Peripheral chemoreflex responsiveness is increased at elevated levels of carbon dioxide after episodic hypoxia in awake humans

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Mateika, Jason H., Chris Mendello, Dany Obeid, and M. Safwan Badr. Peripheral chemoreflex responsiveness is increased at elevated levels of carbon dioxide after episodic hypoxia in awake humans. J Appl Physiol 96: 1197–1205, 2004. First published November 14, 2003; 10.1152/japplphysiol.00573.2003.—We hypothesized that the acute ventilatory response to hypoxia is enhanced after exposure to episodic hypoxia in awake humans. Eleven subjects completed a series of rebreathing trials before and after exposure to eight 4-min episodes of hypoxia. During the rebreathing trials, subjects initially hyperventilated to reduce the partial pressure of carbon dioxide (PETCO2) below 25 Torr. Subjects then breathed from a bag containing normocapnic (42 Torr), low (50 Torr), or high oxygen (140 Torr) gas mixtures. During the trials, PETCO2 increased while a constant oxygen level was maintained. The point at which ventilation began to rise in a linear fashion as PETCO2 increased was considered to be the ventilatory recruitment threshold. The ventilatory response below and above the recruitment threshold was determined. Ventilation did not persist above baseline values immediately after exposure to episodic hypoxia; however, PETCO2 levels were reduced compared with baseline. In contrast, compared with baseline, the ventilatory response to progressive increases in carbon dioxide during rebreathing trials in the presence of low but not high oxygen levels was increased after exposure to episodic hypoxia. This increase occurred when carbon dioxide levels were above but not below the ventilatory recruitment threshold. We conclude that long-term facilitation of ventilation (i.e., increases in ventilation that persist when normoxia is restored after episodic hypoxia) is not expressed in awake humans in the presence of hypocapnia. Nevertheless, despite this lack of expression, the acute ventilatory response to hypoxia in the presence of hypcapnia; long-term facilitation; ventilatory recruitment threshold; modified rebreathing protocol

 decreases are caused in part by exposure to hypoxia independent of other confounding factors (i.e., alterations in acid-base balance, hyperventilation) (16, 38, 44). Additionally, on the basis of animal experiments, it is generally accepted that VAH and the increase in the AHVR after long-term exposure to hypoxia is mediated in part by carotid body adaptations (5, 7, 34, 35). This is further supported by results that have shown that humans with bilaterally resected carotid bodies are unresponsive to hypoxic exposures (15).

Within a much shorter time frame (i.e., 20–30 min), exposure to episodic but not continuous hypoxia elicits gradual increases in respiratory motor activity in animals during successive periods of normoxia that separate the hypoxic episodes (26, 28, 35). Moreover, respiratory activity remains elevated for several minutes to hours after exposure to episodic hypoxia (28). This phenomenon is known as long-term facilitation (LTF), and it is believed to arise from a serotoninergic-dependent (4, 12, 26, 28) central neural mechanism (25, 27). Even though LTF has been observed in animals, it has not been observed in awake humans (18, 24) or non-airflow-limited humans during sleep (2).

Although VAH is accompanied by increases in the AHVR after exposure to chronic episodic hypoxia, it is unknown whether similar increases occur after exposure to episodic hypoxia within a shorter time frame (i.e., 30 min). We hypothesized that the AHVR would be increased despite the lack of expression of LTF in awake humans (18, 24). Our hypothesis was based on the possibility that separate mechanisms may be responsible for LTF and for the increase in the AHVR after exposure to acute episodic hypoxia. Thus one phenomenon could exist despite the absence of the other. This latter suggestion is supported in part by findings in animals that showed that VAH, and presumably the hypoxic ventilatory response, was increased despite the abolition of the serotoninergic mechanism responsible for LTF (23, 31). Conversely, we speculated that the lack of expression of LTF did not preclude its existence in awake humans, because the expression of this phenomenon is dependent on a variety of determinants (27, 28) including levels of carbon dioxide. For example, studies in rats have shown that hypocapnia does not enable the full expression of LTF (32). Thus a single underlying mechanism could be responsible for LTF and the subsequent increase in AHVR.
despite the lack of expression of LTF in awake humans. To test our hypothesis, we examined the ventilatory response to carbon dioxide during rebreathing trials while low or high oxygen levels were maintained before and after exposure to episodic hypoxia (29, 30).

