Long-term facilitation in obstructive sleep apnea patients during NREM sleep

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SLEEP APNEA/HYPOPNEA SYNDROME is characterized by repetitive upper airway obstruction with ensuing cyclical hypoxia. Interestingly, repetitive hypoxia is followed by persistently increased ventilatory motor output; this is referred to as long-term facilitation (LTF). This excitatory mechanism occurs after repetitive stimulation of the carotid bodies as ventilation returns to baseline over a long duration, up to several hours (11). LTF has been observed (to a variable extent) in dogs (4), cats (5, 10), rats (2), and goats (19). We have previously shown that LTF is elicited by repetitive hypoxia during sleep but only in subjects who snore regularly and who have evidence of inspiratory flow limitation during sleep. In our model, LTF manifested as increased inspired minute ventilation (V̇I) and amelioration of inspiratory flow limitation (1). Given the occurrence of repetitive hypoxemia in patients with sleep apnea, we wished to investigate the occurrence of LTF in patients with obstructive sleep apnea/hypopnea syndrome (OSA). The purpose of this study was to test the hypothesis that episodic hypoxic exposure activates LTF in OSA patients during stable non-rapid eye movement (NREM) sleep. To this end, we induced repetitive hypoxia in OSA patients using nasal continuous positive airway pressure (CPAP) to maintain upper airway patency and stable sleep state for the duration of the experiments.

METHODS

Subjects

The Human Investigation Committee of the Wayne State University School of Medicine and the Detroit Veterans Affairs Medical Center approved the experimental protocol. Informed, written consent was obtained from 11 subjects who had recently documented and untreated sleep apnea. Subjects with other medical problems, daytime hypoxemia, or cor pulmonale were excluded. Table 1 shows subject demographics. There were 9 men and 2 women with a mean age of 52.2 ± 10.7 yr (range 31–70), body mass index of 33.9 ± 4.0 kg/m², and apnea/hypopnea index of 43.6 ± 18.7 event/h.

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Breathing Circuit

The breathing circuit is illustrated in Fig. 1. The subject was connected to the circuit with an airtight silicone rubber mask strapped and glued to the face to prevent leaks. The mask was connected to a plateau exhalation valve (Respironics, Pittsburgh, PA) via a heated pneumotachometer. The valve, which provides a continuous leak path in the breathing circuit and serves as an exhaust vent, was connected on the inspiratory side to a nasal CPAP machine (Quantum PSV, Healthdyne Technologies, Marietta, GA). Three cylinders, containing 100% N₂, 8% O₂, or 100% O₂, were connected to the inspiratory line. CO₂ was added to the inspiratory line from an external source, and end-tidal CO₂ (PETCO₂) was maintained at control levels.

Measurements

Electroencephalograms (EEG), electrooculograms (EOG), and chin electromyograms (EMG) were recorded by use of the international 10–20 system of electrode placement (EEG: C₃–A₂ and C₄–A₁; EOG F₇–A₂ and F₈–A₂). Inspiratory airflow was measured by a heated pneumotachometer (model 3700A, Hans Rudolph, Kansas City, MO) that was 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). Airway pressures were measured by using a pressure transducer tipped catheter (Model TC-500XG, Millar Instruments, Houston, TX). Supraglottic pressure (Psg) was measured by positioning the catheter tip in the hypopharynx and observing it disappear behind the tongue. PETCO₂ was measured by using air sampled continuously from the nasal mask by an infrared analyzer (model CD-3A, AEI Technologies, Pittsburgh, PA). Arterial O₂ saturation was measured by a pulse oximeter (Biox 3700, Ohmeda). The signals were displayed on a polygraph recorder (model 7-D, Grass, West Warwick, RI) and recorded by using Biobench data acquisition software (National Instruments, Austin, TX) for further analysis. Surface inspiratory EMG (EMGinsp) was recorded with the use of two surface electrodes (3M Red Dot, 3M, St. Paul, MN) placed 2–4 cm above the right costal margin in the anterior axillary line. One pair was positioned at the percussed dullness at total lung capacity, and another pair was positioned at the point of percussed dullness at functional residual capacity. The electrode pair with the best signal-to-noise ratio was selected for analysis.

