Effect of Benzodiazepine Hypnotic Triazolam on Relationship of Blood Pressure and Paco2 to Cerebral Blood Flow During Human Non-Rapid Eye Movement Sleep

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INTRODUCTION

Benzodiazepine hypnotics, which are ligands acting at the benzodiazepine site of γ-aminobutyric acid type A (GABA_A) receptor, constitute the most extensively used treatment of insomnia and anxiety in psychiatry, neurology, and medicine in general. Benzodiazepine hypnotics are known to induce changes in the architecture of sleep stages, so that stage 1, slow wave (stages 3 and 4), and rapid-eye-movement (REM) sleep decrease while stage 2 sleep increases (Stone et al. 2000; Suzuki et al. 2003). Human positron emission tomography (PET) studies have shown that, during benzodiazepine-induced non-REM sleep, neural deactivation occurs specifically in the prefrontal cortex and basal forebrain (Finelli et al. 2000; Kajimura et al. 2004; Reinsel et al. 2000; Veselis et al. 1997), basal ganglia and thalamus (Finelli et al. 2000; Gillin et al. 1996; Veselis et al. 1997), hippocampus (Gillin et al. 1996; Reinsel et al. 2000), and amygdaloid complexes (Kajimura et al. 2004).

It is also known that, during benzodiazepine-induced sleep, both arterial blood pressure reduction and arterial partial pressure of carbon dioxide (PaCO2) elevation are enhanced compared with placebo-induced sleep (Ford et al. 1990; Schneider et al. 1996). However, it is unclear how these changes affect regional cerebral blood flow (rCBF) and the three primary modes of CBF regulations: 1) pressure autoregulation maintains CBF in spite of changed perfusion pressure; 2) CO2 vasoreactivity has the most potent vasodilatory effect in the brain; and 3) neurogenic regulation mainly consists of the sympathetic and cholinergic influence. This issue potentially has a clinical relevance and should be clarified, because many benzodiazepine hypnotics are associated with neuropsychiatric adverse effects and drug abuse (Schatzberg and Nemeroff 2004).

We hypothesized that, during benzodiazepine-induced sleep, the cerebral white matter has a specific CBF regulation relating to Paco2. This is based on the following evidence. During natural sleep, the cerebral white matter maintains constant CBF (Hiroki et al. 2005) probably due to its specific intolerance to energy deprivation (Brown et al. 2001; Stys et al. 1990) or its gradual, low-flow vulnerability (Tomimoto et al. 2003; Wakita et al. 2002). The white matter lacks a central benzodiazepine receptor (Abadie et al. 1992; Muller 1987). Benzodi-
azepines can increase CO₂ responsiveness to CBF (Forster et al. 1983). We hypothesized that such modulation compensates for altered perfusion parameters especially during stage 2 sleep. This is based on the evidence that sympathetic CBF regulation can be suppressed by benzodiazepine hypnotics (Kenney et al. 2003), while during stage 2 sleep, sympathetic nerve activity increases and arterial blood pressure oscillates in association with sleep spindles (Tank et al. 2003).

Triazolam is a short-acting benzodiazepine hypnotic and is prescribed with a standard dosage of 0.125–0.25 mg. In this study, we investigated the relationship of MAP and PaCO₂ to rCBF, with a focus on brain regions with the smallest decreases in CBF, during human benzodiazepine-induced non-REM sleep.

