Effects of five consecutive nocturnal hypoxic exposures on the cerebrovascular responses to acute hypoxia and hypercapnia in humans

Jon C. Kolb, Philip N. Ainslie, Kojiro Ide, and Marc J. Poulin


Submitted 10 September 2003; accepted in final form 7 January 2004

Kolb, Jon C., Philip N. Ainslie, Kojiro Ide, and Marc J. Poulin. Effects of five consecutive nocturnal hypoxic exposures on the cerebrovascular responses to acute hypoxia and hypercapnia in humans. J Appl Physiol 96: 1745-1754, 2004.——The effects of discontinuous hypoxia on cerebrovascular regulation in humans are unknown. We hypothesized that five nocturnal hypoxic exposures (8 h/day) at a simulated altitude of 4,300 m (inspired O2 fraction = 0.138%) would elicit cerebrovascular responses that are similar to those that have been reported during chronic altitude exposures. Twelve male subjects (26.6 ± 4.1 yr, mean ± SD) volunteered for this study. The technique of end-tidal forcing was used to examine cerebral blood flow (CBF) and regional cerebral O2 saturation (SrO2) responses to acute variations in O2 and CO2 twice before, immediately after, and 5 days after the overnight hypoxic exposures. Transcranial Doppler ultrasound was used to assess CBF, and near-infrared spectroscopy was used to assess SrO2. Throughout the nocturnal hypoxic exposures, end-tidal CO2 decreased (P < 0.001) whereas arterial O2 saturation increased (P < 0.001) compared with overnight normoxic control measurements. Symptoms associated with altitude illness were significantly greater than control values on the first night (P < 0.001) and second night (P < 0.01) of nocturnal hypoxia. Immediately after the nocturnal hypoxic intervention, the sensitivity of CBF to acute variations in O2 and CO2 increased 116% (P < 0.01) and 33% (P < 0.05), respectively, compared with control values. SrO2 was highly correlated with arterial O2 saturation (R2 = 0.94 ± 0.04). These results show that discontinuous hypoxia elicits increases in the sensitivity of CBF to acute variations in O2 and CO2, which are similar to those observed during chronic hypoxia.

In humans, cerebral blood flow (CBF) increases in response to acute hypoxia (11, 17, 28, 43). The magnitude of the CBF response to hypoxia is dependent on the balance between the hypoxemia-induced cerebral vasodilation and the cerebral vasoconstriction secondary to hypocapnia associated with the increased ventilation (40, 41). Over the course of several days, the process of ventilatory acclimatization to the hypoxia of altitude unfolds and the degree of hypoxemia is reduced, whereas the degree of hypocapnia increases (15, 43). Several studies (5, 14, 34) have suggested that the interaction between these opposing vascular reflexes might contribute to individual susceptibility to altitude diseases such as acute mountain sickness (AMS) and high-altitude cerebral edema.

To address the issue of whether the cerebrovascular sensitivity to hypoxia was altered during the process of acclimatization, Jensen et al. (15) quantified the CBF responses in humans during a high-altitude field study. Over a 5-day sojourn at altitude (3,810 m), Jensen and colleagues reported that the sensitivity of CBF to acute variations in isocapnic hypoxia increased 34%, whereas the CBF sensitivity to acute variations in hypercapnia increased 28%. Similar increases in CBF sensitivities to acute variations in O2 and CO2 have recently been reported by Poulin et al. (28) after 48 h of continuous isocapnic and poikilocapnic hypoxic exposures in humans. Whereas these studies and others (14, 15, 26, 43) have examined the effect of chronic hypoxia on cerebral vasomotor reactivity, the impact of discontinuous hypoxia on cerebrovascular events has received little attention. The aim of the present study was to establish a discontinuous hypoxic intervention that would elicit physiological perturbations comparable to those observed during adaptation to chronic hypoxia. We hypothesized that five consecutive overnight normobaric hypoxic exposures (8 h/night) at a simulated altitude of 4,300 m would elicit similar changes in cerebrovascular sensitivities to acute hypoxia and hypercapnia to those reported during chronic altitude exposures. Additionally, there have been reports that alterations in regional cerebral O2 saturation (SrO2) may be associated with the cerebrovascular response to chronic hypoxia (12, 37). Therefore we simultaneously measured SrO2 to gain further insight regarding the changes in cerebral oxygenation before and after the five consecutive nocturnal hypoxic exposures.

This cerebrovascular study was undertaken in conjunction with a study examining the ventilatory responses to nocturnal hypoxia (1).

METHODS

Subjects

Twelve healthy male subjects (26.6 ± 4.1 yr; mean ± SD) participated in this study. The requirements of the study were fully explained to all subjects, with each subject giving written, informed consent before participating in the study. The research project was approved by the University of Calgary Conjoint Health Research Ethics Board. All subjects were nonsmokers, none was taking any medication, and none of the subjects had any history of respiratory, cardiovascular, or cerebrovascular disease. Before commencing the main experimental procedures, each subject made a preliminary visit to the laboratory that served as a familiarization session to the laboratory and apparatus. During this initial visit, measurements of resting end-tidal gases and estimates of hypoxic and hypercapnic sensitivities were conducted. The laboratory is located at an altitude of 1,103 m above sea level, and the average barometric pressure for the duration of the study was 667 ± 14 Torr.

