleeping humans. We used nasal noninvasive positive pressure mechanical ventilation to induce hypocapnic central apnea in 11 healthy participants during stable nonrapid eye movement sleep before and after an exposure to EH, which consisted of fifteen 1-min episodes of isocapnic hypoxia (mean O2 saturation/episode: 87.0 ± 0.5%). The apneic threshold (AT) was defined as the absolute measured end-tidal Pco2 (PETCO2) demarcating the central apnea. The difference between the AT and baseline PETCO2 measured immediately before the onset of mechanical ventilation was defined as the CO2 reserve. The change in minute ventilation (V̇E) for a change in PETCO2 (ΔV̇E/ΔPETCO2) was defined as the hypocapnic ventilatory response. We studied the eupneic PETCO2, AT PETCO2, CO2 reserve, and hypocapnic ventilatory response before and after the exposure to EH. We also measured the hypocapnic ventilatory response, defined as the change in V̇E for a corresponding change in arterial O2 saturation (ΔSaO2) during the EH trials. V̇E increased from 6.2 ± 0.4 l/min during the pre-EH control to 7.9 ± 0.5 l/min during EH and remained elevated at 6.7 ± 0.4 l/min the during post-EH recovery period (P < 0.05), indicative of long-term facilitation. The AT was unchanged after EH, but the CO2 reserve declined significantly from −3.1 ± 0.5 mmHg pre-EH to −2.3 ± 0.4 mmHg post-EH (P < 0.001). In the post-EH recovery period, ΔV̇E/ΔPETCO2 was higher compared with the baseline (3.3 ± 0.6 vs. 1.8 ± 0.3 l/min−1·mmHg−1, P < 0.001), indicative of an increased hypocapnic ventilatory response. However, there was no significant change in the hypocapnic ventilatory response (ΔV̇E/ΔSaO2) during the EH period itself. In conclusion, despite the presence of ventilatory long-term facilitation, the increase in the hypocapnic ventilatory response after the exposure to EH induced a significant decrease in the CO2 reserve. This form of respiratory plasticity may destabilize breathing and promote central apneas.

long-term facilitation; chemoresponse; nonrapid eye movement sleep

ACUTE EXPOSURE to episodic hypoxia (EH) elicits a sustained increase in ventilatory motor output that lasts for up to 90 min after the termination of hypoxia, which is referred to as long-term facilitation (LTF) (12, 27). The occurrence and manifestations of LTF are influenced by the prevailing end-tidal PCO2 (PETCO2) level, experimental paradigm, animal species, and baseline upper airway mechanics (1, 4–6, 10–12, 15, 17–21, 27, 34, 36, 37, 41, 48, 57). Manifestations of LTF in sleeping humans include increased minute ventilation (V̇E; i.e., ventilatory LTF) (4), decreased inspiratory upper airway resistance (1, 4, 5, 7, 48), and increased genioglossus electromyographic (EMG) activity (i.e., upper airway LTF) (15). Ventilatory LTF during sleep may have a role in stabilizing breathing by reducing plant gain, whereby a greater change in ventilation is required to elicit a reduction in PETCO2. Thus, the development of hypocapnia-induced apnea during sleep is less likely (16, 38). Likewise, upper airway muscle LTF might promote breathing stability during sleep by enhancing upper airway patency (1, 45).

However, as recently hypothesized (32), an exposure to acute EH may lead to alterations in chemoreflex characteristics that could potentially counteract the stabilizing impact that ventilatory and upper airway muscle LTF have on breathing during sleep. More specifically, a number of studies (20, 31, 38, 40) in both animals and humans during wakefulness have indicated that chemoreflex sensitivity is enhanced after an exposure to EH. Enhancement of chemoreflex sensitivity could lead to the development of hypocapnia and, ultimately, central apnea (38). In addition to the induction of apnea, hypocapnia could render upper airway muscle LTF ineffective (32), which in due course could lead to the development of an obstructive event (8, 46). Likewise, it is possible that an exposure to EH could decrease the CO2 reserve, which also increases the incidence of an apnea.

Despite these possibilities, no studies to date have ascertained the effect of EH on the susceptibility to develop hypocapnic central apnea in healthy humans during sleep. We hypothesized that an exposure to EH would increase the hypocapnic ventilatory response and decreases the CO2 reserve despite the presence of ventilatory LTF.

