Cerebral blood flow response to isocapnic hypoxia during slow-wave sleep and wakefulness

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1Clinical and Academic Unit of Sleep and Breathing, National Heart and Lung Institute, Imperial College, London SW3 6LY; 2MacKay Institute of Communication and Neuroscience, School of Life Sciences, Keele University, Keele ST5 5BG; 3Sleep and Ventilation Unit, Royal Brompton Hospital, London SW3 6NP, United Kingdom

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Meadows, Guy E., Denise M. O’Driscoll, Anita K. Simonds, Mary J. Morrell, and Douglas R. Corfield. Cerebral blood flow response to isocapnic hypoxia during slow-wave sleep and wakefulness. J Appl Physiol 97: 1343–1348, 2004. First published June 11, 2004; 10.1152/japplphysiol.01101.2003.—Nocturnal hypoxia is a major pathological factor associated with cardiorespiratory disease. During wakefulness, a decrease in arterial O2 tension results in a decrease in cerebral vascular tone and a consequent increase in cerebral blood flow; however, the cerebral vascular response to hypoxia during sleep is unknown. In the present study, we determined the cerebral vascular reactivity to isocapnic hypoxia during wakefulness and during stage 3/4 non-rapid eye movement (NREM) sleep. In 13 healthy individuals, left middle cerebral artery velocity (MCAV) was measured with the use of transcranial Doppler ultrasound as an index of cerebral blood flow. During wakefulness, in response to isocapnic hypoxia (arterial O2 saturation 10%), the mean (±SE) MCAV increased by 12.9 ± 2.2% (P < 0.001); during NREM sleep, isocapnic hypoxia was associated with a 7.4 ± 1.6% reduction in MCAV (P < 0.001). Mean arterial blood pressure was unaffected by isocapnic hypoxia (P > 0.05); R-R interval decreased similarly in response to hypoxia during wakefulness (−21.9 ± 10.4%; P < 0.001) and sleep (−20.5 ± 8.5%; P < 0.001). The failure of the cerebral vasculature to react to hypoxia during sleep suggests a major state-dependent vulnerability associated with the control of the cerebral circulation and may contribute to the pathophysiology of stroke and sleep apnea.

transcranial Doppler ultrasound; middle cerebral artery velocity; cortical blood flow

NOCTURNAL HYPOXIA IS A MAJOR pathological factor associated with cardiorespiratory diseases, including obstructive sleep apnea (OSA) (14) and congestive heart failure (16). Reductions in arterial O2 levels will impose stress on all organ systems; however, the brain is particularly vulnerable to the effects of hypoxia (3). Recently, OSA, a condition in which cognitive function can be substantially impaired, has been associated with pathological loss of cortical gray matter (19, 22), suggesting that the nocturnal hypoxia associated with OSA may be sufficient to damage brain tissue directly.

During any hypoxic insult, protection of the brain will depend on an adequate cerebral vascular response. Normally, perfusion of the brain is dependent on a tight coupling between its O2 supply and the metabolic demand (31). During wakefulness, a decrease in O2 supply results in a decrease in cerebral vascular tone and a consequent increase in cerebral blood flow that will mitigate the effects of the systemic hypoxia. Although the cerebral vascular response to hypoxia is not linearly related to the fall in arterial PO2, like the ventilatory response to hypoxia, it is linearly related to the fall in arterial O2 saturation (SaO2) (15).

The transition from wakefulness to stage 3/4 non-rapid eye movement (NREM) sleep is accompanied by marked alterations in the control of the cerebral vascular system. During stage 3/4 NREM sleep and despite a relative state of hypercapnia (a potent cerebral vasodilator), cerebral blood flow decreases along with cerebral metabolism (36). Our laboratory recently reported that cerebral vascular reactivity to CO2 is markedly reduced during NREM sleep (21), a reduction that would permissively allow cerebral blood flow to fall in this state. In this context, we hypothesized that the cerebral vascular response to hypoxia is similarly reduced during NREM sleep; such a reduction, in hypoxic cerebral vascular reactivity, would severely impair the brain’s ability to defend itself against a nocturnal hypoxic insult. The present study tested the hypothesis that, in normal human subjects, isocapnic hypoxic cerebral vascular reactivity is decreased during stage 3/4 NREM sleep compared with wakefulness.

METHODS

The study was carried out with local ethical approval (Royal Brompton and Harefield Hospital Ethics Committee) and with written, informed consent from subjects. None of the subjects reported history of cardiopulmonary disease, and all had normal lung function determined by forced spirometry. Snorers or light sleepers were excluded. Of 17 volunteers (all men), sufficient data were collected from 13 subjects (mean ± SD: age 22 ± 4 yr, body mass index 22 ± 2 kg/m2). There were no anthropometric differences between included and excluded subjects.

