Effect of the 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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Abstract

We sought to clarify the effect of a short-acting benzodiazepine hypnotic on the relationship of arterial blood pressure and arterial partial pressure of carbon dioxide (Paco2) to regional cerebral blood flow (rCBF) during human non-rapid-eye-movement (REM) sleep. Nine young normal volunteers were treated in a randomized, crossover design with triazolam or placebo and underwent positron emission tomography at night. During wakefulness and stage 2 and slow wave (stages 3 and 4) sleep, we measured mean arterial blood pressure (MAP), Paco2, and absolute CBF. With triazolam compared to placebo, MAP reduced gradually. During stage 2 sleep, Paco2 increased and whole-brain mean CBF decreased. During triazolam, relative rCBF of the left orbital basal forebrain decreased more during stage 2 than slow wave sleep whereas absolute CBF of the occipital cortex and cerebral white matter remained constant. Absolute CBF of the cerebral white matter and occipital cortex correlated more strongly to both MAP and Paco2 during triazolam-induced stage 2 sleep than during placebo sleep. In the frontal white matter, during triazolam-induced stage 2 sleep compared to wakefulness, absolute CBF was significantly better correlated to MAP, but not to Paco2. During triazolam-induced stage 2 sleep, the cerebral white matter may receive a modulated CBF regulation having the strengthened relationship of Paco2 to CBF and, more locally, the frontal white matter may depend precariously on CBF regulation.
INTRODUCTION

Benzodiazepine hypnotics, which are ligands acting at the benzodiazepine site of gamma-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 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 complex (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 (Paco_2) elevation are enhanced compared to placebo-induced sleep (Ford et al. 1990; Schneider et al. 1996). However, it is unclear how these physiological changes affect regional cerebral blood flow (rCBF) and the three primary modes of CBF regulation: 1) pressure autoregulation maintains CBF in spite of changed perfusion pressure; 2) CO_2 vasoreactivity has the most potent vasodilatory effect in the brain; and 3) neurogenic regulation mainly consists of sympathetic and cholinergic influences. 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 Paco_2. 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 of energy deprivation (Brown et al. 2001; Stys et al. 1990) and its gradual, low-flow vulnerability (Tomimoto et al. 2003; Wakita et al. 2002). The white matter lacks benzodiazepine receptors (Abadie et al. 1992; Muller 1987). Benzodiazepines can increase CBF responsiveness to CO₂ (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 outflow 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.

METHODS

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 $[^{15}\text{O}]{\text{H}_2}\text{O}$ PET was conducted, comparing triazolam with placebo. Two nights of study were separated by a 1-week interval. Patients’ wake-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 a week prior to 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-complexes or sleep spindles) 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 datasets of PET and physiological parameters were obtained from nine subjects (age, $21.0 \pm 1.0$ years [range, 20–23]; body weight, $63.8 \pm 6.7$ kg [range, 55–73]; body mass index, $21.3 \pm 2.3$ kg/m$^2$ [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 antebrachial 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, mean arterial blood pressure (MAP) was recorded immediately before tracer injection, and arterial blood for gas analysis was sampled immediately after the scan.

