Effect of hypoxia on the hypopnoeic and apnoeic threshold for CO_2 in sleeping humans

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1. Rhythmic breathing during sleep requires that P_{CO_2} be maintained above a sensitive hypocapnic apnoeic threshold. Hypoxia causes periodic breathing during sleep that can be prevented or eliminated with supplemental CO_2. The purpose of this study was to determine the effect of hypoxia in changing the difference between the eupnoeic P_{CO_2} and the P_{CO_2} required to produce hypopnoea or apnoea (hypopnoea/apnoeic threshold) in sleeping humans.

2. The effect of hypoxia on eupnoeic end-tidal partial pressure of CO_2 (P_{ET,CO_2}) and hypopnoea/apnoeic threshold P_{ET,CO_2} was examined in seven healthy, sleeping human subjects. A bilevel pressure support ventilator in a spontaneous mode was used to reduce P_{ET,CO_2} in small decrements by increasing the inspiratory pressure level by $2\ cmH_2O$ every 2 min until hypopnoea (failure to trigger the ventilator) or apnoea (no breathing effort) occurred. Multiple trials were performed during both normoxia and hypoxia (arterial O_2 saturation, $S_{a,O_2} = 80\%$) in a random order. The hypopnoea/apnoeic threshold was determined by averaging P_{ET,CO_2} of the last three breaths prior to each hypopnoea or apnoea.

3. Hypopnoeas and apnoeas were induced in all subjects during both normoxia and hypoxia. Hypoxia reduced the eupnoeic P_{ET,CO_2} compared to normoxia (42.4 ± 1.3 *vs.* 45.0 ± 1.1 mmHg, $P < 0.001$). However, no change was observed in either the hypopnoeic threshold P_{ET,CO_2} (42.1 ± 1.4 *vs.* 43.0 ± 1.2 mmHg, $P > 0.05$) or the apnoeic threshold P_{ET,CO_2} (41.3 ± 1.2 *vs.* 41.6 ± 1.0 mmHg, $P > 0.05$). Thus, the difference in P_{ET,CO_2} between the eupnoeic and threshold levels was much smaller during hypoxia than during normoxia (-0.2 ± 0.2 *vs.* -2.0 ± 0.3 mmHg, $P < 0.01$ for the hypopnoea threshold and -1.1 ± 0.2 *vs.* -3.4 ± 0.3 mmHg, $P < 0.01$ for the apnoeic threshold). We concluded that hypoxia causes a narrowing of the difference between the baseline P_{ET,CO_2} and the hypopnoea/apnoeic threshold P_{ET,CO_2}, which could increase the likelihood of ventilatory instability.

Rhythmic breathing during sleep is critically dependent on the level of P_{CO_2} in arterial blood (Fink, 1961; Skatrud & Dempsey, 1983; Datta *et al.* 1991). Small reductions of P_{CO_2} to levels that are slightly below the awake, eupnoeic P_{CO_2} consistently produce apnoea in sleeping humans. The importance of this sensitive apnoeic threshold for CO_2 has been implicated in the periodic breathing with central apnoeas that is commonly observed in sleeping subjects at high altitudes. When supplemental CO_2 sufficient to elevate the P_{CO_2} a mere 1–2 mmHg was administered to subjects at high altitudes, and the hypoxaemic arterial P_{O_2} (P_{a,O_2}) was maintained via nitrogen administration, the apnoeas were prevented or eliminated, and the breathing pattern was stabilized (Berssenbrugge *et al.* 1983). Thus, hypocapnia plays an important role in the development and maintenance of breathing instability during hypoxia.

However, several observations suggest that hyperventilation and consequent reduction of eupnoeic P_{CO_2} alone are insufficient to account for the breathing instability seen during hypoxia. For example, chronic hyperventilation does not cause breathing instability during sleep in disease states such as interstitial fibrosis (Perez-Padilla *et al.* 1985) or during the chronic administration of ventilatory stimulants, such as acetazolamide, theophylline and medroxyprogesterone acetate (Skatrud *et al.* 1981; White *et al.* 1982; DeBaker *et al.* 1995; Javaheri *et al.* 1996). On the contrary, these chronic ventilatory stimuli have been shown to have stabilizing effects on ventilation during sleep (Hudgel & Thanakitcharu, 1998). Likewise, during prolonged hypoxia, the susceptibility to periodic breathing decreases during the acclimatization process, such that periodic breathing during sleep is diminished despite an increased ventilation and there is an even greater reduction in arterial P_{CO_2} (P_{a,CO_2}) and alkalization of blood and cerebrospinal fluid (CSF). Administration of acetazolamide at high altitudes also reduces periodic breathing despite ventilatory stimulation (Hackett *et al.*

1987). These findings indicate that the lowering of the eupnoeic P_{CO_2} by a ventilatory stimulant is not the only determinant of whether or not breathing instability will be initiated during sleep.

To reconcile these observations, we considered the possibility that the difference between the eupnoeic P_{CO_2} and the apnoeic threshold P_{CO_2}, rather than the absolute eupnoeic P_{CO_2}, is the critical determinant of breathing instability during sleep. A smaller difference between the eupnoeic and apnoeic threshold would indicate an increase in the sensitivity of the ventilatory control system below eupnoea and thereby, increase the likelihood of ventilatory instability (Carley *et al.* 1988; Khoo *et al.* 1991). Consequently, a given transient reduction of P_{CO_2} will cause a greater reduction in ventilation and result in apnoea during hypoxia. Accordingly, the present study was performed during both normoxia and hypoxia to determine precisely the eupnoeic P_{CO_2} and the P_{CO_2} required to cause hypopnoea or apnoea (hypopnoea/apnoeic threshold). We hypothesized that hypoxia reduces the difference between the eupnoeic P_{CO_2} and hypopnoea/apnoeic threshold in sleeping humans.

