Hypercapnic cerebral vascular reactivity is decreased, in humans, during sleep compared with wakefulness

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THE TRANSITION FROM WAKEFULNESS to stage III–IV non-rapid eye movement (NREM) sleep is associated with a reduction in cerebral metabolism (19). Cortical blood flow is closely coupled to cerebral metabolism (12) and the sleep-related fall in metabolism would be expected to be accompanied by a similar reduction in cortical blood flow. In humans, some studies have reported no change or even an increase in cortical blood flow during stage III–IV NREM sleep (20, 27, 32); however, the majority of studies, using a range of methodologies, report a reduction. Studies using inhalation (29, 35) and injection (18) of radiolabeled Xe showed reductions in cortical blood flow of between 6 and 28% in stage III–IV NREM sleep compared with the awake state. Droste et al. (7), Hajak et al. (11), and Kuboyama et al. (17) also reported similar reductions in mean flow velocities determined by using transcranial Doppler (TCD) ultrasonography of the middle cerebral artery (MCA).

CO2 is a potent cerebral vasodilator and, in humans during wakefulness, cortical blood flow is very sensitive to changes in arterial PCO2. With sleep, ventilation is reduced and arterial PCO2 is increased, (e.g., 4). The reported reduction in cortical blood flow during stage III–IV NREM sleep therefore occurs despite a relative state of hypercapnia. This reduction would not be predicted from the wakefulness effects of CO2 and could, at least in part, be explained by a reduction in the cerebral vascular reactivity to CO2 during sleep.

The present study aimed to test the hypothesis that, in normal human subjects, hypercapnic cerebral vascular reactivity is decreased during stage III–IV NREM sleep compared with wakefulness.

METHODS

This study was carried out with local ethical approval, and all subjects gave written and informed consent. Initial screening excluded subjects who reported to be light sleepers or snorers. None of the subjects reported a history of cardiopulmonary disease and all had normal lung function determined by forced spirometry. Twenty-five normal healthy non-snorers and non-smokers were tested. Sufficient data were collected from 12 subjects (means ± SE: age 23 ± 5, body mass index 23.9 ± 2.5; 9 men). A full data set was not collected in 13 subjects who slept for an insufficient time.

Protocol. The cortical blood flow responses to increasing arterial PCO2 were assessed in each subject during wakefulness (lying supine, eyes open, watching a video) and during the first cycle of stage III–IV NREM sleep. Each CO2 intervention was performed for 5 min. During sleep, if any intervention was associated with an arousal, it was immediately terminated. The intervention was repeated once a stable sleep state had been reestablished. Each subject was studied on only one occasion.

Determination of MCA velocity. A 2-MHz TC22 pulsed Doppler ultrasound system (Scimed) was used to measure the velocity of blood in the left middle cerebral artery (MCAV). The left, and not the right, MCAV was monitored, transcranial Doppler ultrasound; middle cerebral artery velocity; cortical blood flow

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because it was more accessible within our laboratory arrangement. Previous research reported no systematic differences in measures of MCAV measured from the left or right sides by use of similar methodology (16, 17, 23). The MCA was identified by an insonation pathway through the left temporal window, just above the zygomatic arch, by using the search techniques described by Aaslid et al. (1). Optimization of the Doppler signals from the MCA was performed by varying the sample volume depth in incremental steps and at each depth varying the angle of insonance to obtain the best quality signals from the Doppler frequency. We defined optimal velocity measurements as those in which a clearly defined Doppler waveform and envelope were present. When necessary, and to maintain clearly a defined waveform and envelope, the gain setting was carefully adjusted in a limited fashion, therefore ensuring that only optimal velocity data were recorded. A headband (Welder TCD Fixation, Nicolet EME GmbH) was used to hold the ultrasound probe, ensuring optimal insonation position and angle for the duration of the experiment. Measurements and markings of the headband position were made, allowing easy relocation of the probe if movement occurred during sleep. The mean insonation depth was 50 ± 3 mm. The signals were sampled every 10 ms by using a computerized data-acquisition system (Micro 1401, Spike 2, Cambridge Electronics Design). For each cardiac cycle, the mean value for the velocity associated with the maximum frequency of the Doppler shift was calculated. For this purpose, the cardiac cycle was defined as the interval between successive systolic peaks in the velocity signal. Periods in which artifact was present in the Doppler signal or when Doppler waveforms were not clearly outlined by the set envelope were rejected. With these criteria, very limited data were rejected.