Determination of Middle Cerebral Artery Velocity

The velocity of blood in the left middle cerebral artery (MCAV) was determined with the use of pulsed Doppler ultrasonography (Scimed) applied by using existing protocols from our laboratory (21). The mean value of the velocity associated with the maximum frequency of Doppler shift was calculated for each cardiac cycle (21).

Isocapnic Hypoxia

Subjects breathed through an apparatus designed to regulate the fraction of inspired O2 and to maintain the end-tidal PCO2 (PETCO2)
within ±2 Torr of a predetermined level, independent of changes in ventilation ($V_e$) (2, 20).

In each subject, the cortical blood flow responses to four separate conditions were tested during wakefulness (lying supine, eyes open, watching a video) and during the first 90-min cycle of stage 3/4 NREM sleep: 1) eupapnic euoxia (spontaneous air breathing), 2) isocapnic euoxia (air breathing with clamped end-tidal CO$_2$), 3) isocapnic hypoxia (−5% SaO$_2$; hypoxic breathing mixture with clamped $P_{ET \text{CO}_2}$), and 4) isocapnic hypoxia (−10% SaO$_2$; hypoxic breathing mixture with clamped $P_{ET \text{CO}_2}$). To reduce the risk of arousal from sleep and to prevent any overshoot in the stimulus, the level of hypoxia was titrated gradually over a few minutes. Once the target SaO$_2$ was reached, each level of hypoxia was maintained constant for a 5-min period. To ensure a steady state, the data analysis was performed on the last 2 min of this 5-min period.

**General Measurements**

Airflow was measured by using a pneumotachograph (model 3700A, Hans Rudolph). $P_{ET \text{CO}_2}$ and end-tidal $P_{O_2}$ were determined by using rapidly responding gas analyzers (models CD-3A and S-3A, Applied Electrochemistry). Blood pressure was monitored continuously by using a Finapres blood pressure monitor (model 2300, Ohmeda); from this, mean arterial blood pressure (MABP) was derived. Cardiac intervals (R-R) were monitored by using a Lifetrak ECG monitor (HME). SaO$_2$ was monitored by using a pulse oximeter (model N-200E, Nellcor). Electroencephalograms (C3A2, C4A1), electrooculograms (F7A1, F8A1), and a submental electromyogram were recorded (Grass Telefactor) by using the international 10-20 system of electrode placement. Sleep was staged by using the approach of Rechtschaffen and Kales (26), and staging was performed with the investigators blinded to the other physiological data. Subsequently, any intervention associated with an arousal [American Sleep Disorders Association criteria (1)] was excluded from the analysis (21).

**Statistical Analysis**

Results are presented as the group means ± SE. Statistical comparisons between wakefulness and sleep baseline eupapnic euoxic variables were performed by using Student’s paired sample $t$-tests (2 tailed). A repeated-measures ANOVA was used to determine the effect of varying levels of isocapnic hypoxia during wakefulness and sleep on MCAV, $V_e$, MABP, and R-R interval (Systat version 8). Where the ANOVA identified significance, $t$-tests were used to identify any pairwise differences. The significance threshold was set at $P < 0.05$.

**RESULTS**

**Eupapnic Euoxia Baseline Variables During Wakefulness and Sleep (Table 1)**

For the group, the baseline eupapnic euoxic MCAV decreased by 4.9 ± 3.4 cm/s ($P < 0.001$) and the baseline $P_{ET \text{CO}_2}$ increased by 2.2 ± 1.0 Torr from wakefulness to sleep ($P < 0.01$). $V_e$ decreased slightly but not significantly from wake to sleep (−1.7 ± 1.4 l/min; $P > 0.05$). Baseline MABP decreased by an average of 20.5 ± 1.9 mmHg ($P < 0.001$) from wake to sleep; R-R interval did not change ($P > 0.05$). The mean baseline SaO$_2$ decreased by an average of 2.0 ± 0.2% ($P < 0.05$).

**Effects of Isocapnic Normoxia During Wakefulness and Sleep (Table 1)**

As expected, the clamping circuit slightly increased the $P_{ET \text{CO}_2}$ above the baseline eupapnic value during both wakefulness and sleep [wake increase: 1.2 Torr ($P < 0.001$); sleep increase: 0.9 Torr ($P < 0.05$)]. Baseline MCAV, $V_e$, blood pressure, R-R interval, and SaO$_2$ values were not significantly different ($P > 0.05$) during isocapnic euoxia awake or asleep.

