Long-term facilitation of ventilation and genioglossus muscle activity is evident in the presence of elevated levels of carbon dioxide in awake humans

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Harris, Daniel P., Arvind Balasubramaniam, M. Safwan Badr, and Jason H. Mateika. Long-term facilitation of ventilation and genioglossus muscle activity is evident in the presence of elevated levels of carbon dioxide in awake humans. Am J Physiol Regul Integr Comp Physiol 291: R1111–R1119, 2006. First published April 20, 2006; doi:10.1152/ajpregu.00896.2005.—We hypothesized that long-term facilitation (LTF) of minute ventilation and peak genioglossus muscle activity manifests itself in awake healthy humans when carbon dioxide is sustained at elevated levels. Eleven subjects completed two trials. During trial 1, baseline carbon dioxide levels were maintained during and after exposure to eight 4-min episodes of hypoxia. During trial 2, carbon dioxide was sustained 5 mmHg above baseline levels during exposure to episodic hypoxia. Seven subjects were exposed to sustained elevated levels of carbon dioxide in the absence of episodic hypoxia, which served as a control experiment. Minute ventilation was measured during trial 1, trial 2, and the control experiment. Peak genioglossus muscle activity was measured during trial 2. Minute ventilation during the recovery period of trial 1 was similar to baseline (9.3 ± 0.5 vs. 9.2 ± 0.7 l/min). Likewise, minute ventilation remained unchanged during the control experiment (14.4 ± 1.7 vs. 14.7 ± 1.4 l/min). In contrast, minute ventilation and peak genioglossus muscle activity during the recovery period of trial 2 was greater than baseline (minute ventilation: 28.4 ± 1.7 vs. 19.6 ± 1.0 l/min, P < 0.001; peak genioglossus activity: 1.6 ± 0.3 vs. 1.0 fraction of baseline, P < 0.001). We conclude that exposure to episodic hypoxia is necessary to induce LTF of minute ventilation and peak genioglossus muscle activity and that LTF is only evident in awake humans in the presence of sustained elevated levels of carbon dioxide.

GESTIVOGLOSSUS MUSCLE; EPISODIC HYPOXIA

LONG-TERM FACILITATION (LTF) of minute ventilation and/or its components (i.e., tidal volume and breathing frequency) may be elicited during and after short-term exposure to episodic hypoxia (i.e., 20–30 min). Long-term facilitation is characterized by a gradual increase in respiratory motor activity during successive periods of normoxia that separate hypoxic episodes (30, 32, 44) and by respiratory activity that remains elevated for up to 90 min after exposure to episodic hypoxia (32). Long-term facilitation of minute ventilation or phrenic nerve activity (4, 7, 17, 49), as well as genioglossus muscle or hypoglossal nerve activity (3, 14, 24, 29), has been observed in most, but not all (19), studies completed in animals. In contrast, LTF of minute ventilation and genioglossus muscle activity in healthy humans (20, 25, 26, 34) and LTF of minute ventilation in humans with obstructive sleep apnea are not evident during wakefulness (22). Although LTF of minute ventilation has been observed consistently in snorers (2) after episodic hypoxia, primarily because of alterations in upper airway resistance, it has been observed inconsistently in healthy individuals (2, 48) and has not been observed in individuals with obstructive sleep apnea during sleep (1). Additionally, although studies have shown that decreases in upper airway resistance occur in healthy individuals and individuals that snore or suffer from obstructive sleep apnea after exposure to episodic hypoxia (1, 2, 48), no direct evidence of LTF of upper airway muscles in humans during sleep exists presently. Despite the absence of LTF in humans during wakefulness, its existence cannot be discounted as the manifestation of LTF is influenced by a variety of factors, including levels of carbon dioxide. Early on, investigators recognized that reduced levels of carbon dioxide levels may constrain the expression of LTF of phrenic nerve or hypoglossal nerve activity in animals because of disfacilitation of the chemoreceptors (3, 13, 17, 24). Consequently, carbon dioxide levels were often elevated 2–4 mmHg above the apneic threshold (4, 14, 17) during and after episodic hypoxia to promote the expression of LTF of phrenic nerve activity. In some cases, carbon dioxide was maintained at higher levels (i.e., 7 mmHg) to promote the expression of LTF of internal intercostal nerve or upper airway muscle activity (13, 24). The potential impact that carbon dioxide may have on the manifestation of LTF was supported by a study completed in rats during wakefulness, which showed that the full expression of LTF is constrained if accompanied by hypocapnia (38). Thus the purpose of the present investigation was to determine whether LTF of ventilation and genioglossus muscle activity in awake humans is dependent on the underlying level of carbon dioxide during exposure to episodic hypoxia.