Protocol

Figure 2 illustrates the sequence of sleep studies each subject underwent consisting of polysomnography (PSG) in the clinical sleep laboratory to diagnose OSA then a second PSG for nasal CPAP titration to obtain the optimal pressure needed to eliminate apneas, hypopneas, and snoring. At this point and before initiation of treatment with CPAP at home, patients diagnosed with OSA who fit our inclusion criteria were invited to participate in our study protocol, which included three additional sleep studies performed at the research sleep laboratory. During each study, the CPAP was

Table 1. Subjects' demographics

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Means ± SD 52.2 ± 10.7 33.9 ± 4.0 43.6 ± 18.7

BMI, body mass index; AHI, apneas/hypopneas index; F, female; M, male.
retitrated to a pressure needed to eliminate apneas/hyponneas but maintain airflow limitation (suboptimal pressure).

Night 1. Eleven patients were studied on night 1 (N1). The suboptimal pressure was 7.1 ± 2.7 cmH2O. After reaching stage 2 or stage 3 sleep, the subjects breathed room air for 5 min (control period), followed by 3 min of hypoxic gas (8% O2); this sequence was repeated 10 times. Hypoxia was rapidly induced by having the subject breathe one or two breaths of 100% N2 followed by continuous 8% O2 for 3 min to maintain hypoxia (O2 saturation: 80–84%). Care was taken to ensure that isocapnia was maintained throughout the hypoxia period by measuring PETCO2 and 5% CO2 was supplemented as needed. Hypoxia was abruptly terminated with one breath of 100% O2. The breathing pattern was monitored at 5, 20, and 40 min of the recovery period after the 10th exposure to hypoxia.

Night 2. Each patient received a nasal CPAP machine set to the optimal titration pressure and was asked to use it for a minimum of 6 h/night for at least 4 wk. After 4 wk of treatment with optimal pressure CPAP, 8 patients returned for the night 2 (N2) study, which followed the same protocol as the N1 study.

Sham study. Seven patients had a third study during which the CPAP was retitrated to suboptimal pressure (mean = 5 ± 1.4 cmH2O). The pressure was maintained throughout the study night without any hypoxic periods.

Data Analysis

Wakefulness/sleep stage was scored according to standard criteria (12). The subjects were in stable stage 2 or stage 3 (slow wave % = 20–25%) sleep during the hypoxic exposures and data collection, and there were no arousals found during the data collection periods. Inspired VT, inspiration time (Ti), total time for a breath (Tr), breathing frequency, PETCO2, and arterial O2 saturation were calculated breath by breath during stable sleep during the first normoxic period (control period) and at 5, 20, and 40 min after the 10th hypoxic exposure. Breaths for analysis were selected during a period of stable sleep with no evidence of an arousal (17) by an independent observer. A mean value for each variable was computed from 10 consecutive breaths except for control, from which two sets of 10 consecutive breaths were chosen. For the sham study, no hypoxia was induced. Data were sampled after 80 min of sleep; this duration is equal to the total duration of the repetitive hypoxia trials in the N1 protocol. Accordingly, sham recovery periods were selected at 85, 100, and 120 min from the beginning of the control period. The recovery period at 100 min represents 20 min into recovery (R20) for the sham study. All the data were normalized to the control period data for comparison.

To ascertain changes in upper airway mechanics, a pressure-flow loop was plotted for every breath in 20 breaths of the control period, 10 breaths of the first hypoxia, and 10 breaths each at 5, 20, and 40 min of recovery period. All breaths were averaged, and composite pressure-flow loops were plotted for the control and 20-min recovery periods for each subject (Fig. 4). To generate a composite pressure-flow plot of breaths with different duration, pressure and flow were sampled at equally distributed points in inspiration and expiration in each breath. Upper airway resistance (Rua) at a fixed flow (0.2 l/s) and at a maximum linear flow was computed from each loop as a numeric representation of the slope of the linear part of the pressure-flow loop. To ascertain the presence of inspiratory flow limitation in each subject who had a pressure catheter placed, a pressure-flow loop of each breath was used. Flow limitation was defined as plateau in flow despite ≥1 cmH2O decrease in the Psg. However, we used the criteria of Teschler et al. (18) to ascertain the presence of inspiratory flow limitation during the study with suboptimal CPAP. We had previously validated this method against pressure-flow loops in our laboratory (1).