METHODS

Subjects

Seven healthy, non-snoring volunteers (3 male and 4 females) with a mean age of 25 years (range, 19–35 years) and body mass index of 24 ± 2 served as subjects. Women were studied within 7–10 days of their last menstrual period. All were non-smokers and free from any cardiovascular, pulmonary and neurological diseases. This study was approved by the University of Wisconsin Health Sciences Human Subjects Committee. All experimental procedures were performed in accordance with the Declaration of Helsinki and all subjects gave their informed written consent to participate in the study.

Polysomnographic methods

Overnight sleep studies were performed on each subject using standard polysomnographic techniques (Rechtschaffen & Kales, 1968), including electroencephalogram (C3/A2 and O2/A1), electro-oculogram (EOG), electrocardiogram and submental electromyogram with surface electrodes to identify sleep stages and arousals. Ventilation was measured with a pneumotachograph (Hans Rudolph) coupled to a differential pressure transducer (Validyne) positioned between the nasal mask and the ventilator tubing. The flow signal was electronically integrated to provide inspiratory and expiratory volume. The airway pressure was measured from a side port on the mask. Respiratory effort was also monitored by respiratory inductive plethysmography (Respitrace, Ambulatory Monitoring Inc.), calibrated with an isovolume manoeuvre. Arterial oxyhaemoglobin saturation (S_{a,O_2}) was measured by pulse oximetry (Ohmeda Oxicap no. 4700). End-tidal gas was sampled via a catheter in the nostril with a sampling tube passed through a side port of the nasal mask. End-tidal P_{CO_2} and P_{O_2} were measured by a gas analyser (Model CD-3A, AMETEK), which was calibrated by known gases before and after each experiment. All the variables were recorded continuously on a polygraph (Model 78D; Grass Instruments) and on videotape (Vetter).

Protocol

Subjects breathed through a sealed nasal mask attached to a mechanical ventilator (Hamilton Medical). The mouth was taped shut to prevent air leaks. The ventilator was set in the pressure support mode, which allowed an independent adjustment of the inspiratory and end-expiratory pressures. The trigger sensitivity of the ventilator was set at -2 cmH$_2$O. To facilitate sleep and avoid sleep fragmentation, zolpidem (10 mg) was administered orally to all subjects 10 min prior to lights out. During stages 2–4, non-rapid eye movement (NREM) sleep, each trial was initiated with a 3–5 min period of spontaneous breathing obtained while the subject breathed with an inspiratory pressure and end-expiratory pressure of 2 cmH$_2$O, i.e. continuous positive airway pressure (CPAP) at 2 cmH$_2$O. The low level of CPAP was used to minimize the upper airway resistance.

Following the baseline CPAP period, the inspiratory pressure was increased to 6 cmH$_2$O while maintaining end-expiratory pressure at 2 cmH$_2$O. If no apnoea or hypopnoea occurred after 2 min, the inspiratory pressure was increased in 2 cmH$_2$O increments at 2 min intervals with the end-expiratory pressure being maintained at 2 cmH$_2$O. When periodic breathing occurred with recurrent apnoeas and/or hypopnoeas, the subject was returned to baseline CPAP and spontaneous breathing. At least 3–5 min of spontaneous breathing was required between trials to allow the S_{a,O_2} and P_{ET,CO_2} to return to their baseline levels.

Apnoea was defined as a delay in the perceptible inspiratory effort on the mask pressure, Respitrace and flow signals for a length of at least two baseline respiratory cycles, (arrow B in Fig. 1*B*). Hypopnoea was defined as two or more non-triggered efforts detected on the mask pressure tracing associated with a 50% or greater reduction in tidal volume (arrow A in Fig. 1*B*). The pre-apnoea or pre-hypopnoea respiratory variables were determined by averaging data from the three breaths immediately prior to each apnoea or hypopnoea event. Trials that resulted in awakening or arousal were excluded from analysis.

Multiple trials were performed during both normoxia and hypoxia in a random order. For hypoxia trials, subjects inhaled a gas mixture with an inspired oxygen fraction (F_{I,O_2}) of 8–14% to achieve an oxygen saturation (S_{a,O_2}) of 80%. When the S_{a,O_2} stabilized at 80% for 5 min, the pressure-support protocol was initiated as described above. If a normoxia trial immediately followed a hypoxia trial, the pressure-support protocol was not initiated until the S_{a,O_2} returned to the normoxic baseline level.

Data analysis

Sleep stages were scored according to standard criteria (Rechtschaffen & Kales, 1968). Respiratory parameters including tidal volume (V_T), breathing frequency (f), minute ventilation ($\dot{V}_E$), inspiratory time (T_I), expiratory time (T_E), end-tidal P_{O_2} (P_{ET,O_2}) and P_{ET,CO_2} were measured breath-by-breath and expressed as means $\pm$ S.E.M. Ventilatory measurements from stages 2–4 of non-rapid eye movement (NREM) sleep were combined for analysis.

Baseline values for respiratory parameters were determined by averaging all breaths during 3–5 min of stable, spontaneous breathing on CPAP during normoxia and hypoxia. At each level of pressure support, we averaged the respiratory parameters at each inspiratory pressure level for the periods before the occurrence of hypopnoea or apnoea. We then compared the respiratory parameters at each pressure-support level with each individual's baseline respiratory parameters using an ANOVA with repeated measures followed by Bonferroni *t* tests if necessary. We also used least squares linear regression analysis to examine the relationship between

respiratory parameters and the pressure-support level. The mean pre-apnoea and pre-hypopnoea respiratory parameters were compared with each individual's baseline values as well as between normoxia and hypoxia using a two-way ANOVA with repeated measures. When the P value was significant, a Bonferroni t test was performed to localize the difference.