Address for reprint requests and other correspondence: M. J. Poulin, Dept. of Physiology & Biophysics, Faculty of Medicine, Heritage Medical Research Bldg, Rm. 212, Univ. of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1 (E-mail: poulin@ucalgary.ca).

http://www.jap.org 8750-7587/04 $5.00 Copyright © 2004 the American Physiological Society

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Protocol

The experimental design, illustrating the timing of measurements and the sequence of nocturnal hypoxic exposures, is presented in Table 1. One week before the nocturnal hypoxic exposures, each subject participated in a control sleep under normoxic conditions to obtain overnight baseline measurements. Subsequently, each subject reported to the laboratory on two occasions, 5 days apart, at the same time of day after an overnight fast for control measurements of the sensitivity of CBF and SrO2 responses to an acute incremental hypoxic protocol using an end-tidal forcing system to control end-tidal gases. After the final control measurement, each subject underwent five consecutive overnight sleep exposures of normobaric hypoxia at a simulated altitude of ~4,300 m [inspired O2 fraction (FIO2) ~13.8%]. Immediately after the five nocturnal hypoxic exposures, and after 5 days of recovery, subjects reported to the laboratory to repeat the measurements of cerebrovascular sensitivities to acute variations in hypoxia and CO2.

Nocturnal Hypoxic Exposures and Overnight Measurements

Subjects entered the purpose-designed chambers (Hypoxico) at ~2300 and exited at 0700 the following morning. On each night, the ambient environment in the chamber was reduced progressively from 2300 and exited at 0700 the following morning. On each night, the

| Table 1. Timing and sequence of overnight measurements, blood measurements, and acute dynamic measurements |
|-----------------|---|---|---|---|---|---|---|---|
| Overnight measurements | Sao2 | PETCO2 | AMS symptoms | Blood measurements | Sensitivity of CBF to acute variations in hypoxia | Sensitivity of CBF to acute variations in CO2 | SrO2 responses to acute variations in hypoxia | SrO2 responses to acute variations in CO2 |
| CS | C1 | C5 | E1 | E2 | E3 | E4 | E5 | R1 | R5 |
| Overnight measurements | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Blood measurements | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Dynamic measurements | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

Overnight measurements made inside the hypoxic chamber during the nocturnal hypoxic exposures included arterial oxygen saturation (Sao2; pulse oximetry), end-tidal PCO2 (PETCO2), and AMS symptoms (Lake Louise Acute Mountain Sickness Scoring System). Blood measurements for overnight exposures to hypoxia (E1–E5) were made before subjects entered the chamber (normoxia) and in the morning after 8 h of hypoxia before they exited the chamber. Dynamic cerebrovascular measurements were made before and after the 5 consecutive overnight hypoxic exposures using an end-tidal forcing system to control the end-tidal gases. CS, control sleep (normoxia); C1 and C5, control day 1 and control day 5; R1 and R5, recovery day 1 and recovery day 5 after E1–E5; CBF, cerebral blood flow; SrO2, regional cerebral O2 saturation.

The presence and severity of the AMS symptoms of headache, gastrointestinal upset, fatigue or weakness, dizziness, and difficulty sleeping were evaluated with the Lake Louise Acute Mountain Sickness Scoring System questionnaire (33). Each symptom was evaluated on a sliding scale ranging from 0 (no symptom) to 3 (severe). The diagnostic criteria for AMS, as outlined in the scoring system, stipulate the presence of headache and any other symptom(s). A summated score of 3 or more constitutes the incidence of moderate AMS. The questionnaire was completed by each subject at the beginning of each overnight session, after 4 h, and after 8 h before exiting the chamber.

Incremental Step Hypoxic and Hypercaphnic Protocol

Subjects reported to the laboratory at the same time each morning after an overnight fast. On each of these visits, baseline air breathing measurements of the subject’s end-tidal PO2 (PETO2) and PETCO2 values were measured before the experiment, while the subject was sitting quietly and comfortably for ~10 min. The respired gases were sampled via a fine catheter held at the opening of one nostril by an adapted nasal O2 therapy kit. The gas was sampled continuously at a rate of 20 ml/min and analyzed for PO2 and PCO2 by mass spectrometry (AMS 2000, Innovaision, Odense, Denmark). Values for PO2 and PCO2 were sampled by computer every 10 ms. PETO2 and PETCO2 were identified and recorded for each breath with a computer and dedicated software (Chamber v1.00, University Laboratory of Physiology, Oxford, UK). Baseline measurements of mean peak blood velocity (Vp) were recorded simultaneously.