METHODOLOGY

Subjects and experimental procedure

Fifteen healthy, right-handed male university students served as study subjects between September 1999 and October 2000. Written informed consent, approved by the Intramural Research Board of the National Center of Neurology and Psychiatry (Kodaira, Tokyo, Japan), was obtained from each subject before participating in the study. Subjects were evaluated by complete medical and psychiatric histories and physical examinations. None had a history of sleep disorders or serious medical, neurological, or psychiatric problems, alcohol or substance abuse, or use of sleeping pills or other psychoactive medications. A randomized, double-blind, crossover study using [15O]H₂O PET was conducted, comparing triazolam with placebo. Two nights of study were separated by a 1-week interval. Patients’ wakefulness–sleep pattern was strictly monitored before each PET experiment. Since sleep deprivation, often employed in previous studies, has the potential to alter physiological sleep control (Braun et al. 1997; Loewy 1991), each subject was instructed to sleep regularly between the hours of 10:00 P.M. and 7:00 A.M. for at least 1 week before an experiment. Subjects were also prohibited from medications, drugs, or alcohol or, after 5 P.M., drinks containing caffeine. Compliance was monitored by an actigraph and an interview on the day of the experiment. If a lack of compliance with these instructions occurred, that subject was excluded from the study. PET scanning was performed during each period of wakefulness and stage 2 (characterized by the appearance of K-complex or sleep spindle) and slow wave (stages 3 and 4 sleep; characterized by a slow, high amplitude delta wave) sleep under both triazolam and placebo conditions. Consequently, complete data sets of PET and physiological parameters were obtained from nine subjects (age, 21.0 ± 1.0 yr [range, 20–23]; body weight, 63.8 ± 6.7 kg [range, 55–73]; body mass index, 21.3 ± 2.3 kg/m² [range, 18.9–25.9]).

On the night of the experiment, each subject lay on a PET scanner couch with electrodes attached to the head for polysomnography. The head was fixed on the end of the scanner couch with an individually-molded thermoplastic facemask secured to a plastic head holder, and angled so that the subject’s canthomeatal line was parallel to the axial planes of the PET scanner. A venous line was inserted into the right median antecubital vein for tracer injection. An arterial line was inserted into the left radial artery for blood pressure monitoring, arterial blood gas analysis, and radioactivity measurements. A flow-through radioactivity monitor (PICO COUNT, Bioscan, Washington, DC) was used to detect the radioactivity of the arterial blood by automatic sampling throughout the scanning period. The length of the catheter was 30 cm from the radial artery to the flow-through radioactivity monitor. The delay and dispersion were simultaneously corrected using the measured arterial time activity curve and the tissue time activity curve (Lammertsma et al. 1990). Arrival time of the radioactivity between the radioactivity monitor and brain was not corrected. In each PET scan, MAP was recorded immediately before tracer injection, and arterial blood for gas analysis was sampled immediately after the scan.

Electroencephalograms (EEGs) were recorded from disc electrodes placed at F3, F4, C3, C4, P3, P4, Fz, Cz, and Oz with A1 and A2 references. Monopolar electrooculograms were recorded from both canthi and bipolar electromyograms were recorded from the chin. Details of the polygraphic methodology are the same as in a previous study (Kajimura et al. 1995). EEG signals were visually scored per 30-s epoch according to the standardized sleep manual of Rechtschaffen and Kales (1968), and the sleep stage for each 90-s period during PET scanning was determined if that stage appeared in two or three epochs. Final assessment of sleep stage scoring was confirmed later with C3 recording.

PET procedure and image reconstruction

PET scanning started at approximately 9:30 P.M. After scans for wakefulness were obtained in the eye-closed condition, each subject ingested a gelatin capsule containing 0.25 mg of either triazolam or placebo at 10:00 P.M. The lights were turned out at 10:30 P.M. and three scans for each of stage 2 and slow-wave sleep were conducted between 11:00 P.M. and 2:00 A.M. The time frame for obtaining the scans was determined by taking the pharmacokinetics of triazolam into consideration; this drug reaches peak plasma concentration at about 1.3 h after oral administration, and the elimination half-life of triazolam and of its active metabolite is 2.9 and 3.9 h, respectively (Data on File, The Upjohn Company 1988). Considering the effect of the plasma concentration of triazolam on MAP and PaCO₂ or on rCBF (Grillien et al. 1996), the time period from administering triazolam to the start of the PET scan was matched between stage 2 and slow wave sleep. A maximum of eight intravenous injections of 259 MBq (7 mCi) [15O]H₂O were conducted for each subject during periods of relaxed wakefulness and stage 2, and slow-wave sleep under polygraphic monitoring on each night of triazolam and placebo experiments. The whole body exposure was totally mSv, which is the limit recommended by the International Commission on Radiological Protection (Mountford and Temperton 1992). The [15O]H₂O bolus was automatically flushed intravenously for 15 s. With a PET scanner (Siemens ECAT EXACT HR 961; Siemens Medical Systems, Erlangen, Germany) in three-dimensional mode, scanning started manually 1 s after the initial rise of head counts and continued for 90 s. A camera with an axial field of view of 150 mm, acquired data simultaneously from 47 consecutive axial planes. An image resolution of 3.8 × 3.8 × 4.7 mm was obtained after back-projection and filtering (Hanning filter, cutoff frequency 0.5 cycles per pixel), and the reconstructed image was displayed in a matrix of 128 × 128 × 47 voxel format (voxel size, 1.732 × 1.732 × 3.125 mm). A 10-min transmission scan before acquisition of the emission data corrected for tissue attenuation. Functional images of absolute rCBF were produced using arterial time activity data by the autoradiographic method (Herscovitch et al. 1983).