METHODS

Protocol. The Human Investigation Committees of Wayne State University School of Medicine and Detroit Veterans Affairs Medical Center approved the experimental protocol. Eleven healthy subjects (7 males and 4 females), naïve to the purpose of the study, visited our laboratory on three occasions after informed consent was obtained. At least 8 h before each visit, subjects were advised to avoid alcohol and caffeine to preclude any influence that these substances might have on the measured responses. On the first visit to the laboratory, subjects underwent a physical examination. Thereafter, subjects were instrumented (as described below; see Instrumentation) and positioned supine. Subjects were then exposed to two 4-min episodes of hypoxia to become familiar with the experimental apparatus and protocol. During visits 2 and 3, subjects were exposed to episodic hypoxia, while carbon dioxide was maintained at normocapnic levels (trial 1)

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or at a level 5 mmHg above baseline (trial 2). Trials 1 and 2 were introduced randomly.

During trial 1, subjects breathed room air for 15 min before exposure to episodic hypoxia, so that baseline values of minute ventilation, breathing frequency, tidal volume, and carbon dioxide could be determined. Subsequently, subjects were exposed to eight 4-min episodes of hypoxia separated by 5 min of normoxia. During the hypoxic episodes, subjects inspired a gas mixture comprising 8% oxygen/balance nitrogen. At the completion of each episode, hypoxia was abruptly terminated with a single breath of 100% oxygen to rapidly bring the end-tidal partial pressure of oxygen (PETO2) to the normoxic range. After the last exposure to hypoxia (8th episode), respiration was monitored continuously for 15 min during the poststimuli recovery period (Fig. 1). During the hypoxic episodes and recovery periods of trial 1, carbon dioxide levels were actively maintained at the measured baseline normocapnic level (see Instrumentation for method used to actively maintain carbon dioxide levels).

Trial 2 was similar to trial 1 with the following exceptions. In addition to the physiological variables measured during trial 1, peak genioglossus muscle activity was measured. Consequently, before obtaining baseline measures, subjects completed a series of isometric maneuvers to record maximal genioglossus muscle activity. To generate a maximal response, subjects were instructed to force their tongue against the mandibular incisor teeth and to maintain this maneuver for a minimum of 3 s. The maneuver was completed three times to ensure that each subject was exerting a maximum voluntary effort that was reproducible. Subsequently, baseline measures of minute ventilation, tidal volume, breathing frequency, carbon dioxide, and peak genioglossus electromyogram (EMG) activity were monitored for 10 min. Thereafter, the end-tidal partial pressure of carbon dioxide (PETCO2) was increased 5 mmHg above normocapnic baseline values, and measurements of minute ventilation, breathing frequency, tidal volume, carbon dioxide, and peak genioglossus muscle activity were obtained for an additional 15 min before exposure to eight episodes of hypoxia (Fig. 1). Throughout exposure to episodic hypoxia and during the recovery periods separating the episodes, carbon dioxide levels were maintained at the predetermined hypercapnic value. At the end of the 8th hypoxic episode, breathing was monitored for 15 min during the poststimuli recovery period, while PETCO2 was sustained at the hypercapnic level. Thereafter, PETCO2 was reduced and maintained at baseline normocapnic levels, while breathing was observed for 10 additional minutes (Fig. 1). The primary reason for obtaining measurements under normocapnic conditions during baseline and the poststimuli recovery period of trial 2 was to confirm published findings that showed that LTF of peak genioglossus muscle activity was not observed when normocapnic levels were maintained throughout exposure to episodic hypoxia. Secondarily, these measurements were also completed to ensure that evidence of LTF was due to the maintenance of elevated levels of carbon dioxide and not the result of exposure to a stronger respiratory stimulus (i.e., episodic hypoxia/sustained hypercapnia vs. episodic hypoxia/sustained normocapnia). After completion of the protocol, three maximal isometric maneuvers were completed to ensure that the integrity of the electromyographic signal was not altered during completion of the protocol.