We were also interested in determining whether increases in the ventilatory response to carbon dioxide during hypoxic rebreathing trials occurred as a consequence of an increase in slope of the minute ventilation vs. end-tidal carbon dioxide (PETCO2) relationship (i.e., chemoreflex responsiveness) and/or via a decrease in the PETCO2, that demarcates the point during rebreathing when ventilation begins to raise in a linear manner in response to progressive increases in carbon dioxide (i.e., the ventilatory recruitment threshold). Previous studies have suggested that the increase in AHVR after exposure to chronic hypoxia is caused by a change in the slope of the ventilatory response to hypoxia (1, 13, 16, 43, 44). However, because the response to hypoxia was measured at a single level of carbon dioxide, the possibility that alterations in the ventilatory recruitment threshold to carbon dioxide were responsible for the increases in the AHVR could not be discounted (9). This suggestion is supported by Mahamed and colleagues’ findings (21, 22), which showed that increases in the ventilatory response to hypoxia at various levels of carbon dioxide could be fully explained by a decrease in the PETCO2, that demarcates the ventilatory recruitment threshold. This result was obtained in awake humans after exposure to 3 h of continuous hypoxia (21) or 20 min of hypoxia each day for 2 wk (22). Thus we hypothesized that an increase in the ventilatory response to hypoxia after exposure to episodic hypoxia may be mediated primarily by a decrease in the ventilatory recruitment threshold to carbon dioxide.

METHODS

Protocol overview. 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 men and 4 women) visited our laboratory on one occasion after giving informed consent. Eight of these subjects returned on a second occasion to complete additional series of experiments. On the first visit, subjects completed rebreathing trials before and after exposure to episodic hypoxia. On the second occasion, rebreathing experiments were completed before and after exposure to atmospheric air for a duration equivalent to the episodic hypoxia (29, 30).

Four rebreathing experiments, each separated by 20-min of rest, were completed by use of Read’s modified rebreathing protocol before and ~1 h after exposure to episodic hypoxia (21, 22, 29). Thus a total of eight rebreathing experiments were completed on each visit. During each of these experiments, the subjects initially breathed room air for 5 min. Subsequently, the subjects hyperventilated for 5 min while being coached to maintain a PETCO2 between 50 and 25 Torr. This period of hyperventilation was employed to lower the stores of carbon dioxide so that the recruitment threshold for ventilation could be delineated during rebreathing. Furthermore, the reduction in carbon dioxide stores also allowed us to measure ventilation below the threshold.

After the 5-min period of hyperventilation, the subjects were switched from room air to a rebreathing bag. The PETCO2 in the bag during four of the eight experiments completed per visit was 50 Torr (H80), whereas the PETCO2 for the remaining two experiments was 140 Torr (H140). The pressures were maintained throughout the rebreathing experiment, and the PETCO2 (50 Torr vs. 140 Torr) selected for the initial two experiments was random. The PETCO2 in the bag at the start of the rebreathing experiment was 42 Torr. Rebreathing began at the hypoxia condition and was terminated by three rapid and deep breaths that produced rapid equilibration of the carbon dioxide partial pressures in the bag, lungs, and arterial blood to that of mixed venous blood. The observation of a plateau in the PETCO2 was used as verification of adequate equilibration and as a prerequisite for continuing the test. Rebreathing continued until PETCO2 increased to a maximum of 10 Torr above the recruitment threshold. In all subjects, this point of termination occurred before the breathing frequency threshold that has previously been described by Mohan and Duffin (29).

During the rebreathing experiments, the subjects wore nose clips and breathed through a mouthpiece that was connected to a pneumotachograph (model RSS100-HR, Hans Rudolph, Kansas City, MO) that was used to monitor breath-by-breath changes in ventilation. The pneumotachograph was attached to one side of a three-way valve that allowed us to switch the subjects from room air to the rebreathing bag. End-tidal oxygen (model 17518, Vacumed, Ventura, CA) and carbon dioxide (model 17515, Vacumed) were sampled from the pneumotachograph side of the three-way valve. The gas that was sampled for end-tidal monitoring was returned to the bag during rebreathing. The oxygen level in the bag during rebreathing was maintained by a flow of oxygen that was computer controlled. If oxygen decreased below the desired threshold (50 or 140 Torr), oxygen was immediately bled into the bag. Oxygen saturation was monitored by using a pulse oximeter (Biox 3700, Ohmeda, Boulder, CO).

A 16-bit analog-to-digital converter (National Instruments, AT-MIO-16XE-50) digitized the analog signals for online computer analysis by use of software specifically designed for this purpose. The software calculated tidal volumes, breathing frequency, ventilation, PETCO2, and PETCO2 on a breath-by-breath basis.