SPM analysis

Data were analyzed on a Sun Sparc 20 workstation (Sun Computers Japan, Tokyo, Japan) using Analyze v. 7.5.4 image display software (Biodynamic Research Unit, Mayo Foundation, Rochester, MN) and on a PC-compatible computer using statistical parametric mapping (SPM) 99-software (Wellcome Department of Cognitive Neurology, London, UK [http://www.fil.ion.ucl.ac.uk/spm, last accessed 06/12/02]) (Friston et al. 1995) implemented in MATLAB v. 5.3 (The MathWorks, Sherborn, MA) for Windows XP (Microsoft, Redmond, WA). Spatial normalization was employed to fit each individual brain to a standard brain template in three-dimensional space, in order to correct for differences in brain size and shape and to facilitate intersubject averaging. The stereotactically normalized scans con-
tained 68 planes (voxel size, $2 \times 2 \times 2$ mm). Smoothing was done with a Gaussian kernel ($10 \times 10 \times 6$ mm). SPM uses a standard brain from the Montreal Neurological Institute (MNI, Montreal, Quebec, Canada) and the precise anatomical localizations of significant changes were indicated in accordance with the atlas of Talairach and Tournoux (1988) by using a numerical transformation formula supplied by MRC Cognition and Brain Science Unit (Cambridge, UK [http://www.mrc-cbu.cam.ac.uk/Imaging/minispace.html, last accessed 06/12/02]).

Whole-brain mean CBF was obtained by the method of tissue segmentation with the smoothed, spatially normalized PET image. Details of this analysis are the same as in our previous study (Hiroki et al. 2005). In the analysis of the relative rCBF, the whole-brain mean CBF in each image was normalized to $50 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ by the proportional scaling method, which is considered to be appropriate to minimize false-positive areas with the least decreased CBF while gray matter CBF decreases. The gray matter threshold was set at 0.3 to include the white matter. After the appropriate design matrix was specified, estimates of the subject and condition were determined according to a general linear model at each and every voxel. Parameter estimates were compared using linear contrasts. By peak amplitude, the exact level of significance of volumes of difference and magnitude estimates were compared using linear contrasts. By peak amplification, the correlation was characterized respectively between conditions and covariate. Voxels that had peak T values $>3.45$ (uncorrected $P = 0.001$) were considered to show a significant difference, and a cluster threshold was not set in this analysis. In the eigenvalue analysis, a cluster threshold was set at a corrected $P$ value of 0.05.

All of the SPM analysis for relative rCBF was done using a multisubject design. Areas with a significantly different relative rCBF during triazolam-induced sleep compared with placebo-induced sleep and with a significantly decreased relative rCBF during triazolam-induced sleep were identified. Based on these results, we focused on the dorsolateral prefrontal cortex and orbital basal forebrain and compared their magnitude of change from wakefulness during triazolam-induced stage 2 and slow wave sleep. This analysis included all of the first eigenvalues of at most local maxima $>8.0$ mm apart per cluster, extracting data using a spherical voxel-of-interest with 4-mm radius. We also identified areas with the least decreased relative rCBF during the progression of triazolam-induced sleep. Furthermore, using the analysis of covariate correlation, brain regions were identified with significantly negative correlation of MAP to relative rCBF and significantly positive correlation of PaCO$_2$ to relative rCBF, through states of wakefulness and triazolam-induced sleep.

