Determinants of long-term facilitation in humans during NREM sleep

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Babcock, Mark, Mahdi Shkoukani, Salah E. Aboubakr, and M. Safwan Badr. Determinants of long-term facilitation in humans during NREM sleep. J Appl Physiol 94: 53–59, 2003. First published September 6, 2002; 10.1152/japplphysiol.00476.2002.—Long-term facilitation (LTF) is a prolonged increase in ventilatory motor output after episodic peripheral chemoreceptor stimulation. We have previously shown that LTF is activated during sleep following repetitive hypoxia in snorers (Babcock MA and Badr MS. Sleep 21: 709–716, 1998). The purpose of this study was 1) to ascertain the relative contribution of inspiratory flow limitation to the development of LTF and 2) to determine the effect of eliminating inspiratory flow limitation by nasal CPAP on LTF. We studied 25 normal subjects during stable non-rapid eye movement sleep. We induced 10 episodes of brief repetitive hypoxia (inspired O2 fraction = 8%; 3 min) followed by 5 min of room air. Measurements were obtained during control and at 20 min of recovery (R20). During the episodic hypoxia study, inspiratory minute ventilation (V̇I) increased from 6.7 ± 1.9 l/min during the control period to 8.2 ± 2.7 l/min at R20 (122% of control; P < 0.05). Linear regression analysis confirmed that inspiratory flow limitation during control was the only independent determinant of the presence of LTF (P = 0.005). Six subjects were restudied by using nasal continuous positive airway pressure to ascertain the effect of eliminating inspiratory flow limitation on LTF. V̇I during the recovery period was 97 ± 10% (P > 0.05). In conclusion, 1) repetitive hypoxia in sleeping humans is followed by increased V̇I in the recovery period, indicative of development of LTF; 2) inspiratory flow limitation is the only independent determinant of posthypoxic LTF in sleeping human; 3) elimination of inspiratory flow limitation abolished the ventilatory manifestations of LTF; and 4) we propose that increased V̇I in the recovery period was a result of preferential recruitment of upper airway dilators by repetitive hypoxia.

APNEAS AND HYPOPNEAS DURING sleep do not occur in isolation but in cycles of apnea/hypopnea, resulting in episodic hypoxia and followed by hyperpnea. Interestingly, episodic hypoxia has been shown to elicit a prolonged increase in ventilatory motor output subsequent to the removal of the stimulus, referred to as long-term facilitation (LTF). This phenomenon has been observed in some animal models (6, 8, 11, 13, 15, 18, 19, 26) but not others (10). Similarly, studies investigating the occurrence of LTF in humans have shown variable results. For example, the only study in humans during wakefulness found no evidence of LTF (14). In contrast, we have shown that ventilatory LTF for 40–60 min can be evoked by repetitive hypoxia in a subset of sleeping humans, specifically in subjects who snored and who demonstrated inspiratory flow limitation (IFL) while breathing room air (2). In addition, we have shown that ventilatory LTF occurs in association with decreased upper airway resistance in the recovery period (22). Thus ventilatory LTF may be a result of decreased upper airway resistance and unloading of the upper airway.

Despite the consistency of the LTF development in individuals with IFL, it is not clear whether IFL represents a true independent variable or whether it reflects another primary variable. Specifically, upper airway narrowing during inspiration is more common in men, and it correlates with body mass index (BMI) (17). Similarly, upper airway narrowing during sleep may dampen hypoxic ventilatory response. Thus the purpose of this study was twofold: first, to ascertain the relative contribution of IFL vs. other potential determinants of LTF and, second, to determine the effect of eliminating IFL on LTF. To this end, we unloaded the upper airway with nasal continuous positive airway pressure (CPAP) sufficient to eliminate snoring and IFL.

METHODS

Subjects

The Human Investigation Committee of the Wayne State University School of Medicine and the John D. Dingell Veterans Affairs Medical Center approved the experimental protocol. Informed consent was obtained from each participant. We studied 25 healthy subjects free of any cardiopulmonary or sleep disorder. The group consisted of 14 men and 11 women, aged 21–45 yrs, with body mass index 20–28 kg/m2.

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Eleven women with a mean age of 29.7 ± 3.5 yr and BMI of 25.9 ± 2.2 kg/m².