Five of the eleven subjects returned to the laboratory on a fourth occasion (trial 3) to repeat the protocol completed during trial 1 (episodic hypoxia/normocapnia), while peak genioglossus EMG activity was measured. This additional study was completed to eliminate the possibility that similar measures of peak genioglossus EMG activity during the normocapnic baseline and poststimuli recovery period of trial 2 occurred simply because LTF dissipated by the time measures of EMG activity were obtained during the poststimuli recovery period.

Additionally, 7 of the 11 subjects returned to the laboratory on a fifth occasion (trial 4). During trial 4, subjects were exposed to sustained elevated levels of carbon dioxide without concurrent exposure to episodic hypoxia. The duration of exposure and stimulus intensity (i.e., 5 mmHg above baseline carbon dioxide levels) was identical to that used during trial 2. This trial was completed to demonstrate that LTF of ventilation observed during the hypercapnic poststimuli recovery period of trial 2 was caused by exposure to episodic hypoxia rather than prolonged exposure to sustained elevated levels of carbon dioxide.

Instrumentation. To record electromyographic activity from the genioglossus muscle, we inserted two fine-wire Teflon-coated stainless steel recording wires bonded together contained within a 25-gauge needle into the body of the muscle. The electrodes were inserted 20 mm deep and at right angles to the oral mucosa at a point that was 3 to 4 mm lateral to the frenulum and anterior to the lingual salivary duct. The genioglossus EMG activity was amplified and filtered (3 Hz to 1 kHz) (model NL 822 - Neurolog Instruments, Inc.)
Digitimer, Hertfordshire, England), rectified, and integrated (Model # NL 703) with a time constant of 100 ms. The data were acquired using a commercially available analog-to-digital converter and software (DATAQ Instruments, Akron, OH) at a sampling rate of 1,000 Hz.

During completion of the episodic hypoxia protocol, subjects breathed through their nose while wearing a face mask that allowed end-tidal oxygen (model 17518, Vacumed, Ventura, CA) and carbon dioxide (model 17515, Vacumed, Ventura, CA) to be sampled from two separate mask ports. The face mask was connected to a pneumotachograph (model RSS100-HR, Hans Rudolph, Kansas, MO), which monitored breath-by-breath changes in ventilation. The pneumotachograph was attached to a two-way valve. The inspiratory port of the valve was connected to a stopcock. Subjects inspired either room air or the contents from one of two bags attached to the stopcock that contained either 8% oxygen/balance nitrogen, or 100% oxygen. The output from a flowmeter was attached to the stopcock port connected to the inspiratory port of the valve. Gas from two cylinders containing 100% oxygen and 100% carbon dioxide were connected to the flowmeter. Thus supplemental oxygen and carbon dioxide could be added to the 8% oxygen/balance nitrogen to maintain desired levels of PETCO₂ (50 mmHg) and PETCO₂ (normocapnia or hypercapnia). Oxygen saturation was monitored using a pulse oximeter (Biox 3700, Ohmeda, Boulder, CO). A 16-bit analog-to-digital converter (National Instruments, Austin, TX; AT-MIO-16XE-50) digitized the analog signals for online computer analysis using software specifically designed for this purpose. The software calculated minute ventilation, tidal volume, breathing frequency, and PETCO₂ and PETCO₂ on a breath-by-breath basis.

Data analysis. During trial 1 (i.e., episodic hypoxia/normocapnia) average values of minute ventilation, tidal volume, breathing frequency, and PETCO₂ were determined for the last 10 min of the 15-min baseline period recorded immediately before episodic hypoxia. Average values were also obtained for the last 2 min of each hypoxic episode. Additionally, average values were obtained from the last 2 min of the normoxic recovery periods that followed each hypoxic episode and for the initial, middle, and last 5 min of the poststimuli recovery period that followed the 8th hypoxic episode. Average values of minute ventilation, tidal volume, breathing frequency, peak genioglossus muscle activity, and PETCO₂ were determined for identical time points using the data collected during trial 2 (i.e., episodic hypoxia/hypercapnia). The peak genioglossus muscle activity was expressed initially as a percentage of maximum. Subsequently, the averaged values obtained were expressed as a fraction of baseline to normalize the electromyographic data.