**Region of interest analysis**

Absolute rCBF was determined using region of interest (ROI) analysis. Based on the SPM results showing areas with the least decreased CBF during triazolam-induced sleep, ROI was set on the perioral cortex (the primary motor cortex and unimodal somatosensory regions of the parietal cortex), occipital cortex (the primary and secondary visual cortices in the striate and lateral occipital gyri), and cerebral white matter. Using MRicro-software (C Rorden, University of Nottingham, Nottingham, UK [http://www.psychology.nottingham.ac.uk/staff/crl/mricro.html, last accessed 12/20/02]), each ROI was manually drawn on a prior probability MNI image segmented for gray or white matter. The ROI boundary of the cerebral white matter was defined on an upper corona radiata slice, which included all of the white matter regions except for the subcortical white matter adjacent to the cerebral cortex. Among the cerebral white matter, ROI was also set at each of the bilateral frontal and temporooccipital white matter, based on the results of SPM analysis showing areas with significant correlation of PaCO$_2$ to relative rCBF. Finally, absolute value of each of ROI was automatically calculated for every image.

**Statistical analysis**

Using a one-way repeated-measure ANOVA, time period from triazolam administration to the start of the PET scan, MAP, PaCO$_2$, and absolute CBF of the whole-brain mean, the perioral cortex, occipital cortex, cerebral white matter, and frontal and temporooccipital white matter were compared among states of wakefulness and non-REM sleep, and between triazolam and placebo. Post hoc analysis was done with Bonferroni’s procedure. In the eigenvalue analysis, each side of left and right of the dorsolateral prefrontal cortex and orbital basal forebrain was compared using three-way ANOVA: sleep stages (stage 2 vs. slow wave sleep with triazolam); regions of local maxima ($n = 7 \text{ [left]}$ and 5 [right], dorsolateral prefrontal cortex; and $n = 7 \text{ [left]}$ and 6 [right], orbital basal forebrain); and subjects ($n = 9$). Using Pearson’s product-moment correlation coefficient, correlations of MAP and PaCO$_2$ to absolute CBF of the occipital cortex, cerebral white matter, and frontal and temporooccipital white matter were evaluated during each state of wakefulness and non-REM sleep. Using the $z$ test, the difference of correlation coefficients of MAP and PaCO$_2$ to absolute CBF were compared between triazolam and placebo in each of the occipital cortex and cerebral white matter and between the occipital cortex and cerebral white matter. In the frontal and temporooccipital white matter, the correlation coefficients of MAP and PaCO$_2$ to absolute CBF was compared between stage 2 or slow wave triazolam-induced sleep and wakefulness. The level of significance was set at $P < 0.05$.

**R E S U L T S**

**Subjects and time period to PET scan**

Complete datasets of both physiological parameters and PET with triazolam and placebo, respectively, numbered 21 and 20 for wakefulness, 23 and 17 for stage 2 sleep, and 19 and 17 for slow wave sleep. The latencies from triazolam and placebo administration, respectively, to the start of PET acquisition were, 108.5 ± 32.3 and 144.6 ± 88.0 min for stage 2 sleep and 107.8 ± 44.9 and 168.2 ± 66.0 min for slow wave sleep. ($F_{(1,40)} < 0.01, P = 0.954$ stage 2 vs. slow wave sleep with triazolam).
Physiological parameters and whole brain mean CBF

During stage 2 and slow wave sleep with triazolam compared with wakefulness, MAP significantly decreased ($P < 0.001$) and PaCO$_2$ significantly increased ($P < 0.001$) (Table 1). Compared to wakefulness, a significant difference was found between triazolam and placebo in MAP during stage 2 [−9.0 ± 5.9 and −4.7 ± 5.6 mmHg, respectively; $P = 0.026$] and slow wave sleep [−13.8 ± 6.8 and −7.5 ± 6.7 mmHg, respectively; $P = 0.009$] sleep and in PaCO$_2$ during stage 2 sleep [4.3 ± 2.6 and 2.8 ± 1.9 mmHg, respectively; $P = 0.040$]. Whole-brain mean CBF changed significantly with triazolam [$F(2,60) = 7.14$; $P = 0.002$ (stage 2 sleep vs. wakefulness; $P < 0.001$)], and was significantly lower during stage 2 sleep with triazolam compared with placebo [$P = 0.049$] (see Supplementary Fig. 1).$^{1}$