METHODS

Participants

The Human Investigation Committees of Wayne State University School of Medicine and Detroit Veterans Affairs Medical Center approved the experimental protocols. Informed written consent was obtained from 11 healthy participants free of daytime sleepiness, sleep-disordered breathing (i.e., apnea-hypopnea index of <5/h), or other medical disorders.

Breathing Circuit

Each participant was connected to the breathing circuit via a nasal mask. An appropriately sized, airtight silicone nasal mask (Respironics, Murrysville, PA) was glued to the participant’s face to prevent mask leaks. The mask was connected to a plateau exhalation valve (Respironics) via a heated pneumotachometer. The valve, which
provides a continuous leak path in the breathing circuit and serves as an exhaust vent, was connected to the inspiratory line. Participants were restricted to nasal breathing by placing tape over the mouth. During the mechanical ventilation (MV) protocol (see below), hyper-ventilation was achieved using a pressure support ventilator (Quantum PSV, Healthdyne Technologies, Marietta, GA) (44, 63). During the EH Protocol (see below), two cylinders containing 100% N₂ or 100% O₂ were connected to the inspiratory line. To maintain isocapnia, supplemental CO₂ (fraction of inspired CO₂: 0.07, balanced with N₂) (41) was added to the inspiratory line from an external source to maintain PETCO₂ at or near control levels.

Measurements

Electroencephalograms (EEGs), electrooculograms (EOGs), and chin EMGs were recorded using the international 10-20 system of electrode placement (EEG: C3-A2 and C4-A1; EOG: O-A2). Inspiratory airflow was measured by a heated pneumotachometer (model 3700A, Hans Rudolph, Kansas City, MO) attached to a pressure transducer (Validyne, Northridge, CA). The tidal volume (VT) was obtained from the electronic integration of the flow signal (model FV156 Integrator, Validyne). To confirm the central etiology of apnea and to ascertain upper airway mechanics, supraglottic pressure was measured using a pressure transducer-tipped catheter (model TC-500XG, Millar Instruments, Houston, TX) with the tip positioned in the hypopharynx. The hypopharyngeal position was obtained by advancing the catheter tip for 2 cm after it disappeared behind the tongue. PETCO₂ readings were obtained continuously by an infrared analyzer (model CD-3A, AEI Technologies, Pittsburgh, PA) from tubing placed in the nares via a port in the nasal mask. Arterial O₂ saturation (SaO₂) was measured by a pulse oximeter (Biox 3700, Ohmeda). Signals were displayed on a polygraph recorder (Grass model 15, Astro-Med, West Warwick, RI) and recorded using Powerlab data-acquisition software (AD Instruments, Colorado Springs, CO) for detailed analysis.

Experimental Protocols

Overview. The study was conducted during normal nocturnal sleep. Study participants were instructed to limit total sleep time to a maximum of 5 h on the night before the study. A screening polysomnography was performed on night 1 to confirm the absence of sleep apnea. The experimental procedure was performed on night 2 (EH) and night 3 (sham hypoxia). Participants assumed the supine position for the entire experimental protocol, which was conducted during stable nonrapid eye movement (NREM) sleep; hence, all trials were conducted while participants were in stable stage 2 or stage 3 sleep. Eight participants agreed to return for the sham protocols on night 3. The experimental protocol was conducted in three phases during NREM sleep, which included a determination of the apneic threshold (AT) before and after the exposure to EH or a comparable duration of room air representing sham hypoxia.

MV protocol: prehypoxia. A representative polygraph segment obtained from a participant in stage N2 sleep before, during, and after the MV trial is shown in Fig. 1. We used nasal noninvasive positive pressure mechanical ventilation to produce hyperventilation to determine the AT before and after the exposure to EH. MV was applied for 3 min as previously described (44, 45, 63) in the spontaneous-timed mode. In this mode, a backup respiratory rate is preset; timed breaths are delivered if the participant’s respiratory rate falls below the set rate. The ventilator respiratory rate was set at 6–8 breaths/min, which was below the participant’s eupneic rate, to prevent neuromechanical inhibition of the ventilatory motor output. During MV, the inspiratory positive airway pressure was increased gradually in 1- to 2-cmH₂O increments starting from 2 cmH₂O at the beginning of each MV trial while keeping the expiratory positive airway pressure fixed at 2 cmH₂O throughout the MV. MV was terminated after 3 min during expiration by returning the inspiratory positive airway pressure to the baseline expiratory positive airway pressure of 2 cmH₂O. The ensuing hypocapnia resulted in either a hypopnea or central apnea. If an apnea was not induced, additional hyperventilation trials were completed until an apnea was evident. Central apnea was defined as an expiratory
time \( \geq 5 \) s. After the MV protocol, each participant was switched to spontaneous room air breathing for 15 min. Subsequently, the participants were exposed to EH.