Data analysis. Average values of minute ventilation, tidal volume, breathing frequency, and PETCO2 were determined for the last 5 min of the 20-min baseline period recorded immediately before episodic hypoxia, the last minute of each hypoxic episode, the last minute of normoxia that separated each hypoxic episode, and for minutes 0–5, 6–10, and 11–15 of recovery from episodic hypoxia.

Average values of minute ventilation, tidal volume, breathing frequency, and PETCO2 were determined for the baseline periods measured immediately before completion of the rebreathing trials. Thus measures from four baseline periods were obtained before and after exposure to episodic hypoxia. Subsequently, average values obtained from the four baseline periods recorded before exposure to episodic hypoxia were averaged, as were the values from the baseline periods recorded after exposure to make statistical comparisons. A
similar analysis was completed for the time constant values that were obtained from the exponential decline, which was chosen to fit any waning of ventilatory “poststimulus potentiation” that might have occurred after hyperventilation (see below).

The data collected during the rebreathing experiments were analyzed by using a spreadsheet designed for this purpose. Before analysis, the three deep breaths that were required for gas equilibration, in addition to sighs or swallows that were detected by the software during the experiment, were excluded from further analysis. Subsequently, breath-by-breath PET CO\textsubscript{2} was plotted against time and fitted with a least-squares regression line. The equation for this line provided a predicted value of PET CO\textsubscript{2} vs. time, thereby minimizing interbreath variability associated with the measurement of this variable. Thereafter, ventilation was plotted against the predicted PET CO\textsubscript{2}.

Subsequently, each of these plots was fitted with a model made up of the sum of two segments separated by one breakpoint. Model fitting was based on minimizing the sum of least squares for nonlinear regressions by use of commercial software (SigmaPlot 7.0, SPSS). Figure 1 shows an example of the lines fitted to the responses of one subject under the H\textsubscript{SO} condition. The first segment of the response was an exponential decline to a final value (i.e., basal value). This value was taken as a measure of ventilation at the recruitment threshold. The exponential decline was chosen to fit any waning of ventilatory poststimulus potentiation that might have occurred after hyperventilation. However, poststimulus potentiation is often not observed so that the time constant of the response may be <1 s (29, 30).

The second segment was characterized by a breakpoint (Fig. 1) followed by a linear increase in minute ventilation that occurred in conjunction with a rise in PET CO\textsubscript{2}. The first breakpoint was taken as a measure of the recruitment threshold for the minute ventilation response to carbon dioxide. The threshold measured under H\textsubscript{140} conditions was thought to originate from the central chemoreceptors while oxygen was maintained at 50 Torr. Note the measurement of the recruitment threshold for the minute ventilation response to increases in carbon dioxide levels that were 0, 3, and 6 Torr above the recruitment threshold during the H\textsubscript{50} or H\textsubscript{140} trials represented central chemoreflex responsiveness (10), whereas the slope recorded from the H\textsubscript{50} trials represented the combined peripheral and central chemoreflex response (10). On the basis of the modeling of Duffin and others (10, 14, 19, 20), we assumed that the impact of the central and peripheral chemoreflex on ventilation was additive. Thus the mean slope of the H\textsubscript{140} trials was subtracted from the slope of the H\textsubscript{50} trials for each subject to obtain a measure of peripheral chemoreflex responsiveness. In making this assumption, the potential for nonlinear interactions was ignored.

Statistical analysis. Paired t-tests were used to determine whether 1) baseline measures of minute ventilation, tidal volume, and breathing frequency measured before completion of each rebreathing trial were significantly different before vs. after exposure to episodic hypoxia and 2) the time constant of the hyperventilatory poststimulus potentiation observed at the onset of rebreathing trials was different before compared with after exposure to episodic hypoxia. A one-way analysis of variance with repeated measures in conjunction with Holm-Sidak post hoc test was used to determine minute ventilation, tidal volume, breathing frequency, and PET CO\textsubscript{2} recorded during each hypoxic exposure. In each condition, the central or peripheral recruitment threshold was determined, minute ventilation before and after exposure to episodic hypoxia were not significantly different before vs. after episodic hypoxia [the main factors were carbon dioxide level (0, 3, and 6 Torr) and oxygen concentration (H\textsubscript{50} vs. H\textsubscript{140})]; 2) establish whether the change in ventilation after episodic hypoxia at 0, 3, or 6 Torr was significantly different before vs. after episodic hypoxia [the main factors were carbon dioxide level (0, 3, and 6 Torr) and oxygen concentration (H\textsubscript{50} vs. H\textsubscript{140})]; 3) ascertain whether the change in ventilation after episodic hypoxia at 0, 3, or 6 Torr was significantly different [the main factors were carbon dioxide level (0, 3, and 6 Torr) and oxygen concentration (H\textsubscript{50} vs. H\textsubscript{140}) and timing of the rebreathing trials (before vs. after episodic hypoxia)]; 4) determine whether differences in the ventilatory recruitment threshold existed before and after episodic hypoxia (the levels of the main factors were before vs. after episodic hypoxia, and H\textsubscript{50} vs. H\textsubscript{140}); and 5) compare measures of chemoreflex responsiveness before and after episodic hypoxia [the levels of the main factors were timing of the rebreathing experiments (before vs. after episodic hypoxia) and oxygen concentration (H\textsubscript{50} vs. H\textsubscript{140} vs. H\textsubscript{SO}–H\textsubscript{140})]. Data are presented as means ± SE. A value of P < 0.05 was considered significant.