The experimental protocol began with an 8-min period during which the subject breathed normally through a mouthpiece with the nose occluded by a nose clip. Respiratory volumes and flow information were obtained with a pneumotachograph and differential pressure transducer (RSS100-HR, Hans Rudolf, Kansas City, MO). Respiratory flow direction and timing information were measured with a turbine volume transducer (VMM-400, Interface Associates, Laguna Niguel, CA). Accurate control of the end-tidal gases was achieved using the technique of dynamic end-tidal forcing (BreatheM v3.07, University Laboratory of Physiology, Oxford, UK). A controlling computer generated the inspired partial pressure of O2 and CO2 predicted to give the desired end-tidal partial pressures by using a fast gas-mixing system (10, 35). The controlling computer received feed-
back of the measured end-tidal partial pressures on a breath-by-breath basis as the experiment progressed. These measured end-tidal values were compared with the desired values, and the computer then adjusted the initial predicted inspired gas mixture by using an integral proportional feedback algorithm based on the deviations of the measured end-tidal values from the desired end-tidal values (35).

The hypoxic stimulus was varied by holding the PET\textsubscript{O}2 at seven different predetermined levels over the range of 88–45 Torr while the PET\textsubscript{CO}2 was held constant at eucapnia (1.5 Torr above the subject’s initial air-breathing value before the start of the overnight hypoxic exposures). These levels were calculated to provide equal steps in O\textsubscript{2} saturation of the arterial blood, by using the relationship described by Severinghaus (42). An 8-min lead-in period consisted of eucapnic euoxia (PET\textsubscript{O}2 = 88 Torr) followed by six descending steps (PET\textsubscript{O}2 = 75.2, 64.0, 57.0, 52.0, 48.2, and 45.0 Torr), each step lasting 90 s. Immediately after the last step, PET\textsubscript{O}2 was elevated to 300 Torr for 5 min while PET\textsubscript{CO}2 remained at eucapnia. Then, PET\textsubscript{CO}2 was raised an additional 7.5 Torr for 5 min while PET\textsubscript{O}2 remained constant at 300 Torr. The final 10 min of this protocol served to establish the changes in the cerebrovascular responses to hypercapnia.

Measurement of CBF. Throughout the experimental protocol, near-infrared spectroscopy was used to monitor cerebral oxygenation (Sr\textsubscript{O}2) in the brain (INVOS 4100, TYCO Health Care Group Canada, Pointe-Claire, QC, Canada). A light-emitting diode (SomaSensor, Tyco Health Care Group Canada) was carefully placed over the right front-temporal region of the forehead, just above the eyebrow and left of the midsagittal sulcus. The SomaSensor alternately generates two wavelengths of light (730 and 805 nm), which detect oxygenated and deoxygenated states of hemoglobin to estimate an index of O\textsubscript{2} saturation based on internal microprocessing algorithms (18). Analog signals of Sr\textsubscript{O}2 were obtained every 2 s throughout the protocol and stored for later analysis.

Measurement of cerebral blood flow. Backscattered Doppler signals from the right middle cerebral artery were measured continuously during the protocol by use of a 2-MHz pulsed Doppler ultrasound system (TC22, SciMed, Bristol, UK). The middle cerebral artery was identified by an ionization pathway through the right temporal window just above the zygomatic arch by using search techniques described previously (29). The Doppler probe was secured with a headband device (Müller and Moll Fixation, Nicolet Instruments, Madison, WI) to maintain optimal ionization position and angle throughout the protocol. In this study, the peak blood velocity was acquired every 10 ms and averaged over each heart beat (V\textsubscript{p}), and this was used as the primary index of CBF (29).

Sensitivity of CBF and Sr\textsubscript{O}2 responses to acute variations of PET\textsubscript{O}2 and PET\textsubscript{CO}2. The acute hypoxic CBF response was determined by linear regression between the V\textsubscript{p} and Sr\textsubscript{O}2 (100 – Sa\textsubscript{O}2) during the final 20 s of each incremental step of hypoxia. Similarly, the acute hypercapnic CBF response was determined by linear regression between the V\textsubscript{p} and PET\textsubscript{CO}2 during the final minute of the hypoxic-eucapnic and hyperoxic-hypercapnic steps.

Statistical Analyses

The overnight time series parameters (Sa\textsubscript{O}2, PET\textsubscript{CO}2, and blood measurements) and the sensitivity of the CBF measurements before and after the nocturnal hypoxic exposures were analyzed by repeated-measures ANOVA. The coefficient of determination (R\textsuperscript{2}) was used to identify the proportion of variance between Sr\textsubscript{O}2 and Sa\textsubscript{O}2 in response to the incremental steps of hypoxia. Post hoc tests (Bonferroni) were performed to isolate any significant differences. AMS symptom scores were analyzed by using the nonparametric Friedman’s test followed by the Dunn’s multiple-comparison test. Statistical significance was set at P < 0.05 for all statistical tests.

RESULTS

General

All subjects completed the five consecutive overnight exposures to hypoxia. The hypoxic environment was well maintained for all subjects throughout the intervention (Fi\textsubscript{O}2 = 13.83 ± 0.06% ; inspired O\textsubscript{2} fraction = 0.0053 ± 0.0038%). The measurements of the sensitivities of CBF to acute variations in hypoxia and CO\textsubscript{2} were obtained by testing the subjects within a short period (between 1 and 2 h) after removal from the hypoxic environment after the fifth night. The majority of subjects reported mild to moderate discomfort during the first hypoxic exposure (mild headache and difficulty sleeping); however, these symptoms were ameliorated over the subsequent four nights of hypoxia. One subject developed a common cold, which prevented the completion of the acute isocapnic hypoxic protocol immediately after the overnight hypoxic exposures, and was withdrawn from the study; therefore, data from this subject were removed from the final analysis (nocturnal hypoxic intervention, n = 12; acute hypoxia and hypercapnia measurements, n = 11).