Electroencephalograms were recorded from disc electrodes placed at F3, F4, C3, C4, P3, P4, Fz, Cz, and Pz with A1 and A2 reference. 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-second epoch according to the standardized sleep manual of Rechtschaffen and Kales (Rechtschaffen and Kales 1968), and the sleep stage for each 90-second period during PET scanning was determined if that stage appeared in 2 or 3 epochs. Final assessment of sleep stage scoring was confirmed later by using 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 hours after oral administration, and the elimination half-life of triazolam and of its active metabolite is 2.9 and 3.9 hours respectively (Data on file 1988). Considering the effect of the plasma concentration of triazolam on MAP and Paco₂ or on rCBF (Gillin 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)-$[^{15}\text{O}]\text{H}_2\text{O}$ 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 1 mSv, which is the limit recommended by the International Commission on Radiological Protection (Mountford and Temperton 1992). The $[^{15}\text{O}]\text{H}_2\text{O}$ bolus was automatically flushed intravenously for 15 seconds. With a PET scanner (Siemens ECAT EXACT HR 961; Siemens Medical Systems, Erlangen, Germany) in 3-dimensional mode, scanning started manually 1 second after the initial rise of head counts and continued for 90 seconds. A camera, with an axial field of view of 150 mm, acquired data simultaneously from 47 consecutive axial planes. An image resolution of $3.8 \times 3.8 \times 4.7$ 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 $128 \times 128 \times 47$ voxel format (voxel size, $1.732 \times 1.732 \times 3.125$ mm). A 10-minute 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 version 7.5.4 image display software (Biodynamic Research Unit, Mayo Foundation, Rochester, MN) and on a PC-compatible computer using SPM (statistical parametric mapping)-99-software (Wellcome Department of Cognitive Neurology, London, UK, [http://www.fil.ion.ucl.ac.uk/spm](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, Inc., Sherborn, MA, U.S.A.) for
Windows XP (Microsoft Co., USA). Spatial normalization was employed to fit each individual brain to a standard brain template in 3-dimensional space, in order to correct for differences in brain size and shape, and to facilitate inter-subject averaging. The stereotactically normalized scans contained 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 MNI (Montreal Neurological Institute, Montreal, PQ), and the precise anatomical localizations of significant changes were indicated in accordance with the atlas of Talairach and Tournoux (Talairach and Tournoux 1988) by using a numerical transformation formula supplied by http://www.mrc-cbu.cam.ac.uk/Imaging/minispace.html (MRC Cognition and Brain Science Unit, Cambridge, UK, [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). Whole-brain mean CBF in each image was normalized to 50 ml/100 g/min 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 in order 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 correlation was characterized respectively between conditions and between condition and covariate. Voxels that had peak $T$ values greater than 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 multi-subject design. Areas with a significantly different relative rCBF during triazolam-induced sleep compared to 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 stage 2 and triazolam-induced 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 smallest decreases in relative rCBF during the progression of triazolam-induced sleep. Furthermore, using the analysis of covariate correlation, brain regions were identified with significantly negative correlations of MAP to relative rCBF and significantly positive correlations of Paco₂ 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 perirolandic 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 (Rorden C, University of Nottingham, Nottingham, UK [http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html]), each ROI was manually drawn on a prior probability MNI image segmented for gray or white matter. ROI boundary of the cerebral white matter was defined on an upper corona radiata slice, and 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 Paco2 to relative rCBF. Finally, absolute value of each of ROI was automatically calculated for every image.

Statistical analysis

Using one-way repeated measure analysis of variance (ANOVA), time period from triazolam administration to the start of the PET scan, MAP, Paco2, and absolute CBF of the whole-brain mean, perirolandic cortex, occipital cortex, cerebral white matter, and frontal and temporooccipital white matter were compared among states of wakefulness and non-REM sleep, and compared between with triazolam and placebo. Post-hoc analysis was done by 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 versus slow wave sleep with triazolam), regions of local maxima (n = 7 [left] and 5 [right], dorsolateral prefrontal cortex; and n = 7 [left] and 6 [right], orbital basal forebrain), and subjects (n = 9). Using Pearson’s product-moment correlation coefficient, correlations of MAP and Paco2 to absolute CBF of the occipital cortex, cerebral white matter, and frontal and temporooccipital white matter were evaluated during each state of wakefulness–non-REM sleep. Using the z test, the differences of correlation coefficients of MAP and Paco2 to absolute CBF were compared between triazolam and placebo in each of the occipital cortex or cerebral white matter and between the occipital cortex and cerebral white matter. In both frontal and temporooccipital white matter, correlation coefficients of MAP and Paco2 to absolute CBF were compared between stage 2 or slow wave triazolam-induced sleep and wakefulness. The level of significance was set at $P < 0.05$. 
RESULTS

Subjects and latency to first 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 versus slow wave sleep with triazolam].

Physiological parameters and whole-brain mean CBF

During stage 2 and slow wave sleep with triazolam compared to wakefulness, MAP significantly decreased \( (P < 0.001) \) and Paco2 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 mm Hg, respectively; \( P = 0.026 \)) and slow wave (– 13.8 ± 6.8 and – 7.5 ± 6.7 mm Hg, respectively; \( P = 0.009 \)) sleep and in Paco2 during stage 2 sleep (4.3 ± 2.6 and 2.8 ± 1.9 mm Hg, respectively; \( P = 0.040 \)). Whole-brain mean CBF changed significantly with triazolam \( (F_{2,60} = 7.14, P = 0.002) \) [stage 2 sleep versus wakefulness; \( P < 0.001 \)], and was significantly lower during stage 2 sleep with triazolam compared to placebo \( (P = 0.049) \) (see supplementary figure 1).