RESULTS

The mean age, height, and weight of the subjects were 28.6 ± 2.19 yr, 169 ± 2.0 cm, and 69.08 ± 2.59 kg, respectively. LT\textsubscript{TF} of ventilation, tidal volume, and breathing frequency was not evident during the normoxic periods that separated the hypoxic episodes. Conversely, our findings showed that ventilation and tidal volume were above baseline values for the initial 5 min of the recovery period. However, the increase in the average values was due to increases in minute ventilation and tidal volume during the initial 60–90 s of recovery. Thereafter, minute ventilation and tidal volume returned to baseline values for the remainder of the recovery period (Fig. 2). The lack of expression of LT\textsubscript{TF} was accompanied by a reduction in PET CO\textsubscript{2} during the 15-min recovery period compared with baseline (P < 0.05 in all cases) (Fig. 2).

Minute ventilation, tidal volume, and breathing frequency measured before the rebreathing experiments completed before exposure to episodic hypoxia were not significantly different

Fig. 1. Example of the ventilatory response to increases in carbon dioxide while oxygen was maintained at 50 Torr. Note the measurement of the ventilatory recruitment threshold, ventilation below the threshold (basal ventilation), and chemoreflex responsiveness. See text for further details.
from similar measures obtained after exposure to episodic hypoxia (P<sub>ETCO2</sub> 40.42 ± 0.79 vs. 39.39 ± 0.64; minute ventilation 11.31 ± 0.84 vs. 10.40 ± 0.59; tidal volume 809.64 ± 96.10 vs. 739.24 ± 67.96; breathing frequency 15.20 ± 1.13 vs. 15.33 ± 1.21). Similarly, the time constant of the poststimulus potentiation induced by hyperventilation before the rebreathing trials was similar before and after exposure to episodic hypoxia (8.42 ± 2.39 vs. 6.45 ± 2.13 s).

During the rebreathing experiments, ventilation measured at the recruitment threshold was not altered after episodic hypoxia but increased significantly at 3 (P < 0.04) and 6 Torr (P < 0.001) above the recruitment threshold in the H<sub>50</sub> experiments (Fig. 3A). A similar trend was observed in the H<sub>140</sub> experiments at 3 (P = 0.06) and 6 Torr (P = 0.07), but the increases did not reach statistical significance (Fig. 3A). Moreover, Fig. 3B shows that the degree to which ventilation increased was not constant but was dependent on the level of carbon dioxide relative to the recruitment threshold for the H<sub>50</sub> (P < 0.02 in all cases) but not the H<sub>140</sub> conditions. Moreover, the increase in ventilation at 3 and 6 Torr was greater than that observed at similar levels of carbon dioxide during the sham experiments (P < 0.01). The rebreathing experiments, during which increases in ventilation were observed, were completed on average 70 ± 6.8 min after exposure to episodic hypoxia.

The increase in ventilation observed during the rebreathing experiments was not due to changes in the recruitment threshold because this measure remained unchanged after exposure to episodic hypoxia (Figs. 4 and 5A). Instead, the increase in ventilation above the recruitment threshold was due to an increase in chemoreflex responsiveness. An example of this increase recorded from one subject that completed rebreathing trials before and after episodic hypoxia under the H<sub>50</sub> condition is shown in Fig. 4. This example is similar to the average results shown in Fig. 5B, which reveal that the combined central and peripheral chemoreflex responsiveness (H<sub>50</sub>) increased after episodic hypoxia (P < 0.001). Moreover, subtracting the central chemoreflex responsiveness (H<sub>140</sub>) from the sum of the central and peripheral chemoreflex responsiveness (H<sub>50</sub>) revealed that the latter response was due primarily to an increase in peripheral chemoreflex responsiveness (H<sub>50</sub>–H<sub>140</sub>) (P < 0.02) (Fig. 5B).