**EH protocol.** After the completion of the MV protocol, the participants breathed room air for a 5-min control period followed by 15 episodes of 1-min isocapnic hypoxia separated by room air breathing for 1–2 min (41). Hypoxia was induced rapidly by adding 100% N\(_2\) for four to six breaths to the breathing circuit to produce a hypoxic mixture. Supplemental 7% CO\(_2\) was added to the circuit to maintain isocapnia (Fig. 2). The administration of 100% N\(_2\) was terminated when the oxyhemoglobin saturation decreased to 89%; thereafter, the O\(_2\) saturation declined spontaneously an additional 3–6% in the milieu of the hypoxic gas mixture in the circuit. Hypoxia was terminated abruptly at the end of 1 min with two breaths of 100% O\(_2\). Once the O\(_2\) saturation had returned to baseline levels, a short recovery period in room air followed before the next hypoxic episode (41). The number of acceptable EH episodes with a stable sleep stage was 11.8 ± 0.7 hypoxic episodes/subject, with a duration of 63.6 ± 4.3 s and separated by room air breathing during a recovery period of 110.1 ± 4.2 s.

**MV protocol: posthypoxia.** After the 15th hypoxic episode, breathing was monitored for 10 min of recovery (i.e., recovery period) in room air. Thereafter, the participant was returned to an expiratory positive airway pressure of 2 cmH\(_2\)O, and the MV protocol was repeated using a similar protocol as described above in MV protocol: prehypoxia. This portion of the protocol was completed within 60 min of the last hypoxic exposure while stable NREM sleep was maintained, since other forms of respiratory plasticity have reportedly persisted for this length of time after EH (6, 21, 57). The MV trials that produced apnea were repeated once to ensure repeatability of the determination of the AT.

**Sham protocol.** To ensure that the changes during the recovery period were not due to time-dependent phenomena independent of EH, eight of the subjects underwent a sham protocol on a different night with identical measurements but without the hypoxia intervention (night 3). The remaining three subjects were unavailable for the sham protocol. The sham protocol involved switching of gases except that the composition was room air instead of the hypoxia mixture. Overall, the flow of room air was similar to the flow of gases during the hypoxia runs.

**Data Analysis.**

**MV protocols: pre- and post-EH.** Sleep staging (43) and scoring of arousals (56) were completed using standard criteria, analyzing trials with stable NREM sleep, as confirmed by a blinded observer. During the control period, five breaths recorded immediately before the onset of the MV were averaged. Likewise, during the MV period, the last five mechanically ventilated breaths before the return to baseline expiratory positive airway pressure were averaged (Fig. 1). The data analysis methodology has been previously described (44, 45, 63). The AT was defined as the PET\(_{\text{CO}_2}\) that demarcated the central apnea closest to the eupneic PET\(_{\text{CO}_2}\). The CO\(_2\) reserve was defined as the change in PET\(_{\text{CO}_2}\) (ΔPET\(_{\text{CO}_2}\)) between eupneic PET\(_{\text{CO}_2}\) (control) and AT. The “hypocapnic ventilatory response” was defined as the change in VT, between control and a hypopnea or an apnea divided by ΔPET\(_{\text{CO}_2}\) (ΔVT/ΔPET\(_{\text{CO}_2}\) relationship), i.e., this is the slope of the ventilatory response (see Fig. 6). The hypocapnic ventilatory response was calculated for each hypopnea trial and the apnea trial with the smallest CO\(_2\) reserve and then averaged for each individual; the values for each individual subject were used for the analysis.

**EH protocol.** Data recorded during the exposure to EH were used for analysis only if they were measured during stable NREM sleep. Inspired VT, inspiration time, total breath time, breathing frequency, VT, PET\(_{\text{CO}_2}\), and SaO\(_2\) were calculated breath by breath. For each variable, an average value was computed during a control period, during each hypoxic episode, and during the recovery periods that followed each hypoxic episode. Average values during the control period were determined by averaging the data obtained from 20 breaths recorded immediately before the onset of the first hypoxic episode. This analysis has also been described previously (41). During each hypoxic episode, the variables outlined above were averaged...
using 11.5 ± 1.0 breaths recorded; the number of breaths analyzed depended on the duration of hypoxia during a given hypoxic episode. Similarly, average values for the variables were obtained using the last five breaths recorded during each normoxic period that preceded each hypoxic episode. Finally, an average value for each variable was obtained from 10 consecutive breaths after 10 min of recovery from the last hypoxic episode.