Average values for minute ventilation, tidal volume, breathing frequency, peak genioglossus muscle activity, and PETCO₂ were determined for the last 5 min of the normocapnic periods recorded before initiation and after termination of hypercapnia during the baseline and recovery period of trial 2, respectively. Average values for peak genioglossus muscle activity were calculated from the last 10 min of the 15-min baseline period and the last 10 min of the poststimuli recovery period of trial 3. Throughout trial 4, minute ventilation, tidal volume, and breathing frequency were averaged at time intervals identical to those described for trial 2 when carbon dioxide was sustained at hypercapnic levels.Only data from the time points during trial 4 that correspond to the poststimuli recovery period of trial 2 are presented because they reflect the overall lack of increase in minute ventilation and its components observed throughout trial 4. Statistical analysis. A two-way ANOVA with repeated measures in conjunction with Student-Newman-Keuls post hoc test was used to determine whether peak genioglossus muscle activity measured during the hypoxic or recovery periods in trial 2 were significantly greater than baseline. The post hoc analysis was also completed using a less stringent analysis (i.e., Fisher’s Least Square Difference). The data used in the analysis were obtained from the segment in which carbon dioxide was sustained 5 mmHg above baseline. This analysis was also used to determine whether peak activity during the last two hypoxic episodes was increased compared with the initial hypoxic episode. A paired t-test was used to determine whether minute ventilation, tidal volume, breathing frequency, and peak genioglossus muscle activity recorded during the normocapnic baseline and poststimuli recovery period of trial 2 were significantly different. A similar analysis was used to determine whether peak genioglossus muscle activity measured during the poststimuli recovery period of trial 3 was significantly different from baseline.

A two-way ANOVA with repeated measures in conjunction with Student-Newman-Keuls post hoc test was used to determine whether peak genioglossus muscle activity measured during the hypoxic or recovery periods in trial 2 were significantly greater than baseline. The post hoc analysis was also completed using a less stringent analysis (i.e., Fisher’s Least Square Difference). The data used in the analysis were obtained from the segment in which carbon dioxide was sustained 5 mmHg above baseline. This analysis was also used to determine whether peak activity during the last two hypoxic episodes was increased compared with the initial hypoxic episode. A paired t-test was used to determine whether minute ventilation, tidal volume, breathing frequency, and peak genioglossus muscle activity recorded during the normocapnic baseline and poststimuli recovery period of trial 2 were significantly different. A similar analysis was used to determine whether peak genioglossus muscle activity measured during the poststimuli recovery period of trial 3 was significantly different from baseline.

RESULTS

The age, height, weight, and body mass index of the subjects were: 26.3 ± 1.7 years, 172.2 ± 2.3 cm, 75.48 ± 2.5 kg, and 25.5 ± 0.8, respectively. Figure 2 shows an example of breath-by-breath data collected from one subject during the completion of trial 2. Note that minute ventilation, tidal volume, and breathing frequency during the poststimuli recovery period after the eighth hypoxic episode were greater than baseline measures when carbon dioxide levels were sustained 5 mmHg above normocapnic baseline values.

Figure 3 shows the average changes in minute ventilation, tidal volume, breathing frequency, and carbon dioxide during and after exposure to episodic hypoxia in the presence of sustained normocapnic (trial 1, solid circles) and hypercapnic (trial 2, open circles) levels. Note that carbon dioxide levels were relative to baseline throughout the hypoxic and recovery periods in trials 1 and 2 (Fig. 3). During trials 1 and 2, minute ventilation and tidal volume both increased compared with baseline in response to episodic hypoxia (Fig. 3). Significant increases in breathing frequency relative to baseline were observed only during trial 2. The increase in minute ventilation observed during each hypoxic episode was similar during trial 1. Conversely, a progressive increase in the minute ventilation and breathing frequency response to hypoxia was evident throughout trial 2 (Fig. 3). As a result, minute ventilation and breathing frequency during the last two hypoxic episodes were greater than the measures obtained during the first episode of trial 2 (P < 0.001 for minute ventilation and breathing frequency). Tidal volume did not progressively increase from the initial to the last hypoxic episodes during trials 1 and 2.