Results obtained from the sham experiments showed that ventilation above the recruitment threshold was not altered after inspiration of atmospheric air for a time period equivalent to the duration of hypoxic exposure (Fig. 6). Moreover, no alterations in recruitment threshold and most importantly chemoreflex responsiveness occurred during the sham experiments (Fig. 7).

**DISCUSSION**

Our major finding was that the ventilatory response to carbon dioxide during hypoxic rebreathing trials was increased...
after exposure to episodic hypoxia for 30 min when \( P_{ET\ CO_2} \) values were elevated above the recruitment threshold. In contrast, we found that, after exposure to episodic hypoxia, LTF of ventilation did not manifest itself and the ventilatory response to carbon dioxide during hypoxic rebreathing did not increase in the presence of hypocapnia.

**Methodological issues.**

The modified rebreathing technique has been discussed extensively in previous investigations (29, 30). Utilization of the modified rebreathing technique allowed us to directly measure both the ventilatory recruitment threshold to carbon dioxide, while low and high oxygen levels were maintained, and the ventilatory response to carbon dioxide above and below the threshold.

Despite the potential benefits of employing the modified rebreathing technique, it may initiate a number of physiological responses that could influence ventilation after the termination of hyperventilation. The possible responses include the induction of poststimulus potentiation, changes in cerebral blood flow, respiratory muscle fatigue, and changes in arousal state (29). However, the modified rebreathing protocol was applied identically before and after episodic hypoxia. Thus completion of the preepisodic hypoxia rebreathing trials and/or exposure to episodic hypoxia would have to directly impact on one or more of the physiological responses outlined above to account for the ventilatory responses observed during the hypoxic rebreathing trials after exposure to episodic hypoxia. As outlined below, we do not believe that this was the case.

Corne and colleagues (8) implied recently that the ventilatory recruitment threshold measured during modified rebreathing trials represents the point at which the decline in poststimulus potentiation intersects with the increase in end-tidal carbon dioxide. This is unlikely the case because poststimulus potentiation is often not observed. Furthermore, when it is observed after hypocapnic (21, 22, 29) or isocapnic hyperventilation (J. Duffin, personal communication), the time constant is much shorter than the time required attaining the recruitment threshold (21, 22, 29). Nevertheless, even if the hypothesis is correct, our finding that the time constant measures were similar before and after episodic hypoxia suggest that the impact of poststimulus potentiation on the recruitment threshold did not vary throughout the experimental protocol.

The cumulative effect of hyperventilation during rebreathing trials and episodic hypoxia may have influenced the ventilatory responsiveness that was observed throughout the hypoxic rebreathing trials completed after exposure to episodic hypoxia. The ventilatory response may have been altered by respiratory muscle fatigue or by increasing respiratory neuromuscular excitation, possibly via direct increases in afferent feedback from chest wall and lung afferents (38) or indirectly by changes in arousal state (41). We believe it is unlikely that our subjects experienced respiratory muscle fatigue because we were careful to ensure that subjects in both groups received at least a 20-min rest between rebreathing trials to avoid this event. Similarly, if respiratory muscle fatigue occurred, one might expect that the ventilatory response during the hypoxic re-

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**Fig. 3.** A: histograms showing the average minute ventilation measured during rebreathing before (solid bars) and after (open bars) episodic hypoxia while oxygen was maintained at 140 Torr (H140) or 50 Torr (H50) and carbon dioxide levels were 0, 3, and 6 Torr above the recruitment threshold. *Significantly different from values measured before exposure to episodic hypoxia. B: line plots showing the difference in ventilation measured during rebreathing trials completed before and after episodic hypoxia while oxygen was maintained at 140 (●) or 50 Torr (○) at carbon dioxide levels that were 0, 3, and 6 Torr above the recruitment threshold. Note the progressive augmentation of ventilation at 3 and 6 Torr above the recruitment threshold in the rebreathing trials that were completed while oxygen was maintained at 50 Torr. *Significantly different from 0 Torr while oxygen was maintained at 140 or 50 Torr; **significantly different from all other measures while oxygen was maintained at 50 Torr or 140 Torr.

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**Fig. 4.** Example of the ventilatory response to increases in carbon dioxide while oxygen was maintained at 50 Torr in 1 subject before (●) and after (○) exposure to episodic hypoxia. Note that ventilation was increased above the recruitment threshold after exposure to episodic hypoxia.
breathing trials after episodic hypoxia would decrease rather than increase as we observed.