Physiological Responses to the Nocturnal Hypoxic Intervention

The PET\textsubscript{CO}2 fell progressively during each night and rose gradually during the subsequent 16 h of normoxia. The hourly changes in PET\textsubscript{CO}2 during the control night and each of the overnight hypoxic episodes are illustrated in Fig. 1. As shown, there was little change in PET\textsubscript{CO}2 during the initial part of the exposure, and this is likely related to a progressive induction of the hypoxic stimulus and to changes in PET\textsubscript{CO}2 associated with the onset of sleep.

Mean Sa\textsubscript{O}2 values for all subjects during all five hypoxic exposures were significantly lower (P < 0.001) than Sa\textsubscript{O}2 measurements recorded during the control sleep (Fig. 2, top). Mean Sa\textsubscript{O}2 on the fifth hypoxic exposure was significantly higher compared with the first hypoxic night (P < 0.001) and second and third hypoxic exposures (P < 0.05).

The mean PET\textsubscript{CO}2 values for all subjects (Fig. 2, bottom) during each of the overnight hypoxic exposures were significantly lower than control sleep values (P < 0.001). Furthermore, the overnight PET\textsubscript{CO}2 group mean from the first hypoxic night was significantly lower than hypoxic nights 1, 2, and 3 (P < 0.001). The intervention resulted in a significant reduction in baseline air breathing PET\textsubscript{CO}2 immediately after the intervention (R1), with a return toward control values at 5 days after intervention (R5) (Table 2).

Mean values for the selected capillary blood samples, which were analyzed immediately before (evening) and at the conclusion (morning) of both the control sleep and all overnight hypoxic exposures, are presented in Fig. 3. At the conclusion of 8 h of overnight hypoxia (morning measurements), the capillary partial pressure of O\textsubscript{2} (Pc\textsubscript{O}2) values for exposures 1–5 were significantly lower than morning Pc\textsubscript{O}2, after the control sleep (P < 0.001). Similarly, morning capillary partial pressure of CO\textsubscript{2} (Pc\textsubscript{CO}2) measurements at the conclusion of exposures 1–5 were significantly lower than the morning after the control sleep Pc\textsubscript{CO}2 (P < 0.001). Figure 3 illustrates a decline in Pc\textsubscript{CO}2 values that persisted between the intermittent hypoxic exposures, with a significant difference observed between evening
values (normoxia) at the commencement of the control sleep and before exposure 4 \((P < 0.01)\). After 5 days of recovery, \(P_c \text{ CO}_2\) blood-gas measurements had returned to near control values. The hypoxic intervention elicited responses for hemoglobin and hematocrit, illustrated by increasing trends for both evening and morning values (Fig. 3). Significant differences were revealed for morning hemoglobin and hematocrit on exposures 3 and 5 when compared with the control sleep \((P < 0.05)\). After 5 days of recovery, hemoglobin and hematocrit values had returned to control values.

**Symptomatic Responses to the Nocturnal Hypoxic Intervention**

The Lake Louise Acute Mountain Sickness Scoring System revealed a significant increase in symptomatology during the first and second nights of hypoxic exposure \((P < 0.001\) and \(P < 0.01\), respectively) compared with results from the control sleep (Fig. 4). A trend of decreasing symptom severity was observed over the five consecutive nights of hypoxia, and the AMS scores on the first night were significantly higher than those on the third, fourth, and fifth nights \((P < 0.05, P < 0.01,\) and \(P < 0.001\), respectively).

**Baseline Measurements of \(P_{ET CO_2}\) and \(V_p\)**

Baseline measurements of \(P_{ET CO_2}\) and \(V_p\) during air breathing were made twice before the overnight hypoxic exposures (i.e., C1 and C5), immediately after (R1), and 5 days after (R5) the intervention. At R1, \(P_{ET CO_2}\) values were 9.6% lower than control values \((P > 0.001,\) Table 2). At R5, \(P_{ET CO_2}\) values were 4.2% lower \((P > 0.05)\) than control values, suggesting that the recovery from the hypoxic intervention was not complete. Turning to \(V_p\), there were no significant changes in \(V_p\) between control values, R1, and R5 (Fig. 5).