During trial 2, minute ventilation during each recovery period that separated the hypoxic episodes and during the
poststimuli recovery period was significantly greater than baseline ($P < 0.01$ for first recovery period; $P < 0.001$ for remaining recovery periods; Fig. 3). Moreover, from the third recovery period to the last 5 min of the poststimuli recovery period, breathing frequency was significantly greater than baseline ($P < 0.02$ for third recovery period; $P < 0.001$ for remaining recovery periods). During trial 2, increases in tidal volume were more evident during the later recovery periods. Thus tidal volume during the recovery periods after the seventh and eighth hypoxic episodes was greater than baseline ($P < 0.04$ for seventh recovery period; $P < 0.03$ for initial 5 min of poststimuli recovery period; $P < 0.001$ for middle 5 min; $P < 0.01$ for final 5 min) (Fig. 3). In contrast, to trial 2, minute ventilation, tidal volume, and breathing frequency during each recovery time point remained unchanged compared with baseline throughout trial 1 (Fig. 3). This finding is similar to the results that showed that minute ventilation, tidal volume, and breathing frequency were similar during baseline and the poststimuli recovery period of trial 2 when carbon dioxide was maintained at normocapnic levels (Fig. 3, gray circles).

Figure 4 shows that minute ventilation, tidal volume and breathing frequency did not increase in response to exposure to sustained hypercapnia in the absence of episodic hypoxia (Fig. 4, solid bars). Consequently, minute ventilation and its components during the time points that corresponded to the initial, middle, and last 5 min of the poststimuli recovery period of trial 2 was not significantly different from baseline during trial 4. In contrast, because LTF manifested itself during and after
sure to episodic hypoxia throughout the last two hypoxic episodes (Fig. 6). Compared with baseline, the increase in genioglossus muscle activity observed during the fifth (P < 0.02) and sixth (P < 0.01) recovery periods, as well as the initial 5 min (P < 0.01) of the poststimuli recovery period, approached statistical significance. These latter results were found to be statistically significant using a less stringent post hoc analysis.

The changes in peak genioglossus activity that were observed when carbon dioxide was maintained at an elevated level were not evident when carbon dioxide levels were reduced back to baseline values. Thus peak EMG activity measured during the normocapnic portion of the poststimuli recovery period (Fig. 6, gray circles, poststimuli recovery/normocapnia) was similar to the values measured under similar conditions during baseline of trial 2 (Fig. 6). Likewise, peak genioglossus muscle activity throughout the recovery period of trial 3 was comparable to baseline (trial 3, 1.08 ± 0.08 vs. 1.0 fraction of baseline).

**DISCUSSION**

The primary finding of our investigation was that LTF of minute ventilation and peak genioglossus muscle activity is clearly evident when awake humans are exposed to episodic hypoxia in the presence of sustained elevated levels of carbon dioxide. This is not the case when carbon dioxide levels are sustained at normocapnic levels. We also showed that the minute ventilation response to hypoxia is progressively enhanced when carbon dioxide levels are sustained at hypercapnic but not normocapnic levels.

**Carbon dioxide and respiratory plasticity.** Recent findings have suggested that progressive augmentation of the ventilatory response to hypoxia and LTF of upper airway muscle activities and ventilation does not exist in humans that are awake (20, 22, 25, 26, 34). However, expression of these phenomena might be dependent, in part, on the level of carbon dioxide maintained during episodic hypoxia. Early on, investigators recognized that carbon dioxide levels may have a confounding influence on the expression of LTF in animals (3, 13, 17, 24). Consequently, carbon dioxide levels were often elevated above the apneic threshold during and after episodic hypoxia to promote the expression of LTF. The possible impact of carbon dioxide levels on the expression of LTF was subsequently confirmed by a study completed in rats during wakefulness (38), which showed LTF is not fully expressed if accompanied by hypocapnia, because ventilation is constrained by reducing carbon dioxide chemoreceptor feedback.

Given these findings in animals, it is possible that carbon dioxide levels must be elevated above the ventilatory threshold in humans in order for LTF to be fully expressed. In previous studies that examined LTF in humans during wakefulness, carbon dioxide was maintained at normocapnic levels during and after exposure to episodic hypoxia (20, 25, 26, 34). However, unlike sleep, ventilation during wakefulness can be sustained by arousal and/or behavioral stimuli when carbon dioxide levels are below the central and peripheral chemoreflex

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**Fig. 4.** Average values for minute ventilation, tidal volume, and breathing frequency recorded 10 min before and 15 min (average values for the initial, middle, and final 5 min of recovery are shown) after exposure to eight episodes of hypoxia in the presence of sustained levels of hypercapnia (i.e., trial 2 - open bars). Additionally, similar measures were obtained from identical time periods during exposure to sustained levels of carbon dioxide without exposure to episodic hypoxia (i.e., trial 4 - solid bars). Note that minute ventilation and its components after exposure to episodic hypoxia in the presence of sustained levels of carbon dioxide were significantly greater compared with measures obtained when subjects were only exposed to sustained levels of carbon dioxide. *Significantly different from baseline; †Significantly different from trial 4.