Cumulative hyperventilation may have increased respiratory neuromuscular activity. This possibility is supported by the previous findings of Ren and Robbins (38), who showed that passive hyperventilation for 6 h increased the slope of the ventilation-carbon dioxide relationship. However, the increase in slope was modest and the duration of the hyperventilation period far exceeded the duration of hyperventilation employed in our studies. More importantly, if this mechanism was primarily responsible for the increase in the ventilatory response, it would be expected that similar increases would have been observed during both the hyperoxic and hypoxic rebreathing trials. This was not the case. Moreover, Ren and Robbins (38) reported that the ventilatory responsiveness to hypoxia was unaltered by the presence of prior hyperventilation.

Hypocapnia and the consequent respiratory alkalosis induced by episodic hypoxia could potentially be responsible for the increase in the ventilatory response that was observed during hypoxic rebreathing (38). However, this alteration is not likely responsible for our observations because carbon dioxide levels returned to baseline in most of our subjects before completion of the rebreathing trials after episodic hypoxia.

Fig. 5. Histograms showing the average recruitment threshold (A) and chemoreflex responsiveness (B) obtained from the $H_{140}$ and $H_{90}$ rebreathing trials before (solid bars) and after (open bars) exposure to episodic hypoxia. Note that the enhancement of ventilation shown in Fig. 3 was mediated primarily by an increase in peripheral chemoreflex responsiveness ($H_{90}-H_{140}$) (see text for further details).

Fig. 6. Histograms showing the average minute ventilation measured during $H_{140}$ and $H_{90}$ rebreathing trials while carbon dioxide levels were 0, 3, and 6 Torr above the recruitment threshold. These measures were obtained before (solid bars) and after (open bars) subjects inspired atmospheric air for an interval of time equivalent to the episodic hypoxia protocol.

Fig. 7. Histograms showing the average recruitment threshold (A) and chemoreflex responsiveness (B) obtained from the $H_{140}$ and $H_{90}$ rebreathing trials before (solid bars) and after (open bars) exposure to atmospheric air for a duration of time equivalent to the episodic hypoxia protocol. Note that recruitment thresholds and chemoreflex responsiveness were similar before and after exposure to atmospheric air.

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Additionally, previous findings showed that respiratory alkalosis induced by 6 h of hyperventilation had no impact on the responsiveness to carbon dioxide or hypoxia but rather caused a leftward shift (i.e., a change in threshold) in the minute ventilation-Paco2 relationship (38). In our study, we observed a change in responsiveness and no change in the ventilatory recruitment threshold after episodic hypoxia, which supports the likelihood that alterations in acid-base imbalance did not impact on our findings.

It is also possible that exposure to episodic hypoxia could lead to alterations in cerebral blood flow (17, 40) that could ultimately lead to modifications in the difference between end-tidal carbon dioxide and brain tissue (extracellular) carbon dioxide and consequently the ventilatory response to carbon dioxide. Although this is a concern when steady-state methods are used (30) to measure the ventilatory response to carbon dioxide, it is less likely to influence the response when Read’s modified rebreathing method is employed (29, 36). Read’s rebreathing method is designed to minimize the arteriovenous difference in blood gases during rebreathing (29, 36). This ensures that changes in cerebral blood flow do not alter the cerebral arteriovenous difference, and thus the relationship between end-tidal and central chemoreceptor partial pressures of carbon dioxide should be unaffected by changes in cerebral blood flow. The modeling experiments of Read and Leigh (37) support this view. In addition, because the system is closed, blood circulation and pulmonary ventilation should act as mixing forces to establish a homogenous distribution of carbon dioxide.

Additionally, we believe that differences observed after episodic hypoxia were not a consequence of other methodological considerations. Conditions conducive to maintaining quiet wakefulness were strictly adhered to during each rebreathing trial both before and after episodic hypoxia so that environmental factors that are known to influence the state of arousal and consequently ventilation were maintained throughout the experiments (41). It is also possible that the change in the ventilatory response that was observed was a consequence of normal circadian influences over the duration of the experimental protocol, which lasted ~6–7 h. This suggestion is supported by previous findings that have shown that circadian rhythms have an impact on the chemoreflex control of breathing (42). However, we believe that this is unlikely because an equivalent augmentation of ventilation was not observed when rebreathing tests were completed before and after normoxic exposure at a time of day similar to that selected for completion of the episodic hypoxia protocol.