$^{1}$The Supplementary Material for this article (a figure and two tables) is available online at http://jn.physiology.org/cgi/content/full/00114.2005/DC1.
SPM analysis

RELATIVE rCBF DIFFERENCE BETWEEN TRIAZOLAM AND PLACEBO.

During stage 2 sleep with triazolam compared to placebo, significantly lower relative rCBF was found in the left frontal and bilateral temporal neocortical regions, as well as left orbital basal forebrain (Fig. 1A). Significantly higher relative rCBF was found in the pontomedullary region, midbrain, right hippocampus, and bilateral cerebral white matter (Fig. 1B). During slow wave sleep with triazolam compared to placebo, significantly lower relative rCBF was found in the left frontal neocortical, left hippocampus, right basal forebrain, and right amygdaloid complex (Fig. 1C). Significantly higher relative rCBF was not detected in any area (Fig. 1D).

RELATIVE rCBF DECREASE WITH TRIAZOLAM. Compared to wakefulness, significantly decreased relative rCBF was found bilaterally in the frontal, parietal, and temporal neocortical regions; orbital basal forebrain; cingulate gyrus; insular cortex; thalamus; and cerebellar hemisphere during stage 2 sleep with triazolam (Fig. 2A). Similarly, significantly decreased relative rCBF was found in almost the same areas during slow wave sleep as those during stage 2 sleep with triazolam compared to wakefulness. In the dorsolateral prefrontal cortex, the first eigenvalues did not differ during stage 2 sleep compared with slow wave sleep (left, $P = 0.077$; and right, $P = 0.344$) (Fig. 2B). In the left orbital basal forebrain, the first eigenvalues were significantly lower during stage 2 sleep compared with slow wave sleep ($-0.058 \pm 0.007$ and $-0.035 \pm 0.008$); $F_{(1,175)} = 4.97; P = 0.027$; there was no difference on the right, ($P = 0.977$). During slow wave sleep compared with stage 2 sleep, significantly decreased relative rCBF was found in the pontomedullary region and midbrain (Fig. 2C).

RELATIVE rCBF INCREASE WITH TRIAZOLAM. Compared to wakefulness, a significantly increased relative rCBF was found bilaterally in the perirhinal cortex, occipital cortex, and cerebral white matter during stage 2 sleep following triazolam (Fig. 3A). Significantly increased relative rCBF was found in almost the same areas during slow wave sleep as those during stage 2 sleep with triazolam compared to wakefulness (Fig. 3B). During slow wave sleep compared to stage 2 sleep with
triazolam, significantly increased relative rCBF was found restrictedly in the bilateral cerebral white matter (Fig. 3C).

RELATIVE RCBF CORRELATION TO MAP AND PACO2 WITH TRIAZOLAM. Through states of wakefulness and triazolam-induced sleep (D, E), and region of interest (ROI) (F). Details of A–E are the same as in Fig. 1. A: during stage 2 sleep compared to wakefulness, a significantly increased relative rCBF was found bilaterally in the perirolandic cortex, occipital cortex, and cerebral white matter. B: during slow wave sleep compared to wakefulness, a significantly increased relative rCBF was found in almost the same areas as those during stage 2 sleep compared to wakefulness. C: during slow wave sleep compared with stage 2 sleep, a significantly increased relative rCBF was found restrictedly in the bilateral cerebral white matter. D: there were no areas with a significant negative correlation of MAP to relative rCBF. E: a significant positive correlation of PaCO2 to relative rCBF was detected restrictedly in the bilateral cerebral white matter. F: ROI (Region of interest) was drawn bilaterally at the perirolandic cortex (z = 12 mm, top middle), occipital cortex (z = 24 mm, top right), and frontal and temporoooccipital white matter (z = −6 mm, bottom left and right, respectively).