**Effects of Isocapnic Hypoxia During Wakefulness and Sleep**

The original traces from one individual show the different effects of isocapnic hypoxia on the MCAV during sleep compared with wakefulness (Fig. 1). Isocapnic hypoxia progressively increased the mean MCAV during wakefulness: for −5% SaO$_2$ by 8.83 ± 1.7%, and for −10% SaO$_2$ by 12.9 ± 2.16% (Fig. 2A; $P < 0.001$). In contrast, during sleep, isocapnic hypoxia progressively decreased the mean MCAV: for −5% SaO$_2$ by −6.97 ± 1.6%, and for −10% SaO$_2$ by −7.42 ± 1.6% (Fig. 2A; $P < 0.001$). The percent changes in MCAV from baseline isocapnic euoxia to isocapnic hypoxia (−10% SaO$_2$) are presented for each individual in Fig. 2B. Isocapnic was maintained during hypoxia in each state (Fig. 3B). The level time course of the MCAV response over the 5 min of each intervention indicated that a steady state had been achieved (Fig. 4); furthermore, there were no statistically significant differences in MCAV between minutes 4 and 5 of each intervention.

During wakefulness, isocapnic hypoxia (−5 and −10% SaO$_2$) increased $V_e$ by 20.7 ± 4 and 26.3 ± 10% ($P < 0.001$; Fig. 3A), respectively; however, during sleep, the increase in $V_e$ was no longer significant (−5% and −10% SaO$_2$: 10 ± 11% and 10 ± 9%, $P > 0.05$; Fig. 3A). MABP was unaffected by isocapnic hypoxia (−5 and −10% SaO$_2$) during wakefulness or sleep (Fig. 3C; $P > 0.05$). However, during wakefulness and sleep, isocapnic hypoxia (−10% SaO$_2$) decreased the R-R interval from its mean baseline isocapnic euoxic value by an average of 21.9 ± 10.4% during wakefulness (Fig. 3D; $P < 0.001$) and 20.5 ± 8.54% during sleep (Fig. 3D; $P < 0.001$).

**DISCUSSION**

The most important finding of the present study was that the cortical blood flow response to hypoxia was dramatically

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**Table 1. Effect of eupapnic and isocapnic euoxia during wake and sleep on cardiorespiratory variables**

<table>
<thead>
<tr>
<th></th>
<th>MCAV, cm/s</th>
<th>$V_e$, l/min</th>
<th>$P_{ET \text{CO}_2}$, Torr</th>
<th>MABP, mmHg</th>
<th>R-R Interval, s</th>
<th>SaO$_2$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wake eupapnia</td>
<td>60.5 ± 3.7</td>
<td>8.6 ± 1.2</td>
<td>41.5 ± 0.9</td>
<td>95.8 ± 1.9</td>
<td>1.2 ± 0.04</td>
<td>99.6 ± 0.1</td>
</tr>
<tr>
<td>Wake isocapnia</td>
<td>60.7 ± 4.0</td>
<td>10.5 ± 1</td>
<td>42.7 ± 0.9</td>
<td>98.1 ± 1.8</td>
<td>1.2 ± 0.09</td>
<td>99.5 ± 0.2</td>
</tr>
<tr>
<td>Sleep eupapnia</td>
<td>55.6 ± 3.1</td>
<td>6.9 ± 0.8</td>
<td>43.7 ± 1.1</td>
<td>75.3 ± 1.9</td>
<td>1.2 ± 0.06</td>
<td>97.6 ± 0.2</td>
</tr>
<tr>
<td>Sleep isocapnia</td>
<td>55.9 ± 3.1</td>
<td>8.0 ± 0.9</td>
<td>44.6 ± 1.0</td>
<td>68.4 ± 2.7</td>
<td>1.1 ± 0.04</td>
<td>97.5 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE for 13 subjects. MCAV, middle cerebral artery velocity; $V_e$, ventilation; MABP, mean arterial blood pressure; SaO$_2$, arterial O$_2$ saturation; $P_{ET \text{CO}_2}$, end-tidal $P_{CO_2}$. 

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altered during sleep compared with wakefulness. During wakefulness, in response to isocapnic hypoxia, cortical blood flow increased; in contrast, during sleep, in response to the same degree of isocapnic hypoxia, cortical blood flow decreased. Because the brain is particularly sensitive to the effects of hypoxia, the inability of the cerebral vasculature to respond to hypoxic stress during sleep suggests a significant vulnerability of the brain in this state.