The reproducibility of the pre-apnoea and the pre-hypopnoea P_{ET,CO_2} was determined as the coefficient of variation for each subject using 3–7 trials per subject. The difference between the eupnoeic P_{ET,CO_2} and the pre-apnoea or the pre-hypopnoea P_{ET,CO_2} ($\Delta P_{ET,CO_2}$) was determined for each subject. We then compared $\Delta P_{ET,CO_2}$ between normoxia and hypoxia, as well as between hypopnoea and apnoea under the same condition (normoxia or hypoxia), using a two-way ANOVA with repeated measures. Another way of examining the relationship between the reduction of P_{ET,CO_2} and the development of apnoea was to calculate the change in ventilatory response to CO_2 between eupnoea and the apnoea threshold. The ventilatory response was calculated by dividing the eupnoeic $\dot{V}_E$ by the $\Delta P_{ET,CO_2}$ (eupnoeic P_{ET,CO_2} − apnoea threshold P_{ET,CO_2}). The slope of the ventilatory response was compared between hypoxia and normoxia using Student's paired t test. Data are reported as means $\pm$ S.E.M.

RESULTS

The baseline breathing pattern and the effect of pressure-support ventilation in producing hypopnoea and apnoea is illustrated during representative normoxia and hypoxia trials (Fig. 1). During normoxia, a stable breathing pattern was noted during the baseline period. A progressive increase in the pressure-support level (note increase in P_m) resulted in an increase in tidal volume and a reduction in P_{ET,CO_2}, which eventually caused hypopnoea

and subsequent periodic breathing. The hypopnoea threshold was determined as the mean P_{ET,CO_2} of the last three breaths, which immediately preceded the first hypopnoea. During hypoxic breathing, the periodic breathing following the initial hypopnoea included an episode of central apnoea.

Effect of hypoxia on baseline ventilation

During stable NREM sleep, hypoxia increased $\dot{V}_E$ (5.4 $\pm$ 0.3 *vs.* 6.3 $\pm$ 0.4 l min^{-1}, $P < 0.05$) and reduced P_{ET,CO_2} (45.0 $\pm$ 1.1 *vs.* 42.4 $\pm$ 1.3 mmHg, $P < 0.001$) compared to normoxia (Table 1). Spontaneously occurring sleep-disordered breathing was more common during hypoxia compared to normoxia (apnoea/hypopnoea index 9.8 $\pm$ 2.6 *vs.* 0.9 $\pm$ 0.5 events h^{-1}, $P < 0.05$). Hypoxia increased the slope of the ventilatory response to CO_2 in the range between eupnoeic P_{ET,CO_2} and the apnoeic threshold P_{ET,CO_2} (1.7 $\pm$ 0.1 *vs.* 5.5 $\pm$ 0.9 l min^{-1} mmHg^{-1}, $P < 0.05$).

Effect of pressure support on ventilation prior to hypopnoea/apnoea

During room air breathing, prior to producing hypopnoea or apnoea, pressure support effectively increased $\dot{V}_E$ and lowered P_{ET,CO_2} during normoxic breathing (Fig. 2). With the increase in the pressure-support level from 0–10 cmH$_2$O, $\dot{V}_E$ gradually increased (5.4 $\pm$ 0.3 to 7.3 $\pm$ 0.6 l min^{-1}; $r = 0.97$, $P < 0.05$), and P_{ET,CO_2} gradually declined (45.0 $\pm$ 1.1 to 42.1 $\pm$ 1.3 mmHg; $r = -0.94$, $P < 0.05$). The pressure-support ventilation caused an increase in V_T (0.35 $\pm$ 0.02 to 0.57 $\pm$ 0.07 l; $r = 0.96$,

Figure 1. Polygraph record of a single trial during normoxic (*A*) and hypoxic (*B*) breathing

The baseline period shows spontaneous, stable breathing with continuous positive airway pressure (CPAP) of 2 cmH$_2$O. During normoxia, the pressure-support level was increased to 8 cmH$_2$O during inspiration with the end-expiratory level maintained at 2 cmH$_2$O (8/2 cmH$_2$O) and subsequently to 10/2 cmH$_2$O. At 10/2 cmH$_2$O, the reduction in P_{ET,CO_2} was sufficient to cause an initial hypopnoea and consequent periodic breathing. The hypopnoea threshold was determined as the mean P_{ET,CO_2} of the last three breaths immediately preceding the first hypopnoea. During hypoxia, arrow A indicates a hypopnoea with two successive untriggered breaths, and arrow B indicates a central apnoea with no respiratory effort.
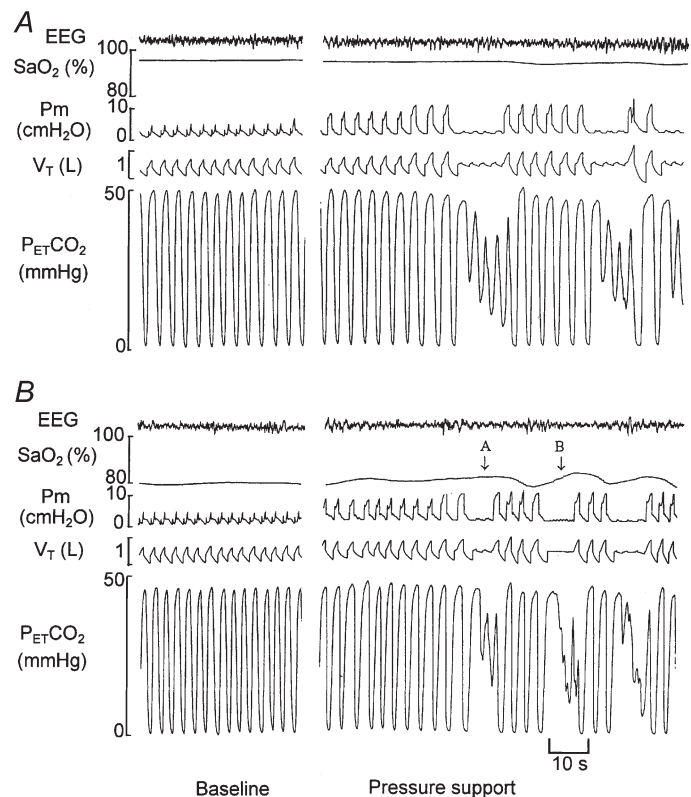
Table 1. Baseline and pre-hypopnoea/apnoeic respiration