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, and 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 region, 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 neocortex, orbital basal forebrain, cingulate gyrus, insular cortex, thalamus, and cerebellar hemisphere during triazolam-induced stage 2 sleep (Fig. 2A). Similarly, significantly decreased relative rCBF was found in almost the same areas during slow wave sleep as during stage 2 sleep with triazolam compared to wakefulness. In the dorsolateral prefrontal cortex, the first eigenvalues did not differ during stage 2 compared to slow wave sleep (left, $P = 0.077$; right; $P = 0.344$) (Fig. 2B). In the left orbital basal forebrain, the first eigenvalues were significantly lower during stage 2 compared to 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 to 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 significant increase in relative rCBF was found bilaterally in the periorlandic 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 during stage 2 sleep 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 non-REM sleep, there was no significant negative correlation of MAP to relative rCBF in any region (Fig. 3D). A significant positive correlation of Paco2 to relative rCBF was found restrictedly in the bilateral cerebral white matter. The frontal white matter tended to be spared (Fig. 3E).

**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 versus 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 the occipital cortex and cerebral white matter during stage 2 sleep and was significantly lower in the occipital cortex during slow wave sleep (Fig. 5, see supplementary table 1). With triazolam compared to placebo, the correlation coefficient of Paco2 to absolute CBF was significantly greater in the cerebral white matter during stage 2 sleep and in both occipital cortex and cerebral white matter during slow wave sleep (Fig. 6, see supplementary table 1).
**Absolute CBF of the frontal and temporooccipital white matter**

ABSOLUTE CBF. 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) (Figs. 3F, bottom left and right; and 4, bottom right). No significant difference was found in any other comparison.

**RELATIONSHIP OF MAP AND PACO2 TO ABSOLUTE CBF 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 CBF 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 brainstem or hypothalamus 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) supports 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 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 brainstem 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 (Schneider et al. 1996). Whole-brain mean CBF decreased specifically 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 methodologies used for 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 cerebral blood flow and metabolism (Roland 1993), the region with the smallest decrease in rCBF during sleep represents the area with the most spared neural activity. 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 smallest decreases in 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 benzodiazepine receptors in 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 triazolam-induced stage 2 sleep, MAP was positively correlated to absolute CBF more strongly in the cerebral white matter than in the occipital cortex. During this period, 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 by the basal forebrain (Hardy and Holmes 1988; Nagai et al. 2004). Sympathetic nerve activity increases and blood pressure oscillates in association with stage 2 sleep K-complexes (Tank et al. 2003). GABA<sub>A</sub> receptor agonists suppress 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 possible that the occipital cortex less undergoes the modulation and thus is not as strongly affected by triazolam..

With triazolam compared to placebo, Paco<sub>2</sub> showed a stronger positive correlation to absolute CBF in the cerebral white matter during stage 2 and slow wave sleep. This corresponds well with evidence that midazolam increases the CBF responsiveness to CO<sub>2</sub> (CO<sub>2</sub> vasoreactivity) in humans (Forster et al. 1983). On the other hand, absolute CBF of the occipital cortex was less well correlated to Paco<sub>2</sub>, similarly to MAP. Accordingly, during stage 2 triazolam-induced sleep, the relationship of absolute CBF of the cerebral white matter
to Paco₂ may compensate for the precarious relationship of MAP to 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.