**Table 2. Air breathing \(P_{ET CO_2}\) and \(P_{ET O_2}\) on control days (average of C1 and C5), R1, and R5**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean of C1 and C5</th>
<th>R1</th>
<th>R5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P_{ET CO_2}) Torr</td>
<td>(P_{ET CO_2}) Torr</td>
<td>(P_{ET CO_2}) Torr</td>
<td>(P_{ET CO_2}) Torr</td>
</tr>
<tr>
<td>1</td>
<td>39.2</td>
<td>34.3</td>
<td>37.0</td>
</tr>
<tr>
<td>2</td>
<td>36.5</td>
<td>30.2</td>
<td>32.7</td>
</tr>
<tr>
<td>3</td>
<td>36.0</td>
<td>33.8</td>
<td>36.0</td>
</tr>
<tr>
<td>4</td>
<td>35.6</td>
<td>33.2</td>
<td>33.3</td>
</tr>
<tr>
<td>5</td>
<td>36.8</td>
<td>32.0</td>
<td>38.2</td>
</tr>
<tr>
<td>6</td>
<td>37.0</td>
<td>33.1</td>
<td>35.0</td>
</tr>
<tr>
<td>7</td>
<td>36.8</td>
<td>33.7</td>
<td>36.0</td>
</tr>
<tr>
<td>8</td>
<td>36.1</td>
<td>32.8</td>
<td>35.0</td>
</tr>
<tr>
<td>9</td>
<td>36.6</td>
<td>33.8</td>
<td>35.6</td>
</tr>
<tr>
<td>10</td>
<td>35.7</td>
<td>31.0</td>
<td>32.0</td>
</tr>
<tr>
<td>11</td>
<td>36.4</td>
<td>32.1</td>
<td>35.0</td>
</tr>
<tr>
<td>Mean</td>
<td>36.6</td>
<td>33.1</td>
<td>35.1</td>
</tr>
<tr>
<td>SD</td>
<td>1.0</td>
<td>2.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

\(P_{ET CO_2}\), end-tidal \(P_{ET CO_2}\). Significant difference from control: \(*P < 0.05, \dagger P < 0.01, \ddagger P < 0.001.\)
Sensitivity of CBF to Acute Variations in Hypoxia

An example of averaged data for P_{ET}O_2 and P_{ET}CO_2, measured breath-by-breath, and V_p, measured beat by beat, during the determination of the CBF response to acute incremental steps of isocapnic hypoxia is presented in Fig. 6. This figure illustrates that the timing and variation of P_{ET}O_2 were well maintained and that the levels of P_{ET}CO_2 were well controlled at the predetermined levels. The degree of the CBF response to...

Fig. 3. Hematological variability during each of the overnight sessions. CS, control sleep (normoxia); E1–E5, hypoxic exposures (F_{IO}_2 = 13.8%); R5, recovery after 5 days postintervention; P_{CO}_2, capillary partial pressure of O_2; P_{CO}_2, capillary partial pressure of CO_2. Closed symbols, evening measurements before subjects entered the chamber; open symbols, morning measurements before subjects exited the chamber. Significant difference between evening values against evening control sleep or significant difference between morning values against morning control sleep: *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 4. Changes in acute mountain sickness (AMS) scores throughout the nocturnal hypoxia intervention. AMS scores were assessed as per the consensus on the Lake Louise Acute Mountain Sickness Scoring System (see text for details). CS, control sleep (normoxia); E1–E5, hypoxic exposures (F_{IO}_2 = 13.8%). Values are means ± SD. Significantly different from control sleep or significantly different from exposure 1 (E1): *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 5. Baseline measurements of mean peak blood velocity (V_p) along with the corresponding values for P_{ET}CO_2. Measurements during quiet air breathing: ○, measurements when P_{ET}CO_2 was regulated 1.5 Torr above prehypoxic exposure resting level and P_{ET}O_2 was regulated at 88.0 Torr. C, average of the control measurements (C1 and C5); R1 and R5, immediately after and 5 days posthypoxic intervention, respectively. Values are means ± SD. Significantly different from C, same condition (*P < 0.05, ***P < 0.001).
the incremental steps of isocapnic hypoxia was determined by the linear regression between mean values of \( V^\rho_p \) and \( 100 - \text{SaO}_2 \). Figure 7 illustrates the individual CBF responses to the incremental steps of isocapnic hypoxia for control, R1, and R5 with the mean response overlain. Figure 8 (top) illustrates that the CBF sensitivity to acute variations in hypoxia significantly increased \((P < 0.01)\) from the mean control value \((0.35 \pm 0.16 \text{ cm}^3\text{s}^{-1}\%^{-1})\) to \(0.77 \pm 0.51 \text{ cm}^3\text{s}^{-1}\%^{-1}\) immediately after five consecutive nocturnal hypoxic exposures. After 5 days of recovery, the sensitivity of CBF to acute variations in hypoxia returned to near control values.