	Room air			Hypoxia		
	Baseline	Pre-hypopnoea	Pre-apnoea	Baseline	Pre-hypopnoea	Pre-apnoea
V_T (ml)	351 ± 15	479 ± 52†	614 ± 63†‡	376 ± 24	402 ± 24†	514 ± 64†‡
f (breaths min^{-1})	16 ± 1	14 ± 1†	12 ± 1†‡	17 ± 1*	17 ± 1*†	16 ± 1*†‡
$\dot{V}_E$ (l min^{-1})	5.4 ± 0.3	6.4 ± 0.6†	7.1 ± 0.5†	6.3 ± 0.4*	6.5 ± 0.4†	7.5 ± 0.6†
P_{ET,CO_2} (mmHg)	45.0 ± 1.1	43.0 ± 1.2†	41.6 ± 1.0†‡	42.4 ± 1.3*	42.1 ± 1.4	41.3 ± 1.2†‡
P_{ET,O_2} (mmHg)	103 ± 3	108 ± 3	113 ± 3†‡	54 ± 2*	59 ± 2*	64 ± 2*†‡

*$P < 0.05$, hypoxia *vs.* room air; †$P < 0.05$, compared to the baseline value; ‡$P < 0.05$, compared to hypopnoea. V_T, tidal volume; $\dot{V}_E$, minute ventilation; P_{ET,CO_2}, end-tidal partial pressure of CO_2; P_{ET,O_2}, end-tidal partial pressure of O_2.

$P < 0.05$) and a decrease in breathing frequency (16 ± 0.7 to 14 ± 0.7 breaths min^{-1}; $r = -0.98$, $P < 0.01$). The T_I was prolonged (1.8 ± 0.1 to 2.2 ± 0.1 s; $r = 0.90$, $P < 0.05$) with no clear effect on T_E (2.2 ± 0.1 to 2.4 ± 0.2 s; $r = 0.84$, $P > 0.05$).

During hypoxia, prior to producing hypopnoea or apnoea, pressure-support ventilation caused a similar increase in V_T (0.37 ± 0.02 to 0.56 ± 0.03 l; $r = 0.95$, $P < 0.05$) and $\dot{V}_E$ (6.3 ± 0.4 to 8.4 ± 0.3 l min^{-1}; $r = 0.92$, $P < 0.05$), a
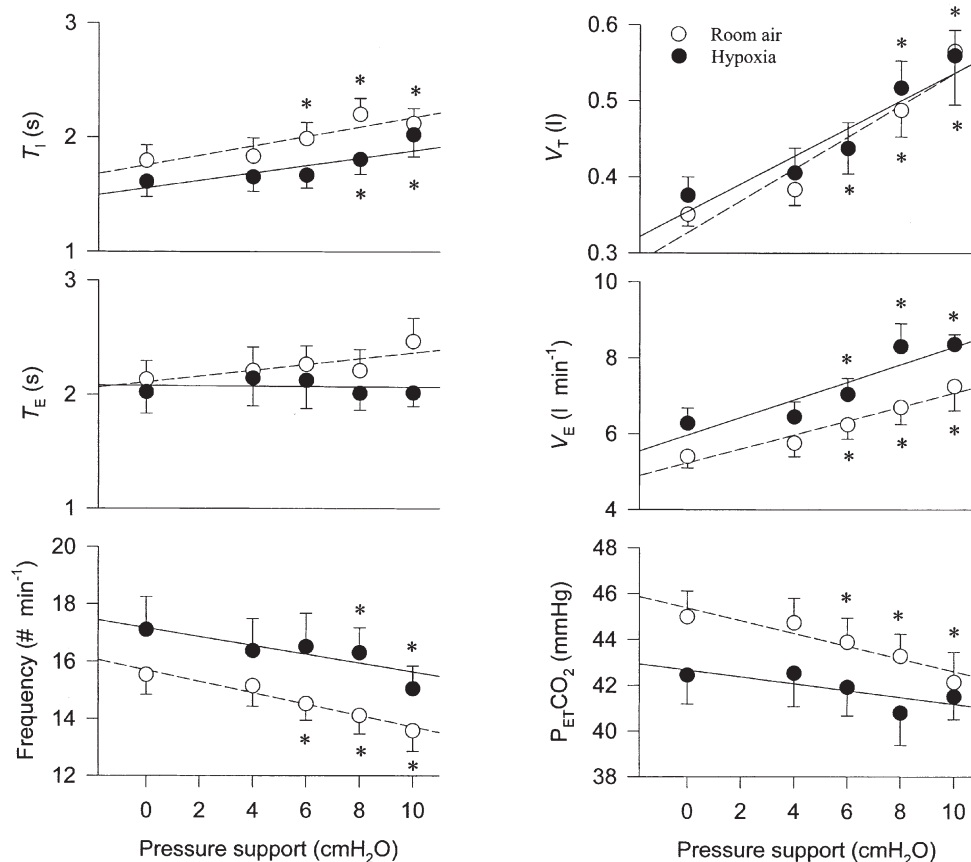

Figure 2. Effect of pressure-support levels on ventilation and respiratory cycle timing during normoxia (○) and hypoxia (●)

The increase of inspiratory pressure support caused an increase in tidal volume and minute ventilation and a decrease in breathing frequency. End-tidal P_{CO_2} gradually decreased. Each point at pressure support levels of 0–8 cmH$_2$O represents the mean data for the entire group of seven subjects. The pressure-support level of 10 cmH$_2$O represents data from all seven subjects during normoxia but only four of seven subjects during hypoxia. During hypoxia, three of seven subjects developed apnoea at pressure support $\leqslant 10$ cmH$_2$O. T_I, inspiratory time; T_E, expiratory time; V_T, tidal volume; $\dot{V}_E$, minute ventilation; P_{ET,CO_2}, end-tidal P_{CO_2}. *$P < 0.05$ compared to the baseline value with zero pressure support.

reduction in breathing frequency (17 ± 1 to 15 ± 1 breaths min^{-1}; $r = -0.88$, $P = 0.05$) and a prolonging of T_I (1.6 ± 0.1 to 2.0 ± 0.2 s; $r = 0.89$, $P < 0.05$). A trend in reduction of P_{ET,CO_2} did not reach statistical significance until apnoea actually occurred (see below and Fig. 1*B*).