To analyze EMGinsp, the raw EMG signal was amplified, filtered with a band-pass filter of 50–10,000 Hz (Grass model 7-D polygraph) and full-wave rectified. Electrocardiogram artifacts were “blanked” with an electrocardiogram blanker (CWE model SB-1). The processed signal was integrated with a moving-time averager with a time constant of 100 ms (CWE model MA-821 RES). Phasic EMG activity was determined from the moving time average.

The effect of CPAP treatment on LTF was determined by comparing the VT at R20 between N1 and N2 (after 4 wk of CPAP treatment with optimal pressure). One-way repeated-measures ANOVA was used to compare values of variables between control, hypoxia, and R20. The Student’s paired t-test was used to compare N1 and N2.

A commercially available computer statistical package was used to analyze the data (Sigma Stat 2.0, SPSS). VT (as percentage of control) at R20 was chosen as the dependent variable because it represents the presence or absence of LTF. The level of significance was set at P < 0.05.

RESULTS

We studied 11 subjects for N1. Figure 3 is a representative polygraph record showing ventilation and upper airway mechanics during control conditions (Fig. 3A), hypoxia (Fig. 3B), and 20 min into the recovery period (Fig. 3C). Hypoxia resulted in increased VT and decreased Rua. During the recovery period, decreased Rua persisted at 5, 20, and 40 min into the recovery period, although VT returned to control. For the group as a whole, hypoxia resulted in increased VT from 11.4 ± 2.6 to 14.8 ± 3.1 l/m (132% of control). Supplemen- tional CO2 maintained PETCO2 at near-normoxic levels.
(PETCO₂ = 42.5 ± 7.9 and 41.7 ± 5.7 Torr during control and hypoxia, respectively, P > 0.05). A representative pressure-flow loop during control and posthypoxic recovery (R20) is shown in Fig. 4. Note the change in slope and the amelioration of the inspiratory flow limitation during the recovery period of N1 (Fig. 4A), but not during the recovery period of the sham study (Fig. 4B). For the group, Rua at a fixed flow (0.2 l/s) and at a maximum linear flow decreased to 58 ± 24 and 64 ± 17% of control, respectively (P < 0.05) (Fig. 5). Decreased Rua was due to diminished magnitude of Psg (from 4.1 ± 2.7 to 2.3 ± 1.4 cmH₂O; P < 0.05, Fig. 6). The decrease in Rua was not matched by changes in ventilation. V̇I during the recovery period was 10.7 ± 2.6 l/min (99% of control; P > 0.05) (Fig. 7). For the group, EMGinsp at R20 was 87% of control (P > 0.05, Fig. 8). There was no change in VT (0.7 ± 0.1 liter; 98% of control; P > 0.05), breathing frequency (15.7 ± 3.4 breaths/min, 98% of control, P > 0.05). Although VT shortened during the recovery period at R20 to 1.8 ± 0.4 s (95% of control), this shortening only approached statistical significance (P = 0.08). The only timing variable with a statistically significant change was T/Tt total breath duration, which decreased slightly but consistently to 0.46 ± 0.04, 92% of control (P < 0.05). We had similar results at 40 min of recovery period (R40). Rua at a maximum linear flow decreased to 60 ± 24% of control in five out of six subjects (P < 0.05), with no changes in ventilation (94 ± 12% of control, P > 0.05).

The findings of the sham study differed from N1 study. Seven subjects underwent sham study including four subjects who had Psg measurements. There was no difference in Rua or V̇I at R20 when compared with control. There were no significant changes between control and recovery period (R20) for V̇I (control = 12.7 ± 3.5 l/min, R20 = 12 ± 2.9 l/min; 98% of control; P > 0.05) and Rua (control = 6 ± 2.2 cmH₂O·l⁻¹·s, R20 = 7.2 ± 2.9 cmH₂O·l⁻¹·s; 120% of control; P > 0.05). Likewise, there was no significant change in VT (control = 0.73 ± 0.16 liter, R20 = 0.69 ± 0.9 liter, P > 0.05), T/Tt (control = 1.8 ± 0.6 s, R20 = 1.9 ± 0.4 s, P > 0.05), T/Tt (control = 0.51 ± 0.16, R20 = 0.53 ± 0.12, P > 0.05), or respiratory frequency (control = 17.5 ± 3.8 breaths/min, R20 = 17.3 ± 3.5 breaths/min, P > 0.05).