Sensitivity of CBF to Acute Variations in CO₂

Figure 6 illustrates the CBF response to hypercapnia in one subject. The magnitude of the sensitivity of CBF to an acute variation in CO₂ was assessed by linear regression between

---

The diagrams and data from figures 6 and 7 are included as visual aids to illustrate the changes in CBF responses to hypoxia and hypercapnia.
mean values of $V_p$ and $P_{ETCO_2}$ measured during the final minute of eucapnic hypoxia and hyperoxic hypercapnia. The individual and mean values for the $P_{ETCO_2}$ intercept of the $V_p$ responses to acute hypercapnia were calculated, revealing no significant differences in the intercepts between the preexposure and postexposure responses ($15.0 \pm 5.1$, $19.4 \pm 4.5$, $15.2 \pm 4.7$ Torr for mean of $C1$ and $C5$, $R1$, and $R5$, respectively). A significant increase ($P < 0.05$) in CBF sensitivity was observed immediately after the hypoxic intervention ($3.04 \pm 0.74 \text{ cm}^3 \text{s}^{-1} \text{Torr}^{-1}$) compared with mean control measurements ($2.23 \pm 0.64 \text{ cm}^3 \text{s}^{-1} \text{Torr}^{-1}$). CBF responses to $CO_2$ subsequently returned to control levels after 5 days of normoxia ($2.40 \pm 0.81 \text{ cm}^3 \text{s}^{-1} \text{Torr}^{-1}$). The overall results are presented in Fig. 8 (bottom). Baseline $V_p$ was determined by averaging CBF measurements over the final minute of the lead-in period before the commencement of the incremental step hypoxic and hypercapnic protocol to establish whether $V_p$ was altered when the end-tidal gases were regulated at constant levels ($P_{ETO_2} = 88$ Torr; $P_{ETCO_2} = 1.5$ above the resting values before the hypoxic exposures). Immediately after the five consecutive overnight hypoxic exposures, there was a trend for baseline $V_p$ to be higher than the preexposure values, but this was not significant (Fig. 5).

**SrO_2 Responses to Acute Variations in Hypoxia and CO_2**

The $Sr\ O_2$ responses to acute variations in hypoxia and CO$_2$ in one subject are illustrated in Fig. 6. The individual and group means for the correlation between $Sr\ O_2$ and $Sa\ O_2$ to the incremental steps of isocapnic hypoxia are presented in Table 3, illustrating remarkable consistency ($R^2 = 0.94 \pm 0.04$ for control, $R1$, and $R5$).

### DISCUSSION

#### Major Findings

To our knowledge, this study is the first to report on the changes in the cerebrovascular responses to acute exposures of isocapnic hypoxia and hyperoxic hypercapnia after a discontinuous intervention of nocturnal hypoxia. The major findings from this study are that 1) there is a significant increase in the CBF sensitivity to acute variations in isocapnic hypoxia after five consecutive overnight exposures of normobaric hypoxia (simulated altitude ~4,300 m); 2) the CBF sensitivity to hyperoxic hypercapnia significantly increases after nocturnal hypoxia; 3) $Sr\ O_2$ was highly correlated with $Sa\ O_2$ in response to acute variations in hypoxia; and 4) the discontinuous hypoxic intervention elicited physiological and symptomatic responses that are similar to those observed during chronic altitude exposure.

This study differs from former studies that investigated the effects of high altitude or hypoxia on CBF in that previous studies have involved continuous periods of hypoxia. This study is fundamentally different in that it involved discontinuous periods of hypoxia. As such, it might be difficult to interpret the relationship of this study to former studies. However, the novel finding of this study is that the sensitivity of CBF to acute isocapnic hypoxia is increased after exposure to recurrent episodes of hypoxia (8 h per night for five nights), each night separated by 16 h of normoxia. These findings are consistent with other studies of continuous hypoxia (15, 28) and support the notion that periods of intermittent hypoxia may elicit cumulative effects, a topic reviewed in detail elsewhere (25, 30).

#### Methodological Considerations

**CBF measurements.** In our study, transcranial Doppler ultrasound was used to measure the velocity associated with the
maximum frequency of the Doppler shift as the primary index of CBF. Studies using levels of hypoxia comparable to ours addressed the issue of the relative changes in the cross-sectional area of the middle cerebral artery by using the transcranial Doppler power signal (28, 29). Those studies reported no significant changes in Doppler power, suggesting that the cross-sectional area of the middle cerebral artery was unchanged. In our study, the Doppler power was analyzed and compared over time (C1, C5, R1, R5) and the Doppler power remained unchanged, suggesting that there was little, if any, change in the cross-sectional area of the middle cerebral artery. Thus, under these conditions, we believe that velocity is an accurate estimate of CBF.

**SrO₂ measurements.** The potential of noninvasive assessments of cerebral oxygenation using near-infrared spectroscopy was first described in 1991 as a new monitoring index of O₂ content in the brain (23). More recently, methodological and comparative studies have validated the accuracy of cerebral oximetry (18, 44). A stepwise hypoxic protocol, similar to the one used in the present investigation, was implemented by Shah and colleagues (44), who compared the performance of the INVOS 4100 cerebral oximeter against direct jugular vein blood-gas measurements in humans. Using hypoxic steps of SaO₂ at 95, 90, 85, and 75%, Shah et al. reported the overall method comparison analysis (2) to have a bias (mean value between the differences of the two methods) of −3.1% and precision (standard deviation of the differences) of 12.1%, and they concluded that SrO₂ is a reliable indicator of changes in brain oxygenation induced by hypoxemia.