## Hypopnoea and apnoea produced by pressure-support ventilation

During normoxia, 42 trials of pressure-support ventilation produced apnoea or hypopnoea without an associated change in sleep stages (Fig. 1*A*). During hypoxia, 37 trials resulted in apnoea or hypopnoea (Fig. 1*B*). Apnoea length was slightly shorter during hypoxia than normoxia (12.1 ± 0.9 *vs.* 13.9 ± 0.9 s, $P < 0.05$). The hypoxic trials were associated with a greater decline of S_{a,O_2} at the end of hypopnoea or apnoea (hypopnoea, -3.8 ± 0.3 *vs.* -1.2 ± 0.2 %, $P < 0.01$; apnoea, -5.3 ± 0.3 *vs.* -2.1 ± 0.5 %, $P < 0.01$). The time delay from the final change in pressure support to the onset of apnoea or hypopnoea was similar during normoxia and hypoxia in terms of either time (19 ± 4 *vs.* 22 ± 5 s, $P > 0.05$) or the number of breaths (4 ± 1 *vs.* 5 ± 1 breaths, $P > 0.05$).

Apnoeas were always preceded by a higher V_T and $\dot{V}_E$ and a lower P_{ET,CO_2} compared to the baseline levels in both normoxia and hypoxia (Table 1 and Fig. 1). Hypopnoeas, during normoxia, were also associated with a significant reduction in P_{ET,CO_2}. However, during hypoxia, the reduction in P_{ET,CO_2} preceding hypopnoeas was small, inconsistent and insignificant. An example of the minimum reduction in P_{ET,CO_2} prior to a hypopnoea and

the slight further reduction in P_{ET,CO_2} associated with an apnoea on the next cycle is shown in Fig. 1*B*.

For each individual, the pre-hypopnoea or pre-apnoea P_{ET,CO_2} was quite reproducible. The coefficient of variation was 2.7 ± 0.2 % for pre-hypopnoea and 1.2 ± 0.5 % for pre-apnoea during normoxia, and 3.2 ± 0.4 % for pre-hypopnoea and 2.8 ± 0.7 % for pre-apnoea during hypoxia. The occurrence of apnoea or hypopnoea during hypoxia was associated with a smaller increase in tidal volume above baseline levels compared to normoxia (V_T, 26 ± 7 *vs.* 128 ± 39 ml, $P < 0.05$ for pre-hypopnoea; 138 ± 44 *vs.* 264 ± 57 ml, $P < 0.05$ for pre-apnoea). Likewise, the minimum pressure-support level which produced hypopnoea or apnoea was lower during hypoxia than during normoxia (5.1 ± 1.1 *vs.* 7.1 ± 0.6 cmH$_2$O, $P < 0.05$).

The difference between the eupnoeic P_{ET,CO_2} and the apnoea or hypopnoea threshold P_{ET,CO_2} ($\Delta P_{ET,CO_2}$) was smaller during hypoxia compared to normoxia (-0.2 ± 0.2 *vs.* -2.0 ± 0.3 mmHg, $P < 0.01$ for hypopnoea and -1.1 ± 0.2 *vs.* -3.4 ± 0.3 mmHg, $P < 0.01$ for apnoea, Fig. 3). No difference was noted in the absolute level of P_{ET,CO_2} at the hypopnoeic threshold (43.0 ± 1.2 *vs.* 42.1 ± 1.4 mmHg, $P > 0.05$) or the apnoeic threshold (41.6 ± 1.0 *vs.* 41.3 ± 1.2 mmHg, $P > 0.05$) between the two conditions. Thus, hypoxia failed to lower the apnoeic threshold P_{ET,CO_2} to as great an extent as it lowered the eupnoeic P_{ET,CO_2}. Moreover, the lower apnoeic threshold compared to the hypopnoeic threshold P_{ET,CO_2} during both normoxia and hypoxia suggests a dose–response phenomenon whereby a greater reduction of
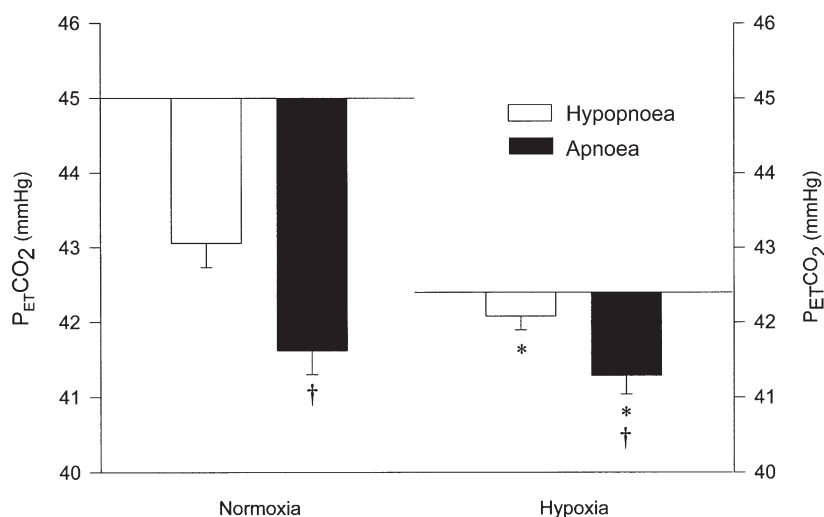
Figure 3. Eupnoeic and threshold P_{ET,CO_2} during normoxic and hypoxic trials