Breathing Circuit

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 (Respiromics, 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 line. Three cylinders, containing 100% N₂, 8% O₂, or 100% O₂, were connected to the inspiratory line. To maintain isocapnia, supplemental CO₂ was added to the inspiratory line from an external source to maintain end-tidal PCO₂ (PETCO₂) at or near control levels.

Measurements

Electroencephalograms, electrooculograms, and chin electromyograms were recorded by using standard methods. Inspiratory airflow was measured by a heated pneumotachometer (model 3700A, Hans Rudolph, Kansas City, MO) attached to a pressure transducer (Validyne, Northridge, CA), and tidal volume (VT) was computed by electronic integration of the flow signal (model FV156 Integrator, Validyne). Supraglottic airway pressure was measured by using a pressure transducer-tipped catheter (model TC-500XG, Millar Instruments, Houston, TX) in 12 subjects. The hypopharyngeal position was confirmed by advancing the catheter tip for 2 cm after it disappeared behind the tongue. To ascertain the presence of IFL in each subject who had a pressure catheter placed, a pressure-flow loop of each breath was used (n = 12). The effect of episodic hypoxia on upper airway resistance in this subgroup has been published separately (22). Flow limitation was defined as plateau in flow despite a decrease in supraglottic (downstream) pressure ≥1 cmH₂O. In the remainder of the subjects, we used the flow profile criteria of Teschler et al. (25) to ascertain the presence of IFL. Flow limitation was determined as a dichotomous variable.

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 (Sao₂) was measured by using pulse oximetry (Biox 3700, Ohmeda). All signals were displayed on a polygraph recorder (model 7-D, Grass, West Warwick, RI) and recorded by using Power Lab digital acquisition software (Power Lab SP Series, AEI Technologies, Pittsburgh, PA). Arterial O₂ saturation from the nasal mask by an infrared analyzer (model CD-3A, Ohmeda) was used to ascertain the presence of IFL. Flow limitation was determined as plateau in flow (22).

Protocol

Subjects lay in the supine position for the entire study, which was conducted during stable non-rapid eye movement (NREM) sleep (stable stage 2 or stage 3 sleep). Two protocols on separate nights were conducted as part of this investigation.

Protocol 1: episodic hypoxia. Twenty-five subjects participated in this protocol, which has been previously described in detail (1, 2, 22). Ten cycles of episodic hypoxia were induced in each subject. 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 (Sao₂, 80–84%). Supplemental CO₂ was titrated to maintain isocapnia during hypoxia guided by PETCO₂ on a breath-by-breath basis. Hypoxia was abruptly terminated with one breath of 100% O₂. This was followed by 5 min of room air breathing. The breathing pattern was monitored for 40 min of recovery after the tenth exposure to hypoxia.

A sham study was also conducted to ascertain the presence of time-dependent changes in ventilation, independent of the experimental protocol. Eleven subjects were studied on a different night. This subgroup consisted of six men and five women with a mean age of 28.8 ± 6.5 yr and BMI of 26.8 ± 4.6 kg/m². The participants were connected to the same breathing circuit with identical instrumentation. However, the subject breathed ambient air for the entire study time (120 min of sleep).

Protocol 2: unloading. To test the effects of inspiratory unloading, a subgroup of subjects who demonstrated ventilatory LTF (n = 6) and IFL on the baseline study (protocol 1) agreed to undergo a repeat episodic hypoxia study with nasal CPAP unloading for the entire study period (120 min). This subgroup consisted of four men and two women with a mean age of 26 ± 4 yr and BMI of 27.1 ± 3.7 kg/m². The level of CPAP was set so that all visual signs of IFL were eliminated. When the subjects reached stable stage 2 or 3 NREM, the episodic hypoxia protocol was performed as outlined above.

Data Analysis

We selected for analysis segments with stable sleep only. Sleep stage was scored according to Rechtschaffen and Kales criteria (20), and transient arousals were scored by using the American Sleep Disorders Association criteria (24). The number and duration of each arousal during hypoxia were calculated for each subject. To ensure that changes in ventilation were not a result of subtle changes in sleep state, an independent observer confirmed the stability of sleep state. The control period consisted of 3 min immediately preceding the first hypoxic exposure. Three minutes of recovery were selected at 20 min of recovery (R20). All breaths in the selected control and R20 segments were used for determination of IFL; the last 20 breaths were used for measurement of ventilation. A mean value for each variable was computed from 20 consecutive breaths. Data are presented as means ± SD. No hypoxia was induced during the sham study; breaths for measurement were selected at 100 min from the beginning of the control period to represent R20. All the data were normalized to the control period data for comparison.