Absolute CBF of the perirolandic cortex, occipital cortex, and cerebral white matter

In the perirolandic cortex (Figs. 3F, top left, and 4, top left), a significant difference of absolute CBF was detected among wakefulness–sleep states with triazolam [F\(_{2,60}\) = 5.12; \(P = 0.009\) (stage 2 sleep vs. wakefulness; Bonferroni’s procedure, \(P = 0.002\)] and during stage 2 sleep with triazolam compared to placebo [F\(_{1,38}\) = 4.79; \(P = 0.035\)]. No significant difference was found among wakefulness–sleep states with either triazolam or placebo in the occipital cortex [F\(_{2,60}\) = 2.23 and F\(_{2,51}\) = 1.17; \(P = 0.116\) and 0.320, respectively] (Figs. 3F, top middle, and 4, top right) or cerebral white matter [F\(_{2,60}\) = 2.58 and F\(_{2,51}\) = 0.08; \(P = 0.084\) and 0.924, respectively] (Figs. 3F, top right, and 4, bottom left).

Relationship of MAP and Paco2 to absolute CBF in the occipital cortex and cerebral white matter

With triazolam compared to placebo, the correlation coefficient of MAP to absolute CBF was significantly greater in both of the occipital cortex and cerebral white matter during stage 2 sleep and was significantly lower in the occipital cortex during
slow wave sleep. The coefficient was significantly greater in the cerebral white matter compared to the occipital cortex during stage 2 sleep with triazolam (Fig. 5; see Supplementary Table 1). With triazolam compared to placebo, the correlation coefficient of PaCO2 to absolute rCBF was significantly greater in the cerebral white matter during stage 2 sleep and in both the occipital and cerebral white matter during slow wave sleep. The coefficient was significantly greater in the cerebral white matter compared to the occipital cortex during stage 2 and slow wave sleep with triazolam (Fig. 6; see Supplementary Table 1).

Absolute CBF of the frontal and temporooccipital white matter

Absolute CBF of the frontal white matter was significantly lower compared to that of the temporooccipital white matter during both stage 2 and slow wave sleep with triazolam \([F(1,44) = 5.60, P = 0.022\) and \(F(1,36) = 9.80, P = 0.004\), respectively] and placebo \([F(1,32) = 4.52, P = 0.041\) and \(F(1,32) = 11.89, P = 0.002\), respectively] (Fig. 3F, bottom left and right and Fig. 4, bottom right). No significant difference was found in any other comparison.

Relationship of MAP and PaCO2 to Absolute rCBF with Triazolam

During stage 2 triazolam-induced sleep compared to wakefulness, the correlation coefficient of MAP to absolute CBF was significantly greater in both the frontal and temporooccipital white matter. The correlation coefficient of PaCO2 to absolute CBF was significantly greater in the temporooccipital white matter, but was not in the frontal white matter. During slow wave triazolam-induced sleep compared to wakefulness, the correlation coefficient of MAP to absolute CBF was significantly greater in the frontal white matter (Fig. 7; see Supplementary Table 2). The correlation coefficient of PaCO2 to absolute rCBF was significantly greater in both the frontal and temporooccipital white matter (Fig. 7; see Supplementary Table 2).