The horizontal line represents the eupnoeic P_{ET,CO_2} during stable breathing with no pressure support. □, the difference between the eupnoeic P_{ET,CO_2} and the hypopnoeic threshold P_{ET,CO_2}. ■, the difference between the eupnoeic P_{ET,CO_2} and the apnoeic threshold P_{ET,CO_2}. *$P < 0.05$, $\Delta P_{ET,CO_2}$ (eupnoeic − threshold P_{ET,CO_2}) during hypoxia compared to normoxia. †$P < 0.05$, $\Delta P_{ET,CO_2}$ required to produce apnoea (eupnoeic − apnoeic threshold P_{ET,CO_2}) compared to the $\Delta P_{ET,CO_2}$ required to produce hypopnoea (eupnoeic − hypopnoeic threshold P_{ET,CO_2}) during normoxia and hypoxia.

P_{ET,CO_2} is required to eliminate respiratory rhythm, as opposed to simply reducing amplitude (Wilson *et al.* 1999).

DISCUSSION

The major observation of this study is that the difference between the eupnoeic P_{ET,CO_2} and the hypopnoea/apnoeic threshold P_{ET,CO_2} was smaller during hypoxia than during normoxia. The closer proximity of the eupnoeic P_{ET,CO_2} to the hypopnoea/apnoeic threshold P_{ET,CO_2} was due to a disproportionate reduction in the eupnoeic P_{ET,CO_2} rather than a difference in the threshold P_{ET,CO_2} between normoxia and hypoxia. The close proximity between the eupnoeic P_{ET,CO_2} and the apnoeic threshold provides a potential explanation for why hypoxia causes breathing instability during sleep.

Mechanisms for failure of hypoxia to lower the hypopnoea/apnoeic threshold

Since all ventilatory stimulants lower the eupnoeic P_{a,CO_2}, a fundamental difference in the effect of hypoxia may be its failure to reduce proportionally the hypopnoea/apnoeic threshold. It is not completely understood why the apnoeic threshold is not lowered during hypoxia. However, compared to other non-hypoxic stimulants, hypoxia uniquely depresses the central nervous system,

dilates cerebral vessels and impairs short-term potentiation. All these effects may work together in changing the overall excitability of the ventilatory control system, which is manifested by a relatively high hypopnoea/apnoeic threshold for P_{CO_2}.

Although hypoxia-induced respiratory depression has not been observed in all preparations (Neubauer *et al.* 1990; Curran *et al.* 2000), when hypoxia lasts longer than 5 min, as it did in our subjects, it may impair neuronal function in the central nervous system, including the bulbar network of respiratory neurons (Natsui & Kuwana, 1992). Extreme hypoxia is associated with the elimination of respiratory neural activity and cessation of respiratory drive (Trippenbach *et al.* 1990; LaManna *et al.* 1996). The mechanism underlying the depressant effect of hypoxia on ventilation is complicated at the cellular and molecular levels and may be related to metabolic impairment causing ATP depletion (LaManna *et al.* 1996; Mironov *et al.* 1998), reduction of synaptic interaction among respiratory neurons (Ramires *et al.* 1998), increased membrane hyperpolarization of respiratory neurons (Neubauer *et al.* 1990) or hypoxia-induced production of neuro-inhibitory substances (Schaeffer *et al.* 1988; Runold *et al.* 1989; Schmidt *et al.* 1995; Richter *et al.* 1999). This hypoxia-produced central neural dysfunction
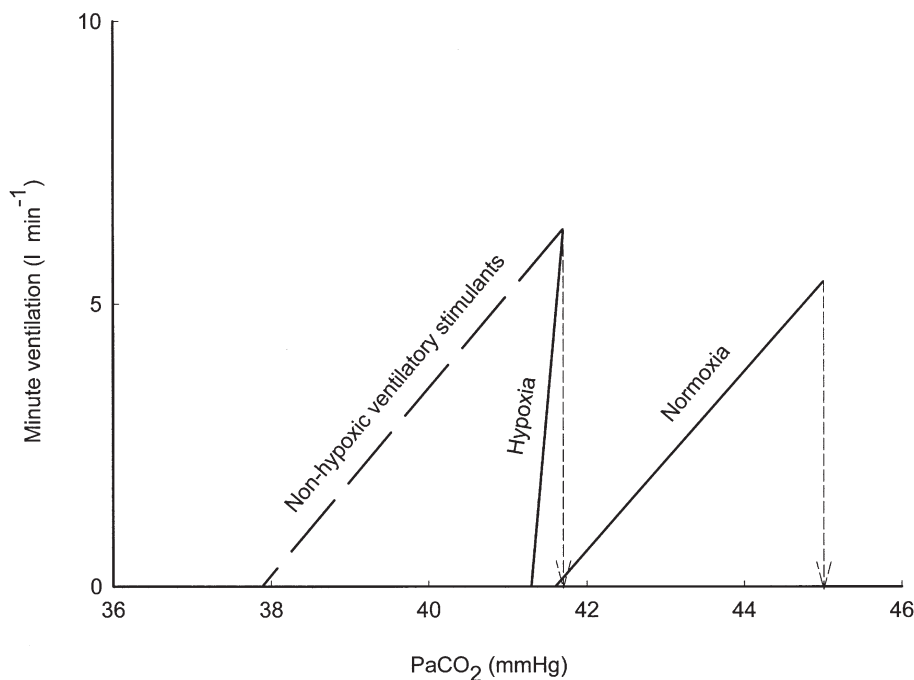
Figure 4. Ventilatory response to CO_2 below the eupnoeic P_{CO_2}
The continuous lines were drawn between the point of eupnoeic ventilation at eupnoeic P_{CO_2} and the point of P_{CO_2} at the apnoeic threshold P_{CO_2} using data from the present study during normoxia and hypoxia. The slope of the ventilatory response below eupnoeic P_{CO_2} was greater during hypoxia compared to normoxia. The dashed line represents a hypothetical non-hypoxic ventilatory stimulant that lowered the eupnoeic P_{CO_2} to the same extent as we observed during hypoxia, but did not change the slope of the ventilatory response below eupnoea compared to room air breathing. Note that a much smaller reduction in P_{CO_2} is required to produce apnoea during hypoxia compared to the non-hypoxic ventilatory stimulant.

may impair the neural switching mechanism from expiration to inspiration and weaken the resistance of the respiratory system to instability (Younes, 1989). Therefore, more input from other sources is needed to initiate inspiration. A modest reduction of CO_2-related drive could more easily lead to hypopnoea/apnoea and would be manifested as a higher P_{CO_2} threshold.