The N2 study was conducted in eight subjects after 4 wk of treatment with optimal nasal CPAP. Because the CPAP machine has a timer to measure hours of usage, we found that the CPAP was used for an average of 4.7 ± 2.1 h/night. Psg was measured in four subjects. There was no difference in the findings between N1 and N2. There was no change in VT in the recovery period compared with control values (control = 10 ±
2.7 l/m, R$_{20}$ = 10 ± 3.6 l/m, $P = 0.98$). Likewise, there was no change in respiratory frequency (control = 15.8 ± 4.1 breaths/min, R$_{20}$ = 15.5 ± 4.7 breaths/min; 98% of control, $P > 0.05$), VT (control = 0.64 ± 0.12L, R$_{20}$ = 0.65 ± 0.14 liter; 101% of control; $P > 0.05$) or T$_{1}$ (control = 1.8 ± 0.4 s; R$_{20}$ = 1.7 ± 0.4 s; 94% of control; $P > 0.05$). The slight but consistent reduction in T$_{1}$/T$_{T}$ (control = 0.44 ± 0.05, R$_{20}$ = 0.42 ± 0.04; 93% of control; $P < 0.05$) was the only timing variable to change to a statistically significant degree (Table 2). Finally, Rua during the recovery period decreased to 83 ± 9% of control (control = 9.6 ± 1.4 cmH$_2$O·l$^{-1}$·s, R$_{20}$ = 8 ± 1.8 cmH$_2$O·l$^{-1}$·s, $P = 0.04$).

DISCUSSION

The aim of this study was to determine whether LTF was present after repetitive hypoxic exposure in patients with OSA during sleep. We have shown reduced Rua in the recovery period after repetitive hypoxia. However, no reciprocal changes in ventilation were noted. This pattern of response persisted after treatment with nasal CPAP for 4 wk.

Limitations of Methods

Several limitations have to be considered for proper interpretation of our findings.

First, changes in sleep state might have caused a misinterpretation of the data. However, we analyzed data only when the sleep was in stable stage 2 or greater, with no evidence of arousal. If a subject did not maintain sleep at stage 2 or deeper for the majority of the 140-min study period, the data of that subject were not used in the analysis. The data reported here were from periods in which there was no difference in sleep state. The changes in sleep state between the experimental nights and the sham night were also similar.

Second, we were unable to maintain precise isocapnia during hypoxia because of the high flow in the nasal CPAP circuit. This may dampen peripheral chemoreceptor output and potentially decrease the magnitude of LTF. However, the reduction in PETCO$_2$ was minimal and not of statistical significance. Furthermore, we were able to demonstrate consistent differential effect between upper airway mechanics and venti-
lation, an indication that LTF may be more pronounced at the level of upper airway dilators (see *Role of LTF in Sleep Apnea Patients*).

Third, the induced repetitive hypoxia in our study may not be representative of gas exchange perturbations due to obstructive apneas. We selected the duration and frequency of hypoxia on the basis of previous studies demonstrating LTF after repetitive hypoxia. Therefore, our model of repetitive hypoxia does not simulate sleep apnea. Whether LTF is elicited by obstructive apneas is yet to be determined.

Fourth, the duration of nasal CPAP therapy may not have been sufficient to reverse all the chronic effects of nocturnal cerebral hypoxia. However, we selected this duration on the basis of previous work showing restoration of load responsiveness after 4 wk of nasal CPAP therapy indicative of central nervous system recovery (6). Furthermore, compliance with therapy may be difficult to attain for longer durations.

Finally, decreased Rua indicates increased upper airway caliber; however, caliber and stiffness are different components of upper airway mechanics. The latter is not addressed by our measurement. Whether LTF is associated with changes in pharyngeal compliance remains to be determined.