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exposure to episodic hypoxia in the presence of sustained hypercapnia, minute ventilation, tidal volume, and breathing frequency during the initial (minute ventilation, P < 0.001; tidal volume, P < 0.001; breathing frequency, P < 0.005), middle (minute ventilation, P < 0.001; tidal volume, P < 0.001; breathing frequency, P < 0.03) and last 5 min (minute ventilation, P < 0.001; tidal volume, P < 0.001; breathing frequency, P < 0.01) of the poststimuli recovery period of trial 2 was greater than the measures obtained during trial 4.

Peak genioglossus muscle activity increased during exposure to episodic hypoxia throughout trial 2 (Fig. 5 and Fig. 6, open circles). Moreover, the EMG response to episodic hypoxia gradually increased from the initial hypoxic episode to the last two hypoxic episodes (P < 0.01). In addition to the increases observed during hypoxia, peak genioglossus EMG activity increased progressively during the recovery periods, so that genioglossus muscle activity during the middle (P < 0.001) and last (P < 0.001) 5 min of the hypercapnic portion of the poststimuli recovery period was greater compared with the hypercapnic baseline segment (Fig. 6).
thresholds (11, 47). Thus maintenance of ventilation during wakefulness is not a strong indicator that concomitant carbon dioxide levels are sufficiently above the thresholds to be the primary stimulus to breathe. On the basis of this possibility, the level of carbon dioxide sustained in previous studies (20, 25, 26, 34) may not have been adequate to promote the manifestation of ventilatory augmentation or LTF.

Progressive augmentation of ventilation. The role of carbon dioxide in the manifestation of progressive augmentation of ventilation is supported by our results, which showed that ventilation did not progressively increase from the initial to the last two hypoxic episodes in the presence of sustained normocapnia. Conversely, when carbon dioxide was elevated and maintained 5 mmHg above baseline measures, the ventilatory response to hypoxia in the presence of sustained hypercapnia did not remain constant but increased gradually. The progressive augmentation of minute ventilation observed was due principally to the amplification of the breathing frequency response to hypoxia, as the frequency response during the middle and last two hypoxic episodes was greater than the first two episodes. This is in contrast to the tidal volume response to hypoxia, which was similar in the presence of sustained normocapnia (trial 1) or hypercapnia (trial 2). The impact of carbon dioxide on the development of progressive augmentation of ventilation is supported by our earlier findings, which showed that the ventilatory response to sustained hypoxia was increased significantly after exposure to episodic hypoxia when carbon dioxide was 3 to 6 mmHg above the chemoreflex threshold (25). Conversely, the ventilatory response to hypoxia when carbon dioxide was at the level of the chemoreflex threshold was unchanged after exposure to episodic hypoxia (25). Although sustained elevated levels of carbon dioxide appear to be necessary for the manifestation of progressive augmentation in humans during wakefulness, this is not the case in goats (49) and ducks (33), as augmentation of the ventilatory response to hypoxia was observed when carbon dioxide was maintained at baseline levels.

Long-term facilitation of ventilation. In addition to the progressive augmentation of the ventilatory response to hypoxia, we also observed the gradual manifestation of LTF of ventilation during the recovery periods that separated each hypoxic episode when carbon dioxide was sustained at elevated levels. This is in contrast to the absence of LTF that was observed when carbon dioxide levels were maintained at normocapnic levels. This is the first time to our knowledge that LTF of ventilation clearly manifested itself in humans during wakefulness. A gradual manifestation of long-term facilitation of ventilation during normoxic recovery periods that separate episodes of hypoxia, or carotid sinus nerve (CSN) stimulation,
has also been observed in rats (17, 38), cats (31), and goats (49). However, in contrast to our findings, these results were obtained while carbon dioxide was maintained at normocapnic levels.