**Baseline measurements of PETCO₂.** After the five nights of hypoxic exposure, there was a persistent hyperventilation that lasted for several days, as shown by the reduction in the baseline resting air breathing postexposure PETCO₂ at R1 and R5 (Table 2 and Fig. 5). In studies of altitude acclimatization, this persistent hyperventilation after descent from altitude has generally been thought to be due to the reduction of cerebral spinal fluid and brain extracellular fluid bicarbonate, a compensatory metabolic acidosis occurring during the periods of cerebral spinal fluid alkalosis associated with hypocapnic hypoxia (6). However, there is also evidence showing that hypoxia per se, not the alkalosis normally accompanying hypoxia, may underlie this slow return of ventilation back to baseline values in the early phases of acclimatization (47). In the present investigation, a process similar to that of altitude deacclimatization may be unfolding after removal from the hypoxic sleeping environment.

**Dynamic cerebrovascular measurements in response to acute variations in O₂ and CO₂.** The determination of a change in the sensitivity (slope) of cerebrovascular responses after discontinuous or chronic hypoxia requires a protocol that has high reproducibility within control conditions over time. Previous observations from our laboratory, using the same protocol as in the present investigation, revealed small coefficients of variation between cerebrovascular measurements in response to acute variations in O₂ and CO₂ (10.4 and 2.9%, respectively), whereas no significant differences were observed between control days (19).

In the acute postsleep testing, the PETCO₂ and PETO₂ were regulated at the preexposure control values, but testing under these conditions did not result in a significant increase in Vp (i.e., CBF) between the control values and those at R1 (Fig. 5). These results are comparable to those of others (28), who reported no change in Vp when the end-tidal gases were regulated (PETO₂ = 100 Torr and PETCO₂ = 1−2 Torr above the initial control value) despite an increase in the sensitivity of Vp to variations in acute isocapnic hypoxia after 48 h of mild continuous isocapnic and poikilocapnic hypoxia, suggesting that the increased sensitivity may be due to hypoxia per se. This study also reports an increase in the sensitivity of Vp to acute isocapnic hypoxia after 5 nights of hypoxia. A potential limitation of the present study is that acute postsleep measurements of hypoxic and hypercapnic sensitivities were not made with the PETCO₂ and PETO₂ regulated at the postexposure air-breathing values. Such measurements might provide further insights to help us better understand the mechanism(s) associated with changes in CBF sensitivity after acclimatization to hypoxia. Additionally, this study does not provide direct evidence in support of any particular mechanism(s) of regulation. To our knowledge, the precise mechanism(s) by which CBF sensitivity is increased after continuous or discontinuous hypoxic exposures remains uncertain.

**Physiological and Symptomatic Responses to the Nocturnal Hypoxic Exposures**

In general, the collective physiological and symptomatic responses observed throughout the nocturnal hypoxic exposures are similar to those observed during chronic high-altitude exposures (7, 30, 41). Numerous studies have investigated the impact of discontinuous or intermittent hypoxia on both the ventilatory response to acute hypoxia (7, 16, 36) and the effect on athletic performance (22, 48), whereas there are no previous reports describing the cerebrovascular responses after discontinuous or intermittent hypoxia. Comparisons between the studies mentioned above are somewhat complicated due to the variability of the chosen levels of hypoxia and the duration of the intermittent hypoxic exposures. However, one recent hypoxic intervention utilized by an Australian group (48) consisted of 20 consecutive overnight (8−10 h/night) exposures to a simulated altitude of 2,650 m, and therefore the first 5 days of their intervention are comparable with our nocturnal hypoxic intervention. Townsend et al. (48) reported that PETCO₂ was significantly lower (P < 0.05) from control values throughout the intervention whereas the sharpest decline in PETCO₂ occurred during the first three nights of hypoxic exposure. In this regard our measurements of PETCO₂ (Fig. 2, bottom) after consecutive bouts of nocturnal hypoxia are in good agreement with those reported by Townsend and colleagues.

Intermittent exposure to moderate simulated altitude (5,000 m) and extreme simulated altitude (>7,000 m) elicits an increased SaO₂ both at rest and during exercise (31, 32) as the acclimatization process unfolds. The increases in SaO₂ observed in the present investigation (Fig. 2, top) are in agreement with those reported by Ricart et al. (31) and Richalet et al. (32), and we concur with these investigators that the increases are primarily a result of ventilatory acclimatization to hypoxia based on respiratory experiments associated with the present study (1). Additionally, a left shift in the oxyhemoglobin dissociation curve owing to hyperventilation and respiratory alkalosis may have contributed to the rise in SaO₂ throughout the nocturnal hypoxic exposures (38). An alternative mecha-
nism for improved $S_aO_2$ over the course of nocturnal hypoxia may have been due to a reduction in periodic breathing during sleep, which has been observed in humans under the condition of sustained hypobaric hypoxia (21).

Turning to the hematology measurements of the present investigation, examination of Fig. 3, top, clearly demonstrates the discontinuous nature of the nocturnal hypoxia episodes through the fluctuation between evening and morning measurements of capillary partial pressure of O$_2$. Also to note in Fig. 3 is the reduction of $PcO_2$ in the capillary blood over the course of the intervention, which mirrors changes in $P_{ET}CO_2$ illustrated in Fig. 2, bottom. Increases in hematocrit and hemoglobin (Fig. 3) are likely a result of hemoconcentration due to hypoxia-induced diuresis, which has been reported to occur during the early stages of acclimatization to altitude (46).