Additionally, we measured the hypoxic ventilatory response (HVR). This was defined as the change in \( V_I \) for a change in \( \text{ Sao}_2 \) during a given hypoxic episode. The ventilation during a given hypoxic episode was compared with the room air control period immediately preceding the hypoxic period (\( \Delta V_I / \Delta \text{ Sao}_2 \)) for each hypoxic episode. HVR was determined during the initial five breaths of the hypoxia episode [acute HVR (AHVR)], representing the immediate effect of hypoxia, and during the nadir \( \text{ O}_2 \) desaturation period at the end of the trial (HVR\(_{\text{nac}}\)), representing the net hypoxic effect.

**Sham protocol.** During the sham protocol, the AT, CO\(_2\) reserve, \( V_I \), and hypocapnic ventilatory response were obtained before and after participants breathed room air for 30 min.

**Statistical Analysis**

Results are presented as means ± SE unless otherwise specified. A commercially available computer statistical package was used to analyze the data (SigmaStat 3.11.0, SPSS). The level of statistical significance was set at \( P \leq 0.05 \).

**MV protocols.** For normally distributed data, paired \( t \)-tests were performed to compare the eupneic \( V_I \), eupneic PET\(_{CO2}\), AT, and CO\(_2\) reserve recorded during the MV protocols completed before and after EH. For non-normally distributed data [hypocapnic ventilatory response (\( \Delta V_I / \Delta \text{ PET}_{CO2} \))], the Wilcoxon signed-rank test was used.

**EH protocol.** For normally distributed data, comparisons between time points for control, hypoxia, and recovery were made using one-way ANOVA with repeated measures followed by a post hoc analysis for all pair-wise comparisons using the Holm-Sidak method. When the normality test failed (for inspiration time, expiratory time, \( \text{ Sao}_2 \), and upper airway resistance), then ANOVA on ranks was performed followed by pairwise multiple-comparison tests again using the Holm-Sidak method. AHVR values from the first three hypoxic episodes were compared with the final three hypoxic episodes using one-way repeated-measures ANOVA. Similarly, HVR\(_{\text{nac}}\) values of the first three hypoxic trials versus the last three hypoxic trials were compared.

**Sham protocol.** For the eight subjects who underwent both the experimental and sham protocols, paired \( t \)-tests were performed to compare the eupneic PET\(_{CO2}\), AT, and eupneic \( V_I \) between the pre- and post-sham MV protocols. To evaluate for the effect of timing in the two groups, the CO\(_2\) reserve and hypocapnic ventilatory response were analyzed using repeated-measures ANOVA with two factors: \( J \) (timing [pre vs. post] \( \times \) 2) protocol (experimental vs. sham) followed by a post hoc analysis for all pairwise comparisons using the Holm-Sidak method. Specifically, for the hypocapnic ventilatory response, the two-way repeated-measures ANOVA was done after log transformation, as the values were not normally distributed.

**RESULTS**

Participant characteristics and the apnea/hypopnea index from their baseline polysomnography experiments are shown in Table 1. All participants were healthy and without sleep-disordered breathing. Results from the EH protocol are shown initially followed by the results obtained from the MV protocols completed before and after the EH and, finally, from the sham protocol.

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26.1 ± 4.2</td>
</tr>
<tr>
<td>Sex</td>
<td>4 men/7 women</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>22.1 ± 2.0</td>
</tr>
<tr>
<td>Apnea-hypopnea index, number/h</td>
<td>0.7 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n = 11 \) subjects.