Statistical analysis. A computer statistical package was used to analyze the data (SigmaStat 2.0, SPSS). The inspiratory minute ventilation (Vi; as percentage of control) at R20 was chosen as the dependent variable for the presence of LTF. Selected independent variables were considered for inclusion in a multiple linear regression model, on the basis of an a priori reasoning that they may contribute to Vi at R20. The level of significance was set at P < 0.05. The following potential variables were tested with univariate regression analyses: 1) percentage of breaths that were flow limited during the control period, 2) gender, 3) BMI, 4) number of arousals during hypoxia, and 5) hypoxic ventilatory response. The latter was defined as the slope of Vi against Sao₂ from the onset of hypoxia until steady state is reached (7) (see Table 2).

The effect of eliminating IFL on LTF was determined from the data of the subgroup of subjects by comparing the Vi at R20 from the regular LTF night and the night when nasal CPAP was used to eliminate IFL. The Student’s t-test was used for the comparison.

RESULTS

Twenty-five subjects participated in the episodic hypoxia protocol, and eleven subjects participated in the sham protocol. Figure 1 is a representative polygraph record from one subject who snored habitually
and demonstrated IFL during the study. The segment depicts ventilation during room air control conditions (A), hypoxia (B), and at R20 (C). Note increased flow and diminished magnitude of supraglottic pressure during hypoxia and R20 relative to control.

During the hypoxic trials, SaO2 decreased to 87 ± 4%. Although sleep state was stable in most of the trials, a few brief episodes of arousal were inevitable. The number of arousals per subject during the entire duration of hypoxia was 3.2 ± 3.0 arousals with sum duration of 4.2 ± 5.2 min. Hypoxia resulted in increased Vt to 9.3 ± 1.9 l/min during room air control (P < 0.05). No significant change in PETCO2 was noted during hypoxia (43.5 ± 5.8 vs. 44.8 ± 5.6 Torr during room air control; P > 0.05).

Table 1 shows the changes in ventilation and timing for episodic hypoxia studies during the recovery period relative to control. Vt at R20 increased to 122% of control (P < 0.05). The increase in Vt was due to increased VT and 120% of control (P < 0.05) as a result of increased VT-to-inspiratory time (TI) ratio to 123% of control (P = 0.01). The aftereffects of repetitive hypoxia on timing parameters were not remarkable, because there was no significant change in TI, expiratory time (TE), or TI-to-total breath duration ratio. The findings of the sham study differed from the repetitive hypoxia study; Vt and VT during the recovery period were 98 and 100% of control, respectively (P > 0.05; Table 2).

Table 2 compares findings between repetitive hypoxia and sham nights in the 11 subjects who underwent both studies. Vt increased at R20 during the repetitive hypoxia but not the sham study. The only change from the control period to R20 during the sham study was the prolongation of TE from 1.91 ± 0.29 to 2.07 ± 0.24 s (P < 0.05; Table 2).

Increased Vt at R20 did not occur in all subjects. To explain the variability in Vt at R20, several possible variables were tested with univariate-regression anal-

<table>
<thead>
<tr>
<th>Table 1. Ventilation and timing during control and posthypoxic recovery</th>
<th>Repetitive Hypoxia (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>R20</td>
</tr>
<tr>
<td>Vt, l/min</td>
<td>6.7 ± 1.8</td>
</tr>
<tr>
<td>VT, liter</td>
<td>0.41 ± 0.13</td>
</tr>
<tr>
<td>f, breaths/min</td>
<td>16.9 ± 2.5</td>
</tr>
<tr>
<td>TI, s</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>TE, s</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>VT/TI, l/s</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>VT/TI, Vs</td>
<td>0.25 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects. Vt, inspiratory minute ventilation; VT, tidal volume; f, breathing frequency; TI, inspiratory time; TE, expiratory time; VT/TI, inspiratory time/tidal breath duration; VT/TI, mean inspiratory flow; R20, 20 min of recovery. *P < 0.05.
Fig. 2. Group data for inspiratory minute ventilation (V̇I) and VT during the recovery period (R20). Note increased VT and VI at R20 during the episodic hypoxia study (solid bars) but not during the sham study (open bars). Values are means ± SE. *P < 0.05.