Fig. 1. Original trace for 1 subject displaying the left middle cerebral artery velocity (MCAV), arterial O2 saturation (SaO2), PCO2, respired O2 fraction (Fo2), and EEG during 4 conditions: wake isocapnic euoxia, wake isocapnic hypoxia –10% SaO2, non-rapid eye movement (NREM) sleep isocapnic euoxia, and NREM sleep isocapnic hypoxia –10% SaO2. During wake, MCAV is seen to increase with isocapnic hypoxia; with sleep this response is absent.

Fig. 2. Response of MCAV to hypoxia. A: MCAV during baseline isocapnic euoxia (0), –5% SaO2 (–5) and –10% SaO2 (isocapnic hypoxia; –10) during wakefulness (black bars) and sleep (gray bars). Values are means ± SE for 13 subjects. Δ, Change. B: percent changes (from baseline isocapnic euoxia to isocapnic hypoxia: –10% SaO2) in MCAV during wakefulness and sleep for each individual and for the group mean (symbol with SE bars).
Methodological Considerations

Validity of transcranial Doppler (TCD) technique. 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 this assumption depends on whether the middle cerebral artery diameter remains constant in response to altered PCO$_2$ and/or blood pressure. This assumption has been challenged (e.g., Ref. 4); however, the majority of research suggests that MCAV is a reliable index of cortical blood flow (24, 29). Poulin et al. (24), using the Doppler signal power as an index of cross-sectional area of the middle cerebral artery, concluded that the caliber of the middle cerebral artery did not change significantly under conditions of moderate hypercapnia. Further support comes from a recent magnetic resonance imaging study by Serrador et al. (29), who demonstrated that middle cerebral artery dimensions (measured to within 0.1 mm) are stable under a wide range of PETCO$_2$ and induced orthostatic stress. In addition, research that used a rodent model suggests that the arterioles and not the larger arteries are responsible for alterations in brain blood flow during slow-wave sleep (12). Although a differential change in the diameter of the middle cerebral artery, in response to hypoxia during sleep compared with wakefulness, may be a theoretical confound, we consider it unlikely that such an effect would nullify the differences in the MCAV responses observed here.

Isocapnic hypoxia. CO$_2$ is a potent cerebral vasodilator, and in humans, during wakefulness, cortical blood flow is very sensitive to changes in arterial PCO$_2$. The clamping circuit used in the present study ensured only a minimal variation in the PCO$_2$ occurred during all testing conditions. The marked differences in hypoxic cerebral vascular reactivity between wakefulness and sleep can, therefore, not be attributed to variations in PCO$_2$.

Arterial blood pressure. In common with others (18, 32), we report a sleep-related reduction in baseline MABP. Cortical blood flow is usually independent of variations in arterial blood pressure, over a wide range, because of autoregulatory mechanisms. Although the presence of autoregulation has not been tested in humans during sleep, it is preserved in lambs (13). Assuming that cerebral autoregulation is present during sleep, a reduction in blood pressure will be met by a reduction in cerebral vascular tone and the maintenance of cerebral blood flow (15). This action will lead to a reduction in cerebral vascular reserve. However, although reduced, the MCAV does increase in response to hypercapnia, indicating that some cerebral vascular reserve does remain during sleep (21). A loss of cerebral vascular reserve could, therefore, not explain the absence of the hypoxic cerebral vascular response during sleep.
In addition, isocapnic hypoxia had no specific effect on MABP either awake or asleep, suggesting that the magnitude of this potential confound would be minor.

Sleep state. To minimize any potential circadian or sleep-state differences that might have influenced our finding, all data were collected during the first cycle of stage 3/4 NREM sleep achieved after a "normal" bed time. In addition, this sleep state was chosen for study because it provides a stable condition in which testing can be performed. Physiologically, stage 3/4 NREM sleep is of interest, because it is the state in which cerebral blood flow is at its lowest. Furthermore, our laboratory has previously shown that hypercapnic cerebral vascular reactivity is reduced during 3/4 NREM sleep (21).

Both cardiovascular and respiratory regulation are altered during rapid eye movement sleep (30); the control of brain blood flow also appears to differ during rapid eye movement, compared with NREM, sleep (36). It is, therefore, possible that differences in cerebral vascular reactivity exist between these states. However, for the present study, to limit the level of discomfort experienced by the subjects it was decided to only test during NREM sleep.