Ventilatory response to carbon dioxide during hypoxic and hyperoxia rebreathing trials. Our findings showed that, despite the absence of LTF, the ventilatory response to carbon dioxide during rebreathing in the presence of hypoxia was enhanced after exposure to episodic hypoxia. Our findings corroborate previous results that showed that the AHVR in humans was increased after exposure to chronic intermittent hypoxia for days or weeks, or continuous hypoxia for 3 or more hours (1, 13, 16, 21, 22, 44). In contrast, our findings differ from the results of Mahamed and Duffin (22), who showed that, after 20 min of continuous exposure to hypoxia, ventilation during rebreathing of carbon dioxide in the presence of hypoxia was reduced compared with baseline. This difference between our results and those of Mahamed and Duffin reflects previous findings observed in awake (32) and anesthetized (3) rats. These findings suggest that exposure to episodic hypoxia has dramatically different effects on ventilation than exposure to continuous hypoxia over a similar time frame.

The increase in ventilation observed during hypoxic rebreathing trials occurred on average >1 h after exposure to episodic hypoxia. This persistent increase is similar to results that showed that the AHVR in awake humans remained elevated up to 1 h after exposure to 3 h of continuous hypoxia (21) and for 24 h after exposure to 48 h of continuous hypoxia (44). Likewise, the increase in the ventilatory response to hypoxia that persisted for >1 h after exposure to episodic hypoxia in our study is similar to the time course of LTF previously observed in awake (32) and anesthetized rats after episodic hypoxia (3).

We also observed a trend toward an increase in the ventilatory response to carbon dioxide during the hyperoxia rebreathing tests. Our finding is positioned between the results obtained from previous studies. Tansley et al. (44) and Ainslie et al. (1) showed that the ventilatory response to carbon dioxide was significantly increased after 48 h of exposure to continuous hypoxia or 8 h of daily exposure to hypoxia for 2 wk, respectively. Alternatively, Mahamed and colleagues (21, 22) showed that no change in the ventilatory response to carbon dioxide occurred after 20 min of daily exposure to hypoxia for 2 wk or after 3 h of continuous exposure to hypoxia. It is possible that the lack of a response observed by Mahamed and colleagues may wholly or in part be due to differences in the duration of exposure to continuous or chronic intermittent hypoxia, because both experiments the duration of exposure was less than that used by Tansley et al. and Ainslie et al. Similarly, it is possible that we would have observed significant increases in the ventilatory response to carbon dioxide during the hyperoxic rebreathing trials if we had employed additional episodes of hypoxia. However, this speculation requires further investigation.

Peripheral chemoreflex responsiveness and the ventilatory response to hypercapnic hypoxia. The increase in the ventilatory response to carbon dioxide during hypoxic rebreathing trials after exposure to episodic hypoxia may have been mediated via increases in peripheral chemoreflex responsiveness. This possibility receives support from animal studies that have shown that the ex vivo rat carotid body sensory response to hypoxia (34) and the AHVR of the goat to hypoxic stimulation of the isolated carotid body (7) was enhanced after exposure to chronic episodic hypoxia or continuous hypoxia. Conversely, sustained central nervous system hypoxia (45) or hypercapnia (without hypoxia) isolated to the carotid body (6) does not lead to VAH. However, whether the increase in responsiveness that we measured reflects increased carotid body sensitivity, enhanced processing of carotid body input to the central nervous system, or enhanced neuromuscular translation into breathing cannot be determined, because each component is incorporated in the quantification of peripheral chemoreflex responsiveness.

The increase in responsiveness that we observed was similar to increases previously observed (1, 16, 44), even though exposure to episodic hypoxia in the present investigation was short compared with the continuous and chronic episodic hypoxic exposures employed previously. Conversely, our results differ from Mahamed and colleagues’ (21, 22) findings, which showed that the increased ventilatory response to carbon dioxide was reduced compared with baseline after 20 min of continuous exposure to hypoxia, ventilation during rebreathing of carbon dioxide in the presence of hypoxia was reduced compared with baseline. This difference between our results and those of Mahamed and Duffin reflects previous findings observed in awake (32) and anesthetized (3) rats. These findings suggest that exposure to episodic hypoxia has dramatically different effects on ventilation than exposure to continuous hypoxia over a similar time frame.
dioxide during hypoxic rebreathing tests after exposure to continuous hypoxia or chronic episodic hypoxia was due primarily to a shift in the peripheral chemoreflex threshold rather than changes in responsiveness. Whether the difference in our and Mahamed and colleagues’ findings are a consequence of the differing time course (32 min vs. 3 h), pattern of hypoxic exposure (acute episodic vs. continuous or chronic episodic hypoxia), and/or hypoxic severity remains to be determined.