Hypoxic respiratory depression may also be related to central alkalosis due to cerebral vasodilatation and increased cerebral blood flow (Cherniack *et al.* 1970; Berkenbosch & DeGoede, 1988). Since central chemoreceptors have a stabilizing effect on respiratory activity, any reduction in the activity of the central chemoreceptors tends to increase the susceptibility to periodic breathing (Khoo *et al.* 1991). Peripheral hypocapnia alone can cause a reduction in tidal volume amplitude, but is not sufficient to trigger apnoea as long as the central chemoreceptors are active (Smith *et al.* 1997). The hypoxia-induced cerebral vessel dilatation may lower the brain P_{CO_2} and thereby, reduce the difference between brain and arterial P_{CO_2}. As a result, a higher arterial and end-tidal P_{CO_2} would be required during hypoxia to maintain the same level of cerebrospinal fluid pH and P_{CO_2} as during normoxia. Consequently, a higher hypopnoea/apnoea threshold would be observed during hypoxia. Given the relatively mild level of hypoxaemia imposed in our study (P_{ET,O_2} $\sim$54 mmHg), we think it unlikely that cerebral blood flow or the arterial-to-CSF P_{CO_2} change was significantly affected (Dempsey *et al.* 1974).

Finally, the higher hypopnoea/apnoeic threshold during hypoxia may reflect a hypoxia-induced defect in short-term potentiation in the respiratory control system (Badr *et al.* 1992). Short-term potentiation stabilizes ventilation following the withdrawal of any ventilatory stimuli. Hypocapnia has a powerful inhibitory influence even in the presence of a normal level of short-term potentiation. During hypoxia, the impairment in short-term potentiation may cause hypopnoea/apnoea to occur following a smaller reduction in P_{CO_2}, which is manifested by a higher hypopnoea/apnoea threshold.

All the aforementioned effects of hypoxia will make the ventilatory control system more sensitive to inhibitory afferents in general, including the effect of neuromechanical inhibition. As a result, the respiratory control system would be expected to become unstable and to demonstrate excessive reductions in tidal volume at levels of P_{CO_2} that would not produce instability during other types of ventilatory stimulation. This effect would be manifested as the failure to lower the apnoeic threshold in proportion to the lowering of the eupnoeic P_{CO_2}. This enhanced effect of non-P_{CO_2}-related inhibitory influences may also be the reason why we did not see the statistically significant reduction in the pre-hypopnoeic P_{CO_2} during hypoxia that we did during normoxia.

Consequences of elevated hypopnoea/apnoeic threshold

The close proximity of the eupnoeic P_{CO_2} and the hypopnoea/apnoeic threshold has important implications for the development of periodic breathing during hypoxia. Our findings confirmed the destabilizing effect of hypoxia on breathing pattern by showing the development of periodic breathing at a lower pressure-support level, a smaller increase in V_T, and a smaller reduction in P_{ET,CO_2}. Even during spontaneous breathing, hypoxia was associated with a greater degree of breathing instability compared to normoxia. How can the observed change in the relationship between eupnoea and the threshold P_{CO_2} account for the greater degree of ventilatory instability?

The mechanism whereby the narrowed difference between the eupnoeic and threshold P_{CO_2} can increase the likelihood of periodic breathing during hypoxia can be considered as an increase in the sensitivity of the ventilatory response to chemical stimuli below the eupnoeic range of P_{CO_2}. Control system theory indicates that ventilatory instability is enhanced under conditions of increased sensitivity of the peripheral chemoreceptors (Lahiri *et al.* 1983; Carley *et al.* 1988; Khoo *et al.* 1991). An appropriate sensitivity is important in maintaining blood gas homeostasis and ventilatory stability. Too high a sensitivity may be associated with central apnoeas because a small reduction in chemical stimuli produces a large reduction in ventilation (Khoo *et al.* 1991; Xie *et al.* 1995; Solin *et al.* 2000). Previous studies have shown that hypoxia increases the ventilatory response to CO_2 above eupnoea (Cunningham *et al.* 1986). Our study provides unique data concerning the ventilatory response to reductions in P_{CO_2} below eupnoea during normoxia and hypoxia. During hypoxia, the smaller difference in P_{CO_2} between eupnoea and apnoeic threshold indicates a steeper slope of the ventilatory response to CO_2 below eupnoea (see Fig. 4). Few previous studies have investigated this response in sleeping humans. Our value of 1.7 l min^{-1} mmHg^{-1} during normoxia is similar to the 1.56–1.87 l min^{-1} mmHg^{-1} previously reported (Henke *et al.* 1988; Meza *et al.* 1998). During hypoxia, our data showed that the slope was increased substantially (5.5 l min^{-1} mmHg^{-1}). A similar effect of hypoxia on the ventilatory response to CO_2 below eupnoea was also found in our previous work where the slope increased from 1.48 l min^{-1} mmHg^{-1} during normoxia to 3.10 l min^{-1} mmHg^{-1} during hypoxia (Skatrud & Dempsey, 1983). As a direct result of this increase in slope below eupnoea, a similar decrease in P_{CO_2} will produce a much greater reduction in ventilation during hypoxia compared to normoxia. Thus, hypoxia enhances the susceptibility to periodic breathing by decreasing the amount of hyperventilation required to produce apnoea.