**CO2 intervention.** The respiratory circuit consisted of a face mask (B & D Electromedical) connected to a pneumotachograph (model 3700A, Hans Rudolf; perpendicular to this was fitted a two-way valve (T-shaped nonrebreath valve, model 2600, Hans Rudolf) and a length of 33-mm corrugated plastic tubing attached to the inspiratory limb, which acted as a reservoir into which CO2 could be bled.

The arterial PCO2 was elevated above the baseline during both wake and sleep by regulating the inspired CO2 load by using a constant flow rate technique (9; H. A. K. Browne, L. Adams, A. K. Simonds, and M. J. Morrell, unpublished observations). Each subject was supplied with a range of flow rates (wake: 0, 150, 360, 600, 840, 1,080 ml/min 79% CO2–21% O2; sleep: 0, 150, 240, 360, 480, 600 ml/min); each flow rate was maintained constant for 5 min. Data analysis was performed on the last 2 min (4th and 5th min) of each 5-min period, on the assumption that a steady state had been achieved. The mean ± SE numbers of CO2 interventions successfully completed per subject during wakefulness and sleep were 5.9 ± 0.3 and 3.75 ± 0.8, respectively.

**General measurements.** The end-tidal P CO2 (PetCO2) was measured by using a rapidly responding CO2 analyzer (Applied Electrochemistry CD-3A; response time <100 ms). A dry line (PK Morgan) was fitted to the sampling tube to minimize moisture buildup. EEGs (C3A2, C4A1), electrocardiograms (F7A1, F8A1), and a submental electromyogram were recorded (Sleep Lab 1000p Jaeger) by using the International 10–20 system of electrode placement. Sleep was staged by using the approach of Rechtschaffen and Kales (26). Wakefulness measurements of MCAV (cm/s), PetCO2 (Torr), and heart rate (beats/min) were made over the last 2 min (4th and 5th) of a 5-min load of CO2 during resting wakefulness. For sleep, measurements were made over the last 2 min (4th and 5th) of the first 5-min period of uninterrupted stage III–IV NREM sleep. The results reflect the mean for the whole 2 min. Arousals were identified by a two-step process. First, during the experiment, the EEG was monitored and any intervention associated with an arousal was immediately terminated. Second, during subsequent analysis, the EEG and/or electrocardiogram from each intervention was screened to exclude interventions associated with arousals. This second screening was performed separately by two investigators blinded to the other physiological data. The cerebral vascular reactivity (cm·s⁻¹·Torr⁻¹) was determined from the slope of the relationship between PetCO2 and MCAV by using linear regression (Microsoft Excel 2000 SR-1).

**Statistical analysis.** Results are presented as the group means ± SE. Statistical comparisons between wakefulness and sleep variables were performed by using Student’s paired-sample t-tests, with the significance threshold being set at P = 0.01 (2-tail).

**RESULTS**

Effects of wakefulness and sleep on PetCO2 and MCAV. The mean baseline PetCO2 during wakefulness was 39.5 ± 0.6 Torr; during sleep this increased significantly, in 11 of the 12 subjects, by an average of 3.4 Torr to 43.0 ± 1.1 Torr (P ≤ 0.001; Fig. 1A).

The mean baseline MCAV during wakefulness was 69.2 ± 8.2 cm/s; during sleep this decreased signifi-
significantly, in 10 of the 12 subjects, by an average of 11.5% to 61.1 ± 1.1 cm/s (P < 0.001; Fig. 1B). Original traces showing the reduction in MCAV from wakefulness to sleep are shown in Fig. 2, A and C.