In the present study, the manifestation of LTF during the early recovery periods that separated the hypoxic episodes was due primarily to increases in breathing frequency. However, both tidal volume and breathing frequency were elevated consistently above baseline during the later recovery periods. The impact of tidal volume and breathing frequency on the manifestation of LTF has varied between studies with some studies reporting that breathing frequency contributed predominantly to LTF in awake goats (49) and rats (27, 38). Morris and Gozal (35) also reported that breathing frequency increased in awake humans after episodic hypoxia. However, no statistically significant findings were evident because individual changes in breathing frequency were submerged in group variance. Conversely, Cao et al. (7) and Zhang et al. (50) reported that LTF in awake dogs and anesthetized rats, respectively, was due to an increase in tidal volume primarily with little change in breathing frequency.

We believe that LTF of ventilation manifested itself as a consequence of exposure to episodic hypoxia and because carbon dioxide levels were sufficiently above the chemoreflex thresholds. Alternatively, it is possible that the appearance of this phenomenon was caused by the application of a more intense respiratory stimulus (hypoxia/sustained hypercapnia vs. hypoxia/sustained normocapnia). However, evidence from studies that used a stimulus comparable to the stimulus we used (i.e., hypoxia/sustained hypercapnia) suggests that ventilatory LTF is not elicited after exposure to episodes of asphyxia. Cummings and Wilson (10) showed using an in vitro rat carotid body preparation that four episodes of asphyxia did not lead to LTF of carotid sinus nerve activity. Conversely, LTF of CSN activity has been observed after exposure to episodic hypoxia, although this phenomenon was evident only after 10 days of exposure to recurrent episodic hypoxia (42). Moreover, O’Halloran and colleagues (36) reported that diaphragmatic EMG activity in rats was not elevated after chronic exposure to intermittent asphyxia, in contrast to the increase in LTF reported after exposure to chronic intermittent hypoxia (23, 28). Our results also support the premise that the maintenance of carbon dioxide levels was more important for the manifestation of LTF rather than the stimulus intensity, as ventilatory LTF was not evident when carbon dioxide levels were reduced from hypercapnic to normocapnic levels during the poststimuli recovery period of trial 2. If the stimulus intensity were principally responsible for the LTF that we observed during trial 2, then we expected that ventilation during the hypercapnic and normocapnic segments of the poststimuli recovery period would be elevated to a similar degree compared with hypercapnic and normocapnic baseline measures. This was not the case.

It is also possible that the LTF we observed was not induced by exposure to episodic hypoxia but rather reflected a gradual drift in ventilation that occurred in response to the step increase (i.e., 5 mmHg) in carbon dioxide that was maintained throughout the protocol. The support for this possibility in the published literature is equivocal. Khamnei and Robbins (21) reported that ventilatory drift was observed in humans in response to a 40-min step increase in carbon dioxide. However, the response was inconsistent since ventilatory drift was not observed in two of the five subjects studied. Indeed, ventilatory roll-off was observed in one subject (21). Georgopoulos et al. (15) reported that the ventilatory response to carbon dioxide was increased after exposure to sustained levels of carbon dioxide; however, the increase was not statistically significant. Thus it is unlikely this finding was evident consistently in all subjects. Reynolds and colleagues (45) reported that ventilatory drift in humans increased in response to step increases in carbon dioxide that ranged from 3 to 7%. Examination of their data revealed little to no ventilatory drift at PETCO2 levels equivalent to those maintained in our study if baseline ventilation were established between 5 and 15 min after the step increase in carbon dioxide (i.e., the time period used to establish baseline ventilation in our study). However, it is difficult to directly compare our results to the work of Reynolds et al. (45), as our subjects were exposed to elevated levels of carbon dioxide for a longer time period. Nevertheless, the sham trial completed in our investigation revealed that no significant increases in ventilation were observed after prolonged exposure to sustained hypercapnia. Our average results are similar to the findings of Engwall and colleagues (12), who reported that no time-dependent increase in carotid body activity occurred when goats were exposed to prolonged normoxic hypercapnia.

Although our results suggest collectively that LTF was induced by exposure to episodic hypoxia, we are unable to comment on whether the intensity of the hypoxic stimulus impacted on the manifestation of LTF, because only one stimulus intensity was used (i.e., 8% oxygen). It is possible that the intensity of the hypoxic stimulus we selected affected our results. McGuire and colleagues (28) reported that five episodes of 10% oxygen elicited LTF in rats, but that exposure to a similar number of episodes of 8% and 12% oxygen did not elicit LTF. Moreover, these investigators showed that 10 episodes of 12% oxygen elicited LTF (28). Thus varying the number of episodes and the intensity of the stimulus might influence the manifestation of LTF. Further studies are required to examine these possibilities.