**EH**

Ventilatory and timing data are shown in Table 2. The oxyhemoglobin saturation decreased to 87.0 ± 0.5% during hypoxia, resulting in increased \( V_T \) and \( V_I \) compared with the room air control; the remaining measured parameters were unchanged. In addition, a significant increase in \( V_T \) and \( V_I \) compared with the baseline persisted into the recovery period after the last hypoxic episode; this was associated with a reduction in PET\(_{CO2}\). (Table 2). However, the AHVR was not altered by repeated hypoxic exposure [AHVR: first 3 hypoxia episodes vs. last 3 hypoxia episodes, 0.38 ± 0.1 vs. 0.38 ± 0.09 l·min\(^{-1}\)·%\(^{-1}\), \( P \) = not significant (NS); and HVR\(_{\text{nac}}\): first 3 hypoxia episodes vs. last 3 hypoxia episodes, 0.26 ± 0.06 vs. 0.24 ± 0.04 l·min\(^{-1}\)·%\(^{-1}\), \( P \) = NS; Fig. 3].

**Pre- and Post-EH AT and CO\(_2\) Reserve**

The eupneic \( V_I \) after the exposure to EH was significantly greater compared with measures obtained before EH (6.3 ± 0.3 vs. 6.1 ± 0.3 l/min, \( P = 0.01 \)). Likewise, the eupneic PET\(_{CO2}\), during the MV protocol after the exposure to EH was significantly less than the eupneic PET\(_{CO2}\) during the MV protocol before EH (38.3 ± 0.9 vs. 39.7 ± 0.9 mmHg, \( P = 0.01 \); Fig. 4). The hypocapnic ventilatory response measured during the MV protocol after the exposure to EH was greater than pre-EH values (3.3 ± 0.6 vs. 1.8 ± 0.3 l·min\(^{-1}\)·mmHg\(^{-1}\), \( P < 0.001 \)). This difference remained significant (2.9 ± 0.4 vs. 1.8 ± 0.3 l·min\(^{-1}\)·mmHg\(^{-1}\), \( P < 0.01 \)) even after one outlier subject (who showed the greatest increase) was excluded from the analysis. Subsequently, the CO\(_2\) reserve decreased significantly after the exposure to EH relative to pre-EH values (−2.3 ± 0.4 vs. −3.1 ± 0.5 mmHg, \( P < 0.001 \); Fig. 4). Notably, the PET\(_{CO2}\), that demarcated the AT was not altered after EH compared with before EH (36.4 ± 0.7 vs. 36.6 ± 0.7 mmHg, \( P \) = NS; Fig. 4). In summary, EH resulted in decreased eupneic PET\(_{CO2}\), an increased hypocapnic ventilatory response, and narrowing of the CO\(_2\) reserve.

**Experimental Versus Sham Protocol**

The eight participants who underwent both the experimental and sham protocols were similar to the remaining three nonparticipants in terms of their individual characteristics. There were no significant changes in eupneic control PET\(_{CO2}\) (39.2 ± 0.9 vs. 38.7 ± 0.7 mmHg, \( P = \) NS), AT (36.1 ± 1.0 vs. 36.5 ± 1.0 mmHg, \( P = \) NS), and eupneic \( V_I \) (5.8 ± 0.2 vs. 5.6 ± 0.2 l/min, \( P = \) NS) during MV completed after sham intervention compared with measures obtained before sham intervention. Comparison of the hypocapnic ventilatory response in these eight subjects revealed a significant interaction between the time of intervention (pre vs. post) and the type of intervention (EH vs. sham, \( P = 0.05 \)), indicating that the change over time in the hypocapnic ventilatory response was not the same for the two levels of the protocol.
Likewise, a comparison of the CO2 reserve revealed a significant interaction between the time of intervention (pre vs. post) and the type of intervention (EH vs. sham, \( P < 0.01 \)). The hypocapnic ventilatory response was significantly higher (\( P < 0.01 \); Fig. 5) posthypoxia within the experimental protocol but not within the sham protocol. Additionally, the CO2 reserve was significantly decreased post- versus preintervention within the experimental protocol (\(-2.1 \pm 0.4 \) vs. \(-2.9 \pm 0.5 \) mmHg, \( P < 0.05 \)) but not within the sham protocol (\(-2.7 \pm 0.6 \) vs. \(-2.7 \pm 0.5 \) mmHg, \( P = \text{NS} \)). Thus, the results clearly indicate that the observed reciprocal changes in the hypocapnic ventilatory response and CO2 reserve after EH were due to the EH intervention per se and were not time-dependent phenomena (unchanged results during bar).