Conclusion

We showed region- and state-specific effects of benzodiazepine hypnotics on the relationship of MAP and Paco₂ to absolute CBF during human non-REM sleep. Triazolam reduced blood pressure 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 CBF regulations are likely based on the deactivation of the basal forebrain specifically induced by triazolam although the underlying mechanisms remain unproven.
REFERENCES


Sakamoto M, Yasumoto M, Ohsumi H, Choi H, Shibata Y, and Kano T. Effects of
midazolam and flumazenil on carotid sinus baroreflex control of circulation in rabbits. *Br J

Schatzberg AF and Nemeroff CB. *The American Psychiatric Publishing Textbook of

Schneider H, Grote L, Peter JH, Cassel W, and Guilleminault C. The effect of triazolam
and flunitrazepam—two benzodiazepines with different half-lives—on breathing during


Stys PK, Ransom BR, Waxman SG, and Davis PK. Role of extracellular calcium in anoxic

Suzuki H, Yamadera H, Asayama K, Kudo Y, Ito T, Tamura Y, and Endo S. Study of
nocturnal sleep and the carryover effects of triazolam and brotizolam using


Tank J, Diedrich A, Hale N, Niaz FE, Furlan R, Robertson RM, and Mosqueda-Garcia
R. Relationship between blood pressure, sleep K-complexes, and muscle sympathetic

Tomimoto H, Ihara M, Wakita H, Ohtani R, Lin JX, Akiguchi I, Kinoshita M, and
Shibasaki H. Chronic cerebral hypoperfusion induces white matter lesions and loss of
oligodendroglia with DNA fragmentation in the rat. *Acta Neuropathol (Berl)* 106: 527–534,
2003.


Figure legends

**FIG. 1.** Transverse sections of brain areas with significantly different relative rCBF during non-REM sleep with triazolam compared to placebo. An SPM (statistical parametric mapping) at a height threshold of $P = 0.001$ with reference to peak $T$ values ($T = 3.45$) is presented. A and B: During stage 2 sleep, significantly lower relative rCBF was found in the left precentral gyrus (BA 44), left superior frontal gyrus (prefrontal cortex) (BA 10), bilateral superior temporal gyrus (BA 38, 22), and left orbital basal forebrain. Significantly higher relative rCBF was found in the pontomedullary region, midbrain, right hippocampus, and bilateral cerebral white matter. C, D: During slow wave sleep, significantly lower relative rCBF was found in the left superior frontal gyrus, left hippocampus, right basal forebrain, and right amygdaloid complex. Significantly higher relative rCBF was not detected in any region.

**FIG. 2.** Transverse sections of brain areas with significantly decreased relative rCBF during triazolam-induced non-REM sleep. Details are the same as in Figure 1. A: During stage 2 sleep compared to wakefulness, significantly decreased relative rCBF was found bilaterally in the precentral gyrus (BA 44), superior frontal gyrus (prefrontal cortex) (BA 10), superior parietal gyrus (BA 7), superior temporal gyrus (BA 38, 22), orbital basal forebrain, anterior and posterior cingulate gyrus, insular cortex (BA 13), thalamus, and cerebellar hemisphere. B: During slow wave sleep compared to wakefulness, significantly decreased relative rCBF was found in almost the same areas as those during stage 2 sleep compared to wakefulness. The change was significantly less extensive in the orbital basal forebrain. C: During slow wave sleep compared to stage 2 sleep, significantly decreased relative rCBF was found in the pontomedullary region and midbrain.

**FIG. 3.** Transverse sections of brain areas with a significantly increased relative rCBF
during triazolam-induced non-REM sleep (A–C) and with a significantly correlated rCBF to mean arterial blood pressure (MAP) and Paco2 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 Figure 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 to 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. The frontal white matter tended to be spared. F: ROI (region of interest) was drawn bilaterally at the perirolandic cortex (z = 42 mm, top left), occipital cortex (z = 12 mm, top middle), cerebral white matter (z = 24 mm, top right), and frontal and temporooccipital white matter (z = –6 mm, bottom left and right, respectively).

FIG. 4. Absolute CBF of region of interest. Data shown are means ± SD. One-way repeated measure ANOVA; *P = 0.002 and †P = 0.035 (perirolandic cortex), and *P = 0.022, †P = 0.041, ‡P = 0.004, and §P = 0.002 (frontal and temporooccipital white matter).

FIG. 5. Relationship of mean arterial blood pressure (MAP) to absolute rCBF. With triazolam compared to placebo, 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 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 Paco$_2$ to absolute rCBF. With triazolam compared to placebo, correlation coefficient of Paco$_2$ to absolute CBF was significantly greater in the cerebral white matter during stage 2 sleep and in both of 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 Paco$_2$ 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 temporoparietal white matter. The correlation coefficient of Paco$_2$ to absolute CBF was significantly greater in the temporoparietal 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 CBF was significantly greater in the frontal white matter (Fig. 7, see supplementary table 2). The correlation coefficient of Paco$_2$ to absolute CBF was significantly greater in both of the frontal and temporoparietal white matter. (Fig. 7, see supplementary table 2).