In protocol 2, subjects who had shown evidence of LTF during protocol 1 (n = 6) were restudied by using nasal CPAP to eliminate IFL. All were snorers with evidence of IFL during eupneic breathing. The level of pressure assist that was used to eliminate IFL was 2.9 ± 0.9 cmH2O. The nadir level of hypoxia was 84.1 ± 2.8%, which was not different from the baseline LTF study night (81.6 ± 3.2%). V̇I at R20 for the unloaded study was not different from prehypoxia control (96.9 ± 10.2%; Table 4). Comparison of the V̇I at R20 between the loaded and unloaded studies for each subject revealed a significant difference between the two studies (P < 0.02; Fig. 4).

### Table 3. Determinants of long-term facilitation

<table>
<thead>
<tr>
<th>Variable</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>V̇I %con vs.</td>
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</tr>
<tr>
<td>Age, yr</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>0.08</td>
</tr>
<tr>
<td>Breaths with IFL, %</td>
<td>0.32*</td>
</tr>
<tr>
<td>HVR, l/min</td>
<td>0.01</td>
</tr>
<tr>
<td>ΔPETCO₂, Torr</td>
<td>0.01</td>
</tr>
<tr>
<td>SaO₂ H, %</td>
<td>0.23</td>
</tr>
<tr>
<td>VT %con vs.</td>
<td>0.01</td>
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<tr>
<td>Age, yr</td>
<td>0.16</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
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</tr>
<tr>
<td>Breaths with IFL, %</td>
<td>0.06</td>
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<tr>
<td>HVR, l/min</td>
<td>0.004</td>
</tr>
<tr>
<td>ΔPETCO₂, Torr</td>
<td>0.23</td>
</tr>
<tr>
<td>SaO₂ H, %</td>
<td>0.23</td>
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</tbody>
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Our study revealed three important findings: 1) post-hypoxic hyperpnea occurred after repetitive hypoxia during sleep, 2) the only independent determinant of increased $V_t$ during the recovery period was the magnitude of IFL in the control period, and 3) alleviation of IFL eliminated posthypoxic hyperpnea.

**Methodological Considerations**

The validity of our results is dependent on stability of sleep state, which was confirmed by an independent observer. Similarly, passive mechanical changes such as changes in head or neck position may cause an increase in $V_t$ or $V_{ti}$. This is unlikely because no changes were noted in the head or neck position. Furthermore, the consistent difference between ventilation during the hypoxia and the sham studies is unlikely to be due to passive mechanical changes.

Nasal CPAP may have other effects on lung volumes or respiratory muscle activity in addition to “unloading” the upper airway and elimination of IFL. However, we used the lowest possible level to eliminate flow limitation. We believe that the effects noted on the CPAP might are a result of unloading per se and not of effects on respiratory or upper airway muscle activity. We selected several variables for inclusion in a linear regression model on the basis of our previous finding (2) that LTF was seen only in individuals who snored and demonstrated evidence of IFL. However, the magnitude of hypoxia and the hypoxic responsiveness were also included as potential independent variables, reflecting the intensity of the “signal” from the peripheral chemoreceptors and potentially the magnitude of LTF.

We collected the recovery data after 20 min of the termination of the last hypoxic exposure. This duration was used in many previous LTF studies. Furthermore, longer recovery periods may not be attainable in all subjects because we were not certain that sleep would remain stable for longer periods.

**LTF in Sleeping Humans**

The occurrence of posthypoxic hyperpnea indicates that hypoxia elicits LTF of ventilatory motor output (LTF). This corroborates previous studies demonstrating evidence of LTF in the aftermath of repetitive hypoxia. Similarly, our study has demonstrated increased $V_t$ manifested mainly as increased $V_{ti}$ without significant change in respiratory frequency.