### DISCUSSION

#### Summary of Findings

Our study revealed several significant findings after EH during NREM sleep. First, EH was followed by a period of sustained increase in \( V_i \) and a reduction in the eupneic PETCO2, indicative of ventilatory LTF. Second, EH was associated with an increased hypocapnic ventilatory response and narrowing of the CO2 reserve; this was not observed after the sham expo-

Table 2. Results of episodic hypoxia presented as grouped data for timing and ventilation during the three periods: room air control, hypoxia, and recovery periods

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Hypoxia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inspiratory time, s</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Expiratory time, s</td>
<td>2.3 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>Frequency of breathing, breaths/min</td>
<td>15.4 ± 0.9</td>
<td>16.0 ± 0.9*</td>
<td>15.3 ± 0.9</td>
</tr>
<tr>
<td>End-tidal PCO2, mmHg</td>
<td>40.1 ± 0.9</td>
<td>39.5 ± 0.9</td>
<td>38.9 ± 0.9*</td>
</tr>
<tr>
<td>Arterial O2 saturation, %</td>
<td>97.7 ± 0.1()</td>
<td>87.0 ± 0.5</td>
<td>97.9 ± 0.2()</td>
</tr>
<tr>
<td>Tidal volume, liters</td>
<td>0.397 ± 0.07</td>
<td>0.497 ± 0.03()</td>
<td>0.446 ± 0.028()</td>
</tr>
<tr>
<td>Minute ventilation, l/min</td>
<td>6.0 ± 0.4</td>
<td>7.9 ± 0.5()</td>
<td>6.7 ± 0.4()</td>
</tr>
<tr>
<td>Upper airway resistance, 1/s \·cmH2O()</td>
<td>9.6 ± 3.1</td>
<td>6.2 ± 1.3</td>
<td>6.5 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 11 \) subjects. *\( P < 0.05 \) vs. control; †\( P < 0.05 \) vs. hypoxia.

Fig. 3. Acute and net hypoxic ventilatory response (HVR) expressed as the change in minute ventilation (\( V_i \)) for a corresponding change in \( S_aO_2 \) (see text). No significant changes were noted between the first 3 hypoxia episodes (solid bar) and the last 3 hypoxia episodes (shaded bar). NS, not significant.

Fig. 4. Schematic representation of the observed eupneic CO2, apneic threshold (AT) PETCO2, and CO2 reserve (eupneic PETCO2 minus AT PETCO2) during the pre-episodic hypoxia (pre-EH; solid bars) and post-EH (grey bars) periods (\( n = 11 \)). The top horizontal line represents the eupneic CO2, the bottom horizontal line represents the AT, and boxes between these two lines represent the CO2 reserve [pre-EH (solid box) and post-EH (shaded box)]. The CO2 reserve was significantly smaller post-EH as a result of the significantly lower eupneic PETCO2, without a change in the AT.

Fig. 5. Comparison of the hypocapnic ventilatory responses under the two conditions: \( \) the EH protocol [pre-EH (solid bar) vs. post-EH (shaded bar)] and \( \) the sham protocol [before (hatched bar) vs. after (cross-hatched bar) the sham intervention]. The major finding was that the hypocapnic ventilatory response was significantly increased after isocapnic EH but not after the sham protocol (see text for explanation).
apnea, including arousals, increased sympathetic nervous system activity, changes in intrathoracic pressures, and increased blood pressure. Additionally, our study is focused on acute EH and does not address the effects of chronic intermittent hypoxia (25). Third, we used moderate sleep curtailment on the night preceding the sleep study to facilitate natural, unaided sleep. We excluded the use of hypnotics, given the potential effects on ventilatory control. Our previous experience demonstrated no differences in the findings between subjects who obtained normal versus curtailed nocturnal sleep. The available literature supports our observations (49). In addition, eight subjects underwent similar sleep curtailment for both the EH and sham nights, so any potential alteration in chemoresponsiveness would be present in both nights and, thus, would not alter our conclusions. Finally, mechanical ventilation may be associated with neuromuscular inhibition. However, such experiments (24) used larger VT than our study, inducing neuromechanical inhibition, even in the absence of hypocapnia, but only when VT was increased to two times the eupneic VT (1.2 liters). Therefore, we doubt that neuromechanical inhibition was responsible for our findings. Indeed, if present, neuromechanical inhibition would have been applicable to both the experimental and sham protocols of the study and would not explain the differential response seen post-EH.