Potential mechanisms of sleep-related changes in hypoxic reactivity. The mechanisms underlying hypoxic cerebral vasodilatation during wakefulness remain unclear. Low Po2 may directly act on smooth muscle to induce relaxation and, indirectly, may induce the release of vasodilator metabolites. Adenosine- and ATP-sensitive potassium channels have been suggested, although the relative importance of each remains unclear (10, 27). There is increasing evidence that the mechanisms of action of hypoxia may involve the production of nitric oxide (NO). During wakefulness, NO is reported to mediate hypoxic cerebral vasodilatation in humans (33). During sleep, Zoccoli et al. (35) has also shown that vasodilatory action of NO plays a major regulatory role within the cerebral circulation of lambs. In humans, circulating blood NO levels are reported to be lowest during the night and in the early hours of the morning (8). This leads us to the speculation that a sleep-related reduction in both endothelial and/or neuronal NO production would reduce the vasodilatory capabilities of the cerebral vasculature and consequently reduce the cortical blood flow response to hypoxia.

A sleep-related reduction in vasodilatory capacity alone could not explain the reduction in cortical blood flow below baseline levels that occurred with hypoxia during sleep in the present study. This hypoxia-related reduction in blood flow would suggest the presence of additional hemodynamic factors or hypoxia-related vasoconstrictors that reduce cortical blood flow when the vasodilatory response to hypoxia is minimized during sleep. Hypoxic hyperventilation reduces mean pleural pressure and thus reduces both central venous and cerebral spinal fluid pressure, thereby increasing the hemodynamic gradient. Because the hypoxic ventilatory response is greater during wakefulness than sleep (5), this would tend to produce a greater blood velocity response to hypoxia during this state. It is, therefore, possible that alterations in hypoxic ventilatory response could in part explain the reported findings; however, the changes in Vt were slight, making it unlikely to be the sole contributor. A study in goats, in which the hypoxic ventilatory response was controlled, reported no difference in the hypoxic cerebral blood flow response between wakefulness and NREM sleep (28). However, significant differences in the experimental approach (i.e., the use of carbon monoxide, to both induce the state of hypoxia and depress the hypoxic ventilatory response) make a direct comparison between this study and ours difficult.

Hypoxia constricts the pulmonary circulation, increasing the pulmonary artery pressure and hence increasing central venous pressure (6). The increase in central venous pressure will reduce the perfusion pressure gradient and therefore tend to reduce cortical blood flow (7). During wakefulness, this effect will be present but is clearly insufficient to mask the predominant vasodilatory action of hypoxia on the cerebral circulation. During sleep, with the loss of the hypoxia-related vasodilatation, the effect of the decreased perfusion gradient to reduce cerebral blood flow may become evident.

Alternatively, or in addition, the hypoxia-related reduction in cerebral perfusion may reflect a more predominant action of vasoconstrictor substances during sleep. One potential candidate, for a hypoxia-related vasoconstrictor substance, is endothelin-1. It has a potent constrictor action on the cerebral circulation (9), and, in animals, intermittent hypoxia induced by sleep apnea increases systemic endothelin-1 levels (17). However, it is not known whether hypoxia induces endothelin-1 synthesis or release in the cerebral circulation. A recent study investigating the circadian variation of endothelin-1 reports that its levels are highest during the night and in the early hours of the morning (8). Whether these circadian variations in endothelin-1 contribute to the altered hypoxic cerebral vascular reactivity during sleep is speculative.

Clinical Implications

Nocturnal hypoxia and hypercapnia are characteristics of cardiorespiratory diseases such as OSA and congestive heart failure. Our laboratory’s studies have shown that the cerebral vascular responses to both hypercapnia (21) and isocapnic hypoxia are drastically reduced or even abolished in healthy subjects during sleep. Failure of the cerebral circulation to respond to hypoxia and hypercapnia would result in hypoperfusion of the brain, leading to impaired neural function and increased risk of cerebral ischemia and stroke (25), a condition with an increased frequency during the early hours of the morning. Such sleep-related reductions in cerebral vascular reactivity may be partly responsible for the pathological loss of gray matter and cognitive dysfunction reported within OSA (19, 22) and heart failure (34). It is noteworthy that daytime hypercapnic cerebral vascular reactivity is reported to be impaired in both OSA (23) and congestive heart failure (11); these pathophysiological changes in the control of the cerebral vascular circulation might be further risk factors for these groups.

In conclusion, the present study reports that, in healthy men, the compensatory increase in cortical blood flow in response to hypoxia is reversed during stage 3/4 NREM sleep compared with wakefulness. These findings suggest a major state-dependent vulnerability associated with the control of the cerebral circulation. The sleep-related mechanisms underlying these responses remain to be elucidated; they may include direct effects of sleep on the mechanisms regulating the response of cerebral vessels to hypoxia. Alternatively, or in addition, indirect effects of sleep on the cardiorespiratory system may limit the cerebral blood flow response to hypoxia.

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