Effects of wakefulness and sleep on heart rate. Heart rate was slightly but not significantly lower during sleep compared with wakefulness (wake: 59.5 ± 1.9; sleep: 57 ± 3.1 beats/min, P = 0.056).

Effects of hypercapnia during wakefulness and sleep. The cerebral vascular reactivity to CO₂ was markedly less in all 12 individuals during sleep compared with wakefulness; the effects of CO₂ on MCAV are shown for one individual in Fig. 2, B and D, and for all subjects in Fig. 3.

The mean cerebral vascular reactivity during wakefulness was 2.48 ± 0.29 cm·s⁻¹·Torr⁻¹; this fell during sleep by an average of 70.1% to 0.74 ± 0.23 cm·s⁻¹·Torr⁻¹ (P < 0.001; Fig. 4).

The increase in heart rate in response to CO₂ was significantly lower during sleep than wakefulness (P < 0.003; wakefulness: 0.86 ± 0.2, sleep: 0.09 ± 0.17 beats·min⁻¹·Torr⁻¹).

DISCUSSION

The notable findings of the present study are that, first, there was a 70% reduction in cerebral vascular reactivity to CO₂ during stage III–IV NREM sleep,
Correlation coefficients (mean, range) for the slopes of the lines were 0.9 (0.51–0.99) and during sleep 0.61 (0.18–0.98).

The cerebral vascular reactivity to CO2 from wake to sleep is reduced in each individual. Open symbols, individual values; solid symbols, group mean values (± SE). Female subjects: •, ○, ◦. Correlation coefficients (mean, range) for the slopes of the lines were awake 0.9 (0.51–0.99) and during sleep 0.61 (0.18–0.98).

compared with during wakefulness. Second, the data confirm previous observations that there is a reduction in cortical blood flow during stage III–IV NREM sleep compared with wakefulness. Our reported 11.7% reduction in cortical blood flow agrees with previous observations that report decreases of between 6 and 28% compared with wakefulness (7, 11, 17, 18, 29, 35).

Validity of TCD technique. The study was performed by use of TCD ultrasonography. The basic assumption with this methodology is that relative changes in MCAV directly represent relative changes in blood flow within this artery. The validity of these relative changes depends on whether the middle cerebral artery diameter remains constant in response to altered PCO2 and/or blood pressure. Previous research has challenged this assumption (5); however, the majority of research suggests that MCAV is a reliable index of cortical blood flow (25, 31). Poulin and Robbins (25), using the Doppler signal power as an index of cross-sectional area of the MCA, concluded that the caliber of MCA did not change significantly under conditions of moderate hypercapnia. Further support comes from a recent MRI study by Serrador et al. (31), who demonstrated that MCA dimensions (measured to within 0.1 mm with MRI) are stable under a wide range of PETCO2 and induced orthostatic stress.

Hypercapnic cerebral vascular reactivity testing during sleep. In the present study, the cerebral vascular reactivity to hypercapnia during wakefulness and stage III–IV NREM sleep was measured by regulating the arterial PCO2 by use of a steady-state, constant-flow technique (9). The advantage of this technique is that it enables subjects to establish a steady state sooner compared with the inhalation of a fixed fraction of CO2 (Browne et al., unpublished observations).

Blood pressure considerations. A change in perfusion pressure could result in an alteration in cortical blood flow if a failure existed within brain cerebral autoregulation. During sleep, mean arterial blood pressure is reported to fall by 5–11 mmHg compared with wakefulness (15, 33). It is unlikely that such a slight fall in the blood pressure would influence cortical blood flow because mean arterial blood pressure can be altered over a wide range, ~50–150 mmHg, without affecting cortical perfusion (12). A recent study in lambs by Grant et al. (10) provided the first evidence that the mechanisms that underlie autoregulation of the cerebral circulation are functional during sleep. These data suggest that small changes in blood pressure during sleep will have no effect on the cortical blood flow in the present study.