The strong association between the presence of IFL and the development of LTF intrigued us. We considered that subjects who manifest LTF might be intrinsically different from non-LTF subjects. This interpretation was suggested by the fact that the activation of LTF is a serotonin-dependent central nervous system phenomenon (3). Theoretically, phenotypic differences in the density of serotonergic neurons or ability to increase serotonin level in the raphe nuclei may contribute to LTF variability among subjects. Accordingly, peripheral chemoreceptor stimulation sends excitatory signals to the brain stem integrative centers and to the raphe nuclei; the density of serotonergic neurons and hence the intensity of LTF might be different among different subjects. However, the elimination of LTF with acute unloading with nasal CPAP argues against phenotypic differences as an explanation for the variability of LTF.

Another possible explanation is that the development of LTF in some subjects only may reflect an augmentation of the magnitude of LTF after prior conditioning with a stimulus; this is referred to as metaplasticity (16). Examples of metaplasticity include cervical dorsal rhizotomy, which enhances LTF in rats (12), or chronic intermittent hypoxia, resulting in induction of hypoglossal LTF in a rat substrain that does...
not express LTF (27). Thus chronic snoring with ensuing repetitive arousals or transient hypoxia may condition the ventilatory control system to elicit LTF after repetitive hypoxia. The elimination of LTF with unloading also argues against metaplasticity and suggests that the manifestation of ventilatory LTF is influenced by upper airway mechanics (see below).

Mechanisms of Ventilatory LTF

The development of ventilatory LTF may be due to increased thoracic pump or upper airway-dilating muscle activity. There is evidence in several animal models that repetitive hypoxia elicits phrenic LTF diaphragmatic or intercostal inspiratory activity (3, 5, 15, 16). However, we have recently found that ventilatory LTF was noted without a corresponding increase in diaphragmatic activity as measured by surface electrodes (22). Although this observation suggests that thoracic inspiratory activity is not necessary for the development of LTF, it does not specifically exclude either muscle group given the low sensitivity of surface recordings.

The development of ventilatory LTF may also be explained by active upper airway dilatation due to LTF of upper airway-dilating muscle activity. This interpretation is supported by animal studies demonstrating that repetitive hypoxia elicits LTF of ventilatory motor output to upper airway dilators. Mateika and Fregosi (13) have shown, in vagotomized cats, that repetitive hypoxia is followed by increased activity of the genioglossus and the alae nasae but not the diaphragm. Similarly, we have shown that repetitive hypoxia in sleeping humans is followed by decreased upper airway resistance in the recovery period, indicative of upper airway dilatation (22). Accordingly, increased VT during the recovery period would be due to decreased upper airway resistance or unloading of the pharyngeal airway.

The aforementioned observations combined with the association between baseline IFL and the development of LTF suggest that ventilatory LTF is due to preferential recruitment of upper airway dilators with ensuing unloading of the upper airway. The magnitude of increased VT in the recovery period is consistent with the magnitude of hyperpnea after upper airway unloading with nasal CPAP in our study (Table 3) or previous unloading studies with CPAP (9) or He-O2 mixture (23). Thus the present study suggests that LTF unloaded the upper airway almost to the same level as nasal CPAP. The notion that upper airway dilators are more amenable to the development of LTF compared with the diaphragm is also supported by empirical evidence from animal studies as well (13).

The preferential recruitment of upper airway dilators cannot solely explain the absence of ventilatory LTF in subjects without IFL, nor can it explain the elimination of LTF with nasal CPAP unloading. Instead, ventilatory LTF may reflect a mechanical consequence of upper airway dilator recruitment. Specifically, mechanical and ventilatory changes are more pronounced in snorers (subjects with IFL) relative to nonsnorers (no IFL) for a given magnitude of neurovascular activation. For example, electrical stimulation of the genioglossus results in reduced upper airway resistance only in the presence of upper airway narrowing (21). Conversely, reduced ventilatory motor output causes worsening of flow limitation in subjects with high upper airway resistance and IFL only and not in normal subjects (4). Thus a narrow upper airway may be required for ventilatory manifestations of LTF.

In summary, we have shown that episodic hypoxia during sleep in normal subjects elicits ventilatory LTF manifested by increased VT and VI in subjects with evidence of IFL during eupneic breathing.

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