**LTF and the ventilatory response to hypercapnia and hypoxia.** Our finding that the ventilatory response during hypoxic rebreathing is enhanced after episodic hypoxia is of interest because it occurred even though LTF did not manifest itself, as previously reported in awake humans (18, 24). Thus it is possible that increases in peripheral chemoreflex responsiveness independent of the LTF mechanism were principally responsible for the ventilatory response observed. This possibility is similar to previous findings that showed that ventilatory acclimatization in animals (23, 31), and presumably enhanced carotid body sensitivity, to continuous hypoxia may occur despite the depletion of serotonin, which is a neurotransmitter that mediates LTF (4, 12, 26, 28).

Conversely, previous work in animals suggests that continuous hypoxia leads to carotid body adaptations (5, 7, 34, 35), whereas episodic hypoxia elicits LTF, which is a central neural mechanism (25, 27). Thus, given the pattern of exposure that we employed, it is possible that LTF may have contributed to the increase in the ventilatory response to carbon dioxide during the hypoxia rebreathing trials. If this is the case, we expected that LTF would have been clearly evident. However, ventilation was only increased above baseline during the initial 60–90 s of recovery from episodic hypoxia, which is a time course that reflects the short-term potentiation phenomenon (11, 35). It is possible that the expression of LTF was not evident after episodic hypoxia because of the accompanying hypocapnia (28). This suggestion is supported by a recent study completed in rats during wakefulness, which showed systematically that the full expression of LTF is prevented if accompanied by hypocapnia (32), because ventilation was constrained as a consequence of reducing carbon dioxide chemoreceptor feedback.

This finding suggests that the impact of LTF on the ventilatory response to carbon dioxide during hypoxic rebreathing trials may be more evident as the levels of carbon dioxide increase. This hypothesis is supported by our results that showed that basal ventilation (i.e., a measure of ventilation below the recruitment threshold) measured from the rebreathing experiments remained unchanged after episodic hypoxia. In contrast, when carbon dioxide levels were sufficiently above the recruitment threshold (i.e., 3 or 6 Torr), increases in ventilation compared with baseline was clearly evident. Moreover, the degree to which the ventilatory response increased did not remain constant but appeared to increase as the level of carbon dioxide increased relative to the recruitment threshold.

Given the possible impact of carbon dioxide on the expression of LTF, the relationship between baseline measures of carbon dioxide and the recruitment threshold for the ventilatory response to carbon dioxide during wakefulness may be of paramount importance. Carbon dioxide levels that are below the recruitment threshold during wakefulness in humans have little or no impact on ventilation. However, this reduction often does not lead to the abolition of ventilation, as it might during sleep, because ventilation is often sustained by arousal and/or behavioral stimuli. Consequently, baseline measures of carbon dioxide, in the absence of recruitment threshold measures, does not necessarily imply that this stimulus is adequate to control resting breathing or promote the manifestation of LTF. Moreover, even if the baseline measures of carbon dioxide are above the recruitment threshold, further elevations in carbon dioxide may be necessary to ensure the manifestation of LTF in awake humans. Thus it is possible that the level of carbon dioxide maintained during episodic hypoxia in previous studies completed in humans during wakefulness (18, 24) and sleep (2) might not have been adequate to ensure the manifestation of LTF.

**Physiological significance.** We believe that the increase in the ventilatory response to carbon dioxide observed during hypoxic rebreathing trials during wakefulness may have important implications for the control of breathing during sleep. Increases in the ventilatory response to hypoxia after exposure to episodic hypoxia during sleep could stabilize respiration by virtue of increased ventilatory motor output. If so, the occurrence of multiple episodes of apnea and hypopnea during sleep might be followed by periods of relative breathing stability. This may be the case if the pathogenesis of sleep apnea is simply anatomic narrowing of the upper airway secondary to loss of upper airway dilator activity. Alternatively, increases in the ventilatory response to hypoxia after exposure to episodic hypoxia might promote the occurrence of apneas and hypopneas. Disproportionate increases in ventilation (i.e., hyperventilation) for a given level of carbon dioxide could subsequently drive carbon dioxide levels below the apneic threshold, ultimately resulting in a reduction in central respiratory drive to chest wall and upper airway muscles. If, as shown in awake humans, the ventilatory response to hypoxia does not manifest itself in the presence of hypocapnia, then the activation of this phenomenon would have little influence in sustaining ventilation when carbon dioxide levels were below the apneic threshold. Thus the activation of this phenomenon might promote rather than mitigate apnea during sleep. However, this speculative interpretation awaits experimental support.

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