The arterial P_{CO_2} represents only one of the many inputs to respiratory rhythm generation. Thus, the apnoeic

threshold is changeable and varies according to other afferent inputs (Boden *et al.* 1998). This may explain why other ventilatory stimulants that lower the eupnoeic P_{CO_2} do not enhance ventilatory instability but may actually stabilize the breathing pattern. For example, patients with pulmonary fibrosis have a low P_{a,O_2} and P_{a,CO_2} but do not have an abnormal degree of periodic breathing during sleep (Perez-Padilla *et al.* 1985). Pharmacological ventilatory stimulants such as theophylline, acetazolamide and progesterone have a stabilizing effect on ventilation during sleep despite a reduction in eupnoeic P_{a,CO_2} (DeBaker *et al.* 1995; Javaheri *et al.* 1996; Hudgel & Thankitcharu, 1998). The propensity of these ventilatory stimuli to stabilize the breathing pattern during sleep may be due to a proportional reduction in the eupnoeic P_{CO_2} and apnoeic threshold. For example, if we assume that the non-hypoxic ventilatory stimulants have a sensitivity to the reduction of P_{CO_2} below eupnoea that is similar to normoxia, then the unchanged slope is associated with an increased difference between eupnoea and the apnoeic threshold (Fig. 4). Thus, the stabilizing influence of non-hypoxic ventilatory stimuli may be related to the decreased propensity to lower P_{CO_2} to the apnoeic threshold following periods of transient hyperpnoea. Alternatively, an unusually low level of eupnoeic P_{CO_2} would require a large change in alveolar ventilation to further reduce the P_{CO_2} towards the apnoeic threshold. In any case, our hypoxic data indicate the importance of a reduced difference between the eupnoeic and threshold P_{CO_2} rather than the absolute level of the eupnoeic P_{CO_2} in predicting the susceptibility to breathing instability.

Critique of methods

The use of mechanical ventilation to determine the apnoea/hypopnoeic threshold is associated with several pitfalls. First, the premature delivery of a ventilator breath could pre-empt spontaneous respiratory rhythm and cause T_E prolongation independently of P_{CO_2} (Knox & King, 1976; Satoh *et al.* 2001). However, our use of the subject-assisted, pressure-support mode, assured that the subject initiated the inspiratory activity and precluded the possibility of pre-emption of spontaneous breaths. Second, the downregulation of inspiratory muscle activity by neuromechanical inhibition can occur independently of hypocapnic inhibition (Wilson *et al.* 1999). Vagal or other inhibitory influences related to the mechanical ventilation could have contributed to the observed hypopnoea and apnoea, especially during hypoxia, when the P_{a,CO_2} of our subjects was already close to the hypocapnic apnoeic threshold. Our methodology minimized this effect since the use of pressure-support ventilation, with a gradual increase in the pressure levels, ensured that only the minimal increase in tidal volume was used to cause a reduction in P_{CO_2} and that only the minimal positive pressure was supplied to produce hyperventilation. Since plots of ventilation measurements against pressure show that ventilation responses at a given level of pressure support are similar in hypoxia and normoxia trials, comparable pressure-support effects and neuromechanical influences are expected in hypoxia *vs.* normoxia trials. In fact, pressure-support ventilation may have produced even less neuromechanical inhibition during hypoxia compared to normoxia, since lower pressures were used and smaller increases in tidal volume were required during hypoxia. Therefore, the greater susceptibility to hypopnoea and apnoea during hypoxia cannot be attributed to a disproportionate degree of neuromechanical inhibition during hypoxia, unless hypoxia increased the subject's sensitivity to neuromechanical inhibition as discussed above.

End-tidal P_{CO_2} was used as a non-invasive surrogate for arterial P_{CO_2}. We have previously demonstrated a predictable relationship between end-tidal and arterial P_{CO_2} during hypoxia (Badr *et al.* 1992) and mechanical ventilation (Wilson *et al.* 1999). Since P_{ET,CO_2} can only be measured during the ventilatory phase, we used the three breaths immediately preceding the hypopnoea or apnoea as indicative of the hypopnoea/apnoeic threshold P_{CO_2}. We did not find a difference in P_{ET,CO_2} among the three breaths prior to the hypopnoea/apnoea. The reproducibility of hypopnoea/apnoeic threshold within a subject confirmed that the P_{ET,CO_2} was a good representation of the threshold P_{CO_2}. Although a systematic error is still possible, it should not affect the difference between eupnoeic P_{CO_2} and threshold P_{CO_2}, which was the measurement of interest.

The use of zolpidem was necessary to facilitate sleep and reduce arousability in the laboratory. Zolpidem has been shown to have no effect on ventilation, oxygen saturation or breathing stability during normoxic or hypoxic sleep (Beaumont *et al.* 1996). However, its effect on the apnoeic threshold is unknown. Even if zolpidem did change the threshold, the effect would be present in both normoxic and hypoxic conditions and thus, would not change our conclusions.

Conclusions

Hypoxia distinguishes itself from other respiratory stimulants by showing a decrease in the proximity between the eupnoeic P_{CO_2} and the hypopnoea/apnoeic threshold P_{CO_2} as a result of the failure to reduce the threshold P_{CO_2} in a parallel manner to the reduction in eupnoeic P_{CO_2}. The relatively high apnoeic threshold may reflect impairment of the ventilatory control system by hypoxia. Consequently, a greater level of afferent input from the periphery, as manifested by a higher apnoeic threshold for P_{CO_2}, is required to maintain rhythmic breathing. The combination of a low P_{CO_2} and a relatively high threshold makes it easier for transient reductions of P_{a,CO_2} to reach the apnoeic threshold and cause breathing instability during sleep.

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