*Role of LTF in Sleep Apnea Patients*

We have shown that repetitive hypoxia results in decreased Rua indicative of upper airway dilatation. Decreased Rua may be explained by passive or active events. Passive dilatation of the upper airway as seen with increased caudal traction was unlikely given that Vt did not increase during the recovery period (20). Reduced Rua during the recovery period may also be due to passive persistence of dilatation after hypoxia. Several studies have demonstrated decreased Rua with hypoxia (3, 9). Accordingly, upper airway hysteresis (the upper airway is easier to keep open than to open for the first time) would maintain upper airway dilatation in the recovery period. However, we doubt sustained persistent dilatation would last for 20–40 min as seen in our study. Thus decreased Rua cannot be adequately explained by passive events.

We interpret decreased Rua as an indication of active upper airway dilatation due to LTF of ventilatory motor output to upper airway dilators. There is evidence in the literature that repetitive hypoxia elicits LTF of ventilatory motor output to upper airway dilators in both humans and animals. Mateika and Fregosi (10) have shown that repetitive hypoxia in vagotomized cats is followed by increased activity of the genioglossus and the alae nasae but not the diaphragm. Similarly, our laboratory has shown that repetitive hypoxia in snorers results in amelioration of inspiratory flow limitation for 40–60 min after termination of hypoxic exposure during NREM sleep (1).
Thus our present findings are consistent with previous literature, suggesting that repetitive hypoxia leads to LTF of upper airway motoneurons, helping to maintain upper airway patency. However, this hypothesis requires testing with an experimental protocol including EMG measurements from upper airway dilators.

The salutary effects of decreased $R_{ua}$ were not matched by increased inspiratory muscle activity or minute ventilation. This finding suggests lack of LTF of the phrenic nerve motor output. This is also consistent with the work of Mateika and Fregosi (10), who did not find LTF of the diaphragm in the aftermath of repetitive hypoxia. However, the lack of increased minute ventilation despite decreased $R_{ua}$ and “unloading” of the upper airway intrigued us. We have previously shown that LTF in normal snoring subjects manifested as amelioration of inspiratory flow limitation and increased $V_{ti}$. One may speculate that LTF in sleep apnea patients is limited to upper airway motoneurons, whereas normal subjects have the ability to activate LTF to upper airway and to the phrenic motoneurons.

The aforementioned discussion does not take into consideration that unloading per se, without invoking phrenic LTF, is associated with increased $V_{t}$ and $V_{i}$. Skatrud et al. (16) have shown that unloading of the upper airway with $He-O_2$ mixture during NREM sleep results in reduced total pulmonary resistance by 38% and increased $V_{t}$ and $V_{i}$ despite shortening of $T_{i}$. We interpret the difference between our present study and the unloading work of Skatrud et al. by the difference in upper airway dimensions between normal snorers and sleep apnea patients. Decreased $R_{ua}$ in snorers would increase inspiratory flow and hence $V_{i}$. In contrast, upper airway caliber is smaller in patients with sleep apnea relative to snorers (13, 15). Consequently, changes in resistance were not associated with increases in inspiratory flows. In fact, the noted decrease in resistance in our subjects was due to decreased $P_{sg}$ without a significant increase in inspiratory flow (see Fig. 6).

The development of LTF after repetitive hypoxemia may be particularly relevant to the PSG manifestations of sleep apnea syndrome. Repetitive upper airway obstruction may activate LTF of upper airway motoneurons and dilate the upper airway after a cluster of respiratory events. This may account for the observation that apnea and hypopnea occur in clusters with lucid periods free of abnormal respiratory events. Thus LTF may be an important mechanism to restore upper airway patency, albeit briefly, after repeated episodes of apnea. It is plausible that recruitment of LTF may explain the improvement of sleep apnea/hypopnea after treatment with serotonergic agents such as selective serotonin reuptake inhibitors (SSRIs) or L-tryptophan that may increase the concentration of serotonin in the central nervous system and hence in the raphe nucleus (7, 8, 14). However, this explanation is speculative in the absence of studies investigating the effect of SSRIs on the manifestations of LTF.

In summary, we have shown that episodic hypoxia during sleep in OSA patients elicits LTF of ventilatory motor output manifested by decreased $R_{ua}$ without an increase in $V_{i}$, suggesting that the thoracic pump muscles do not demonstrate LTF and nasal CPAP did not alter the ability of OSA patients to elicit LTF at the thoracic pump muscle.

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