CO2 is reported to evoke a stimulatory effect on blood pressure as well as cortical blood flow. The inhalation of 5–7% CO2 increases blood pressure by 7–12 mmHg (8, 13). However, cerebral vascular reactivity is reported to be independent of blood pressure, provided that the blood pressure changes occur within the normal range for autoregulation (8, 13). The influence of blood pressure was only relevant when cortical blood flow was compromised in patients with carotid artery disease (13). Although we cannot exclude the effect of blood pressure, it is unlikely that the reported 70.1% reduction in cortical vascular reactivity during sleep can be solely, or substantially, attributed to it.

Regulation of cortical blood flow and cerebral vascular reactivity to CO2 during stage III–IV NREM sleep. From the waking relationship between PCO2 and cortical blood flow, one would predict an increase in cortical blood flow with sleep due to the existence of a relative state of hypercapnia. This prediction is supported by the animal work of Santiago et al. (30), who reported that, during stage III–IV NREM sleep, cortical blood flow increased in line with the sleep-related increase in arterial PCO2. However, subsequent studies in both human and nonhuman species indicate that cortical blood flow decreases during stage III–IV NREM sleep, despite an increased PCO2 (7, 11, 17, 18, 29, 35). Qualitatively, this decrease in cortical blood flow is consistent with the reduction in cerebral metabolism that also occurs in this sleep state (19). The mechanisms underlying the sleep-related reduction in cortical blood flow are uncertain but may include a reduction in the cerebral vascular reactivity to CO2 during sleep. One previous investigation into this was performed by Parisi et al. (24) who reported a small but nonsignificant decrease in cerebral vascular reactivity to CO2 during stage III–IV NREM sleep. Our present observations indicate that, in humans, the reduction in cerebral vascular reactivity to CO2 is of greater significance. The possible mechanisms that mediate the change in cerebral vascular reactivity are considered further.

Biochemical control of cerebral vascular reactivity. Alterations in brain extracellular pH mediated by CO2 indirectly affect smooth muscle tone via a number of second-messenger systems (3). Under conditions of prolonged hypercapnia (several hours), a time-dependent reduction of cortical blood flow toward baseline values, due to a buildup of brain extracellular bicarbonate and an increase in pH, is reported (36). Whether a sleep-
related increase in brain extracellular bicarbonate and pH levels could, in part, explain the reduced sensitivity of the cerebral circulation is unknown. Previous animal studies monitoring pH during sleep do not support this theory, reporting a decrease in pH (28, 30) with the depth of sleep or no change at all (10, 37).

**Neural regulation of cerebral vascular reactivity to CO₂.** In addition to potential biochemical mechanisms, cerebral vascular reactivity to CO₂ may be mediated by centrally located neural mechanisms (22). Central catecholaminergic and cholinergic systems located within the brain stem have been shown to modulate changes in cerebral vascular reactivity to CO₂. When such systems are disrupted by the destruction of the locus ceruleus or by destruction of the ascending reticular activating system, changes in regional cerebral vascular reactivity to CO₂ are reported (2, 34). These brain stem areas are intimately involved in sleep/wake functions, and it is possible that changes in sleep state acting via these areas modulate the changes in cerebral vascular reactivity reported here.

Nitric oxide (NO) is reported to be a permissive mediator in CO₂ induced cerebral vasodilatation (14). A recent study by Zoeccoli et al. (37) has shown that vasodilatory action of NO has a major regulatory role within the cerebral circulation of sleeping lambs. It is therefore speculated that a sleep-related reduction in both endothelial and/or neuronal NO production could account for the reduced cerebral vascular reactivity to CO₂.

In conclusion, the present study reported that in normal human subjects hypercapnic cerebral vascular reactivity is decreased during stage III–IV NREM sleep compared with wakefulness. This marked fall could, in part, explain the reduction in cortical blood flow during stage III–IV NREM sleep. In susceptible individuals, a reduced cerebral vascular reactivity may contribute to the elevated incidence of strokes and transient ischemic attacks during sleep and in the early hours of the morning (21).

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**REFERENCES**