Discussion

MAP, Paco2, and Whole Brain Mean CBF

During non-REM sleep MAP decreased more after triazolam than after placebo. This may be due to an inhibitory effect on the arterial baroreflex (Sakamoto et al. 1994), sympathovagal outflow (Tulen et al. 1998), or on brain stem and hypothalamic activity (Cao and Morrison 2003; Kitajima et al. 2004). Alternatively, the observed decrease in MAP may result from a primary peripheral mechanism (Drugan 1996; Galindo et al. 2001). Our results showing that the basal forebrain is deactivated during both stage 2 and slow wave sleep with triazolam (Fig. 2, A and B) support the idea that inhibition of the hypothalamus contributes to blood pressure reduction during
triazolam-induced sleep, PaCO₂ significantly increased during stage 2 sleep relative to wakefulness. Although the principal mechanism of the PaCO₂ increase induced by benzodiazepines is considered to be the depression of the respiratory center in the medulla oblongata (Murciano et al. 1993), our results show higher relative rCBF in the lower brain stem during stage 2 sleep with triazolam compared to placebo. Therefore a peripheral mechanism such as an increase in upper airway resistance may contribute to the increase in PaCO₂ during stage 2 sleep with triazolam compared to placebo. Therefore a peripheral mechanism such as an increase in upper airway resistance may contribute to the increase in PaCO₂ during stage 2 sleep with triazolam, although our previous study showed no significant difference (Kajimura et al. 2004). This is likely due to the different methodology used for the CBF calculation. Tissue segmentation, applied in the present study, disregards noises in the extracerebral spaces and probably causes the whole brain CBF to better reflect brain activity.

The least decreased absolute CBF and its relationship to MAP and PaCO₂

Based on the coupling between neural activity and energy and between CBF and cerebral metabolism (Roland 1993), the region with the smallest decrease in rCBF represents the area with the most spared neural deactivation. In order to maintain neural activity in the face of changed physiological parameters the least deactivated brain region may need to alter its CBF regulation. The brain regions with the least decreased rCBF during triazolam-induced sleep were the occipital cortex and cerebral white matter (Fig. 4). The relative sparing of these regions may result from lack of a central benzodiazepine receptor in the cerebral white matter (Abadie et al. 1992) as well as the poor capacity of oligodendrocytes to change metabolism or tolerate energy deprivation (Roland 1993). In this respect it is notable that oligodendrocytes and myelin are rich in the occipital cortex (Fatterpekar et al. 2002).

During stage 2 triazolam-induced sleep, MAP was positively correlated to absolute CBF more strongly in the cerebral white matter than in the occipital cortex. During this period, the cerebral white matter may be at least partially spared the effect of pressure autoregulation. The cerebral cortex is subject to the effect of pressure autoregulation which is modulated by the sympathetic nervous system and ultimately controlled at the basal forebrain (Hardy and Holmes 1988; Nagai et al. 2004). Sympathetic nerve activity increases in association with stage 2 sleep K-complexes blood pressure oscillates (Tank et al. 2003). GABA_A receptor agonists suppress the sympathetic nerve activity mediated by the basal forebrain (Kenney et al. 2003). We showed that the basal forebrain is deactivated predominately during triazolam-induced stage 2 sleep. In sum, during stage 2 sleep, triazolam may affect the modulation of pressure autoregulation and induce the precarious relationship of MAP to absolute CBF of the cerebral white matter. It is

FIG. 5. Relationship of MAP to absolute rCBF. With triazolam compared to placebo, correlation coefficient of MAP to absolute rCBF was significantly greater in both the occipital cortex and cerebral white matter during stage 2 sleep and significantly lower in the occipital cortex during slow wave sleep (see Supplementary Table 1). Compared to the occipital cortex, the cerebral white matter had a significantly greater correlation coefficient during stage 2 triazolam-induced sleep (see Supplementary Table 1).
FIG. 6. Relationship of PaCO₂ to absolute CBF. With triazolam compared to placebo, correlation coefficient of PaCO₂ to absolute CBF was significantly greater in the cerebral white matter during stage 2 sleep and in both the occipital cortex and cerebral white matter during slow wave sleep (see Supplementary Table 1). Compared to the occipital cortex, the cerebral white matter had a significantly greater correlation coefficient during both stage 2 and slow wave triazolam-induced sleep (see Supplementary Table 1).

FIG. 7. Relationship of MAP and PaCO₂ to absolute CBF among the cerebral white matter with triazolam. During stage 2 triazolam-induced sleep compared to wakefulness, correlation coefficient of MAP to absolute CBF was significantly greater in both of the frontal and temporooccipital white matter. The correlation coefficient of PaCO₂ to absolute rCBF was significantly greater in the temporooccipital white matter, but was not in the frontal white matter. During slow wave triazolam-induced sleep compared to wakefulness, correlation coefficient of MAP to absolute rCBF was significantly greater in the frontal white matter (see Supplementary Table 2). The correlation coefficient of PaCO₂ to absolute rCBF was significantly greater in both the frontal and temporooccipital white matter (see Supplementary Table 2).
likely based on the deactivation of the basal forebrain specifically induced by triazolam although the underlying mechanisms remain unproved.