**EH, Ventilatory LTF, and the CO2 Reserve**

The susceptibility to hypocapnia-induced central apnea is a function of multiple variables including background ventilatory drive, chemoreflex sensitivity, and sleep state (16, 35, 47). Figure 6 shows the resting levels of ventilation and PetCO2 along with the CO2 reserve and slope of the hypocapnic ventilatory chemoreceptor response before and after EH in our study. The combination of a higher V˙I and lower eupneic PetCO2 after EH would shift the resting PCO2 upward on the isometabolic curve and lead to an increased CO2 reserve. Thus, if all other factors remain constant, increased respiratory motor output produces a reduction in the eupneic PetCO2, and a reduced plant gain (16). Decreased plant gain stabilizes respiration as a greater change in ventilation is required to induce a given change in PetCO2 (decreased ΔPetCO2/ΔV˙I). Accordingly, a decrease in PetCO2 after EH could mitigate the propensity to central apnea and lead to an increased CO2 reserve if the hypocapnic ventilatory response remained unchanged (16, 23). This is the physiological response noted when hyperventilation is elicited by stimulation of the central chemoreceptors with metabolic acidosis or stimulation of the peripheral chemoreceptor with almitrine (38).

**Hypocapnic Ventilatory Response**

Our study revealed that the ventilatory response to hypocapnia increased after EH (steeper slope), resulting in a closer proximity of the hypocapnic AT to the eupneic PetCO2, (narrowed CO2 reserve, as shown by the solid arrow in Fig. 6) and, therefore, a greater propensity to develop hypocapnic central apnea, despite an increase in the resting ventilation (point A on the graph in Fig. 6). This finding is similar to the decreased CO2 reserve noted after an exposure to hypoxia in a canine model (38) and in patients with congestive heart failure (50, 59). Interestingly, in the canine model (38), continuous hypoxia, not EH, sensitized the ventilatory responsiveness to CO2 below eupnea and narrowed the CO2 reserve. Thus, an increased hypocapnic ventilatory response promotes the development of central apnea because the decrease in ventilation for a given reduction in PetCO2 is enhanced (13, 14, 23). Despite a decrease in plant gain, an increased hypocapnic ventilatory response was noted after EH, thereby increasing the tendency for central apnea.

**Mechanisms of Decreased CO2 Reserve After EH**

The increase in the hypocapnic ventilatory response after EH might represent an increase in central or peripheral chemoreflex sensitivity. Collectively, the results from previous studies in humans during wakefulness have indicated that peripheral chemoreflex sensitivity is enhanced after exposure to both EH (20, 31, 40) and sustained hypoxia (2, 22, 28, 29, 54). Conversely, studies have also indicated that central chemoreflex sensitivity remains unaltered after relatively short exposures to EH (31) or sustained hypoxia (28, 29).

**Lack of peripheral chemoresponsiveness.** Enhancement of the peripheral chemoreflex sensitivity would potentially be reflected by a progressive increase in the HVR from the beginning to the end of the EH protocol (31, 32). However, we noted that no change in the HVR was evident throughout the EH protocol. This finding is in contrast to the increased peripheral chemoreflex sensitivity after EH in healthy subjects during wakefulness (20, 32, 58). Interestingly, this increase was observed 1 h after EH and only when PetCO2 levels were at least 3 mmHg above the ventilatory recruitment threshold (20, 58) and not under hypocapnic conditions. Subsequent findings revealed that the expression of an enhanced HVR during and after exposure to EH under conditions of wakefulness is clearly dependent on the level of CO2 sustained throughout the hypoxic exposure (20, 31, 41, 58). Thus, manifestation of the enhancement is absent under hypo- and isocapnic conditions (20, 31) and only becomes clearly evident when CO2 levels are sustained slightly above baseline values. This has also been noted in animal studies (51–53). Although these findings are limited to the wakefulness condition, it is possible that the absence.
of an enhanced HVR in the present study was in part related to the absence of hypercapnia throughout the protocol.