That AMS may develop in humans exposed to simulated altitude (24, 39) in the natural environment when the altitude gained and rate of ascent are rapid (3, 8, 45) has been well documented. However, in most cases, the symptoms of AMS (headache, nausea, fatigue, and dizziness) are generally self-limiting and are often ameliorated over 1–2 days if the hypoxic stress is not increased (9). In the present study, we observed similar time-course changes in AMS symptomatology after discontinuous nocturnal hypoxia as those reported for both simulated altitude (24, 39) and chronic altitude exposure in the natural setting (3, 8, 45).

Cerebrovascular Responses to Acute Variations of Hypoxia and CO$_2$

Oxygen delivery to the brain is dependent on both O$_2$ content in the cerebral arteries and CBF. In response to hypoxia, the cerebral circulation also depends on the balance between hypoxemia-induced vasodilatation and hypocapnia-induced vasoconstriction. Thus the ventilatory response to hypoxia suggests that CBF should increase and subsequently decrease over days or weeks of chronic hypoxia. Severinghaus and colleagues (43) reported the first description of the CBF response during acclimatization to the hypoxia of high altitude. Over the time course of 5 days at an altitude of 3,810 m, CBF measurements revealed an abrupt increase (24%) during the initial hours of exposure, followed by a gradual decline that remained 13% above sea level control values on the fifth day. The time domain of this biphasic CBF response to chronic hypoxia in the natural altitude setting has been subsequently supported by others (4, 15, 26).

The sensitivity of CBF to acute variations in hypoxia and hypocapnia increases after 5 days of continuous residence at an altitude of 3,810 m (15). Poulin et al. (28) reported similar increases in sensitivities of CBF after 48 h of continuous isocapnic and poikilocapnic hypoxia in a purpose-built chamber. The group from Oxford (28) described a rise of 103% in the CBF sensitivity to acute variations in hypoxia and a rise of 19% in the sensitivity of CBF to an acute variation in CO$_2$. The major new finding from the present investigation is that discontinuous nocturnal hypoxia elicits similar increases in the sensitivities of CBF to acute variations in hypoxia and CO$_2$ as has been reported in chronic hypoxia studies. Our data support the observations of Jensen et al. (15) and Poulin et al. in that we found an increase of 116% in the sensitivity of CBF to acute variations in hypoxia, whereas an increase in 33% was observed in the sensitivity of CBF to acute variations in CO$_2$. Despite the increase in slope of the CBF sensitivity to CO$_2$, there were no significant differences in the intercepts between the preexposure (i.e., mean of C1 and C5) and postexposure responses. To our knowledge, there is only one previous study that has published data on the $P_{ET}CO_2$ intercept of the V$_P$ responses to acute hypocapnia (27). Our control data are comparable to those reported by Pandit and colleagues (27).

The $SrO_2$ responses to acute variations in hypoxia did not change after our nocturnal hypoxic protocol. However, individual $SrO_2$ values during the dynamic measurements (Fig. 6) are representative of those reported in clinical settings (18) and in the natural altitude setting (13) for equivalent levels of $P_{ET}O_2$ and $P_{ET}CO_2$. The observation that there were no changes in the $SrO_2$ responses and recognition that $SrO_2$ follows changes in systemic O$_2$ saturation (18) suggest that the technique of end-tidal forcing employed for our dynamic measurements was well controlled and that the desired levels of $P_{ET}O_2$ and $P_{ET}CO_2$ were replicated over the different experimental sessions.

In summary, cerebrovascular responses to acute hypoxia and hypocapnia have been measured before and after five consecutive nocturnal hypoxic exposures. Our results indicate that discontinuous hypoxia elicits increases in CBF sensitivities to O$_2$ and CO$_2$, which are similar to those observed during chronic exposures to hypoxia. Although the physiological measurements and symptomatic observations we have reported during the nocturnal hypoxic episodes are similar to the level and time course observed during chronic hypoxia, the time domain for which changes in CBF occur after discontinuous hypoxia remains to be investigated.

ACKNOWLEDGMENTS

We thank Chantel Debert for assistance with blood sampling and assays and Dr. David Smith, Dr. Stephen Norris, and the technicians from the Human Performance Laboratory (University of Calgary) for assistance in the design and implementation of this study. We extend our gratitude to Professor P. A. Robbins for assistance in setting up the dynamic end-tidal forcing technique in Calgary.

GRANTS

This project was supported by the Alberta Heritage Foundation for Medical Research (AHFMR); the Heart and Stroke Foundation of Alberta, NWT, and Nunavut; the Canadian Institutes of Health Research (CIHR); the Olympic Oval Endowment Fund; and the Calgary Olympic Development Association. P. N. Ainslie was supported by a Focus-on-Stroke postdoctoral fellowship (Heart and Stroke Foundation of Canada, the Canadian Stroke Network, CIHR, and AstraZeneca Canada), and K. Ide was supported by an AHFMR postdoctoral fellowship. M. J. Poulin is a CIHR New Investigator and AHFMR Medical Scholar.

REFERENCES