TABLE 2. Summary of main effects of triazolam on human non-REM sleep

<table>
<thead>
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<th>Stage 2 Sleep vs. placebo</th>
<th>vs. wakefulness</th>
<th>Slow Wave Sleep vs. placebo</th>
<th>vs. wakefulness</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP</td>
<td>DLPFC ↓, BF ↓</td>
<td>DLPFC ↓, BF ↓</td>
<td>BF ↓, amygdala ↓</td>
<td>DLPFC ↓, BF ↓</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>Brain stem ↑</td>
<td>Brain stem ↑</td>
<td>Brain stem →</td>
<td>Brain stem →</td>
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<tr>
<td>Whole-brain mean cerebral blood flow</td>
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<td>Relative rCBF</td>
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Correlation coefficient to the least decreased absolute rCBF
- MBP to occipital cortex: ↑
- PaCO₂ to occipital cortex: ↑
- MBP to cerebral white matter: ↑
- PaCO₂ to cerebral white matter: ↑
- MBP to frontal white matter: ↑
- PaCO₂ to frontal white matter: ↓

*Change following wakefulness. * More extensively decreased compared to during slow wave sleep. * Higher compared to the occipital cortex. * Higher compared to the occipital cortex (z = -2.46, P < 0.05) and compared to during slow wave sleep (z = -3.05, P < 0.005) (see Supplementary Tables 1 and 2). DLPFC, dorsolateral prefrontal cortex; BF, basal forebrain.

possible that the occipital cortex less undergoes the modulation and thus is not strongly affected by triazolam.

With triazolam compared with placebo, PaCO₂ showed a stronger positive correlation to absolute CBF in the cerebral white matter during stage 2 and slow wave sleep. This corresponds well with the evidence that midazolam increases the CBF responsiveness to CO₂ (CO₂ vasoreactivity) in humans (Forster et al. 1983). On the other hand, absolute CBF of the occipital cortex was less well correlated to PaCO₂, similarly to MAP. Accordingly, during stage 2 triazolam-induced sleep, the relationship of PaCO₂ to absolute CBF of the cerebral white matter may compensate for the precarious relationship of MAP to the absolute CBF.

During triazolam-induced stage 2 sleep the strongest positive correlation relationship between MAP and absolute CBF is found in the frontal white matter. This may be due to the inhibitory effect of triazolam on the sympathetic nervous system, which mainly affects the lateral prefrontal cortex (Hardy and Holmes 1988; Nagai et al. 2004). Notably, we found that the correlation of PaCO₂ to absolute CBF within the frontal white matter was weaker during triazolam-induced stage 2 sleep compared to wakefulness. The frontal cortex receives cholinergic innervation from the substantia innominata (Russchen et al. 1985). This cholinergic innervation regulates CBF sensitivity to CO₂ (Dauphin et al. 1991). Therefore during stage 2 triazolam-induced sleep, the predominant deactivation of the orbital basal forebrain may weaken the relationship of PaCO₂ to absolute CBF of the frontal white matter while the cortical region is deactivated.

In conclusion, we showed a region- and state-specific effects of benzodiazepine hypnotics on the relationship of MAP and PaCO₂ to absolute CBF during human non-REM sleep (summarized in Table 2). Triazolam reduced blood pressure gradually and increased PaCO₂ during stage 2 sleep, while keeping absolute CBF constant in the occipital cortex and cerebral white matter. During triazolam-induced stage 2 sleep, the cerebral white matter had a stronger positive correlation of MAP to the absolute CBF, and may compensatorily receive a modulated CBF regulation with the strengthened positive correlation of PaCO₂ to absolute CBF in the region except for the frontal white matter. These modulated CBF regulations are

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