**Long-term facilitation of genioglossus muscle activity.** In addition to the progressive augmentation and LTF of ventilation that was observed during trial 2, we also observed changes in peak genioglossus muscle activity. Peak genioglossus muscle activity gradually increased from the first hypoxic episode to the last hypoxic episode. Furthermore, long-term facilitation of genioglossus muscle activity slowly increased from the initial to the poststimuli recovery period. Consequently, peak genioglossus muscle activity during the middle and last 5 min of the poststimuli recovery period was significantly greater than baseline. Long-term facilitation of hypoglossal nerve activity was recorded initially in rats (3) and subsequently from the genioglossus muscle in cats (24); however, despite previous efforts, this is the first time it has been observed in humans during wakefulness. Although we observed LTF in humans during wakefulness, Aboubakr et al. (1) and Shkoukani et al. (48) showed that upper airway resistance was reduced after exposure to episodic hypoxia during sleep, which suggests that LTF of upper airway muscle activity may be induced during this reduced state of arousal.

As was the case with our interpretation of the ventilatory data, we believe that LTF of genioglossus muscle activity was evident in our study but not in previous investigations because...
the carbon dioxide levels were sustained at hypercapnic levels. This suggestion is supported by our results that show that peak genioglossus muscle activity during the normocapnic segment of the poststimuli recovery period of trial 2 was comparable to measures obtained during baseline. Moreover, we believe that the similar measures of genioglossus muscle activity during normocapnic baseline and recovery of trial 2 were not simply because LTF dissipated by the time measures of EMG activity was obtained during recovery, as similar findings were obtained when peak genioglossus muscle activity during recovery of trial 3 was compared with baseline.

The increase in peak genioglossus muscle activity during recovery compared with baseline was higher in some subjects compared with others. This variability may have been inherent to the subjects that we studied. Conversely, evidence of LTF may have been more robust in all subjects if carbon dioxide levels were sustained at higher levels. We chose to maintain carbon dioxide levels at 5 mmHg above baseline normocapnic levels because we assumed that the genioglossus and ventilatory thresholds were similar and that the level of carbon dioxide selected, which was 47 mmHg on average, exceeded these thresholds. The observation that genioglossus and diaphragmatic activation occurs simultaneously in awake adults exposed to increases in carbon dioxide above normocapnic levels (39, 41) suggests that the thresholds of these two muscles are similar. However, further support for this contention is required, as attempts to measure the ventilatory and genioglossus muscle thresholds directly and concomitantly as carbon dioxide increases from low (i.e., hypocapnia) to high levels (i.e., hypercapnia) has not been attempted. It is possible that the level of carbon dioxide that demarcates the threshold of genioglossus muscle activity is greater than the ventilatory threshold as this occurrence has been observed in a variety of animals (6, 16, 18, 40), sleeping humans (43), and human infants (8). Given this latter observation, it is possible that the selected level of carbon dioxide was not sufficiently above the threshold to ensure the optional manifestation of LTF in all subjects. This contention remains unconfirmed because we did not determine the genioglossus threshold.

Physiological significance and future direction. Long-term facilitation of upper airway and/or ventilatory motor output may serve to stabilize respiration in humans (5). If so, multiple episodes of hypoxemia, associated with apnea during sleep, might be followed by periods of relative breathing stability. However, a small number of clinical studies suggest that if these periods of stability exist, their impact is minimal, as the severity of apnea increases from the beginning to end of the night, independent of sleep stage (9, 37, 46). If breathing stability increased significantly after numerous apneic episodes, it would be expected that apnea severity would improve throughout a given night. Thus LTF of ventilation and/or upper airway muscle activity may not have a predominant role in influencing apnea severity within a given night of sleep. However, the possibility that LTF is not normally manifested during sleep does not preclude its existence. Rather, given our findings, the emergence of LTF may be linked intimately with the level of carbon dioxide. Treating individuals with obstructive sleep apnea by artificially elevating carbon dioxide levels may promote the manifestation of LTF, leading to increased breathing stability after exposure to episodic hypoxia associated with recurrent apnea.

GRANTS

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REFERENCES

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