**Potential contribution of cerebrovascular CO2 reactivity.**

Cerebrovascular reactivity and the ventilatory response to arterial PCO2 are tightly linked, so that the regulation of cerebral blood flow has an important role in stabilizing breathing during fluctuating levels of chemical stimuli, including hypoxia and hypercapnia (3). One study (42) demonstrated that the cerebral blood flow response to step changes in CO2 in humans was much faster (6-s delay) than that documented in previous reports. Studies have indicated that the cerebrovascular responsiveness to CO2, primarily via its effects at the level of the central chemoreceptors, is an important determinant of eupneic ventilation and the hypercapnic ventilatory response in otherwise healthy humans during wakefulness (60), sleep (61), and at high altitude (3, 62). Hypoxia per se is a cerebral vasodilator that induces a rise in cerebral blood flow in proportion to the severity of isocapnic hypoxia (62). Conversely, the hypoxia-induced activation of peripheral chemoreceptor activity leads to a hyperventilation-induced lowering of arterial PCO2 and subsequent cerebral vasoconstriction. Specifically, reductions in the normal cerebral vascular response to hypoxia may increase the susceptibility to apneas and breathing instability during sleep (61). Thus, it is possible that alterations in cerebrovascular reactivity to CO2, specifically a reduced cerebrovascular reactivity rather than an increase in peripheral chemoreflex sensitivity, might be responsible for the observed changes in ventilation in our study, since no significant change in the HVR was observed. In our study, there was an increased responsiveness to CO2, probably via increased chemoreflex sensitivity due to the hypoxia. A change in the hypocapnic ventilatory response may potentially occur without a change in peripheral chemoresponsiveness if cerebral vascular reactivity is decreased, resulting in an increased gradient between cerebral and arterial CO2. Increased central chemoreflex sensitivity is another possible mechanism for the increased hypocapnic ventilatory response. However, evidence for increased central chemoreflex sensitivity after EH is lacking (28, 29, 31).

**Other potential mechanisms.** Sensory LTF may be due to enhanced production of ROS after EH. There is no available evidence that acute EH, per se, elicits sensory LTF of the carotid body; however, acute EH does produce phrenic LTF (18, 25). ROS formation is necessary for the induction and/or maintenance of phrenic LTF (26). Acute EH may increase the production of ROS, enhancing excitatory neurotransmission and facilitation of ventilatory plasticity (an indicator of phrenic LTF). Whether ROS played a role in the increased hypocapnic ventilatory response after acute EH in our protocol is unconfirmed at this time. Additionally, enhanced ventilatory chemoreflex sensitivity could be a result of sensory LTF that manifests as a long-lasting activation of sensory discharge from the carotid bodies, activated by acute EH after preconditioning with chronic intermittent hypoxia (25, 26). However, our model did not address chronic intermittent hypoxia. Finally, studies have identified regions of the caudal hypothalamus and rostral ventrolateral medulla, in addition to the pons, that are directly excited by hypoxia (39) and that, when activated, increase sympathetic and respiratory activity; enhanced chemoreflex sensitivity may occur at the level of the integration of the afferent output at these central nervous system sites via oxygen-sensing neurons and the subsequent translation of chemoreceptor afferent information to appropriate ventilatory changes (11). All of the above are potential explanatory mechanisms; however, our protocol and findings do not permit us to definitively identify a specific mechanism to explain the increased chemoreflex sensitivity after EH.

**Physiological Significance**

The aforementioned findings have significant clinical implications regarding the pathophysiology of central apnea. Increasing the hypocapnic ventilatory response and narrowing the CO2 reserve in the aftermath of EH renders the ventilatory control system more susceptible to recurrent central apnea and may perpetuate instability, even after the initial perturbation is removed. Likewise, this susceptibility to apnea may be evident in the presence of ventilatory LTF, indicating that LTF may be a marker for ventilatory instability after EH. Therefore, enhanced susceptibility to central apnea after EH may contribute to increased apnea severity across the night in patients with sleep apnea. We believe that the increased hypocapnic ventilatory response and the concomitant decrease in the CO2 reserve observed in the present study were consequences of the exposure to EH, since these did not change significantly after the sham intervention. There is evidence that LTF may be mediated via serotoninergic receptors (6, 25, 26), N-methyl-D-aspartate receptors (33), or ROS (26), at least in animal models, and these may provide potential targets for pharmacological manipulations. Our study allowed us to clarify the role of LTF on breathing stability in humans during sleep. Elucidation of the components and mechanisms of LTF in humans during sleep potentially provides an incremental understanding toward the development of pharmacological therapies for sleep apnea.

In summary, our findings confirm our hypothesis: that EH in sleeping humans promotes breathing instability and the development of central apnea by narrowing the CO2 reserve. While ventilatory LTF, per se, may mitigate central apnea by decreasing plant gain, the increased ventilatory chemoreflex sensitivity offsets the protective effect.

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**DISCLOSURES**

No conflicts of interest are declared by the author(s).

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