**Supplementary figure 1.** Whole-brain mean CBF during wakefulness-sleep state with triazolam and placebo. *P < 0.001 and †P = 0.049.
### TABLE 1.  **Physiological parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wakefulness</th>
<th>Stage 2 sleep</th>
<th>Slow wave sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triazolam</td>
<td>82.6 ± 9.6</td>
<td>73.4 ± 8.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>68.2 ± 6.6&lt;sup&gt;a,c,e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Placebo</td>
<td>85.7 ± 9.1</td>
<td>78.8 ± 9.2</td>
<td>76.7 ± 9.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Paco&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triazolam</td>
<td>41.5 ± 1.7</td>
<td>46.0 ± 2.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>46.2 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Placebo</td>
<td>42.1 ± 2.1</td>
<td>44.6 ± 2.4</td>
<td>45.5 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. One-way repeated measure ANOVA; difference among wakefulness-sleep states (<sup>a</sup><i>P < 0.001</i> and <sup>b</sup><i>P < 0.005</i>) and between with triazolam and placebo (<sup>c</sup><i>P < 0.005</i>), and difference in the change following wakefulness between with triazolam and placebo (<sup>d</sup><i>P < 0.05</i> and <sup>e</sup><i>P < 0.01</i>).
<table>
<thead>
<tr>
<th></th>
<th>Stage 2 sleep</th>
<th></th>
<th>Slow wave sleep</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vs. placebo</td>
<td>vs. wakefulness</td>
<td>vs. placebo</td>
<td>vs. wakefulness</td>
</tr>
<tr>
<td>MBP</td>
<td>→</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Paco₂</td>
<td>↑&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑</td>
<td>→</td>
<td>↑</td>
</tr>
<tr>
<td>Whole-brain mean CBF</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Relative rCBF</td>
<td>DLPFC ↓, BF ↓</td>
<td>DLPFC ↓, BF ↓&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BF ↓, amygdala ↓</td>
<td>DLPFC ↓, BF ↓</td>
</tr>
<tr>
<td></td>
<td>Brainstem ↑</td>
<td>Brainstem →</td>
<td>Brainstem →</td>
<td>Brainstem →</td>
</tr>
</tbody>
</table>

Correlation coefficient to the least decreased absolute rCBF

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>MBP to occipital cortex</td>
<td>↑</td>
<td>↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paco₂ to occipital cortex</td>
<td>→</td>
<td>↑</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBP to cerebral white matter</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Paco₂ to cerebral white matter</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↑↑&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBP to frontal white matter</td>
<td>↑↑&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Paco₂ to frontal white matter</td>
<td>→</td>
<td></td>
<td></td>
<td>↑</td>
</tr>
</tbody>
</table>
Change following wakefulness. \textsuperscript{b} More extensively decreased compared to during slow wave sleep. \textsuperscript{c} Higher compared to the occipital cortex. \textsuperscript{d} 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.
Supplementary table 1. *Correlation coefficient of mean arterial blood pressure and Paco₂ to absolute CBF of the occipital cortex and cerebral white matter and difference of the correlation coefficient between under triazolam and placebo administration and between the cerebral white matter and occipital cortex*

<table>
<thead>
<tr>
<th></th>
<th>Occipital cortex</th>
<th>Cerebral white matter versus occipital cortex</th>
<th>Cerebral white matter versus Triazolam</th>
<th>Triazolam Placebo versus placebo (z)</th>
<th>Triazolam Placebo versus placebo (z)</th>
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<tr>
<td></td>
<td>Triazolam</td>
<td>Triazolam</td>
<td>Triazolam</td>
<td>Triazolam</td>
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<tr>
<td></td>
<td>(r)</td>
<td>(r)</td>
<td>(r)</td>
<td>(r)</td>
<td>(r)</td>
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<tr>
<td>Mean arterial blood pressure</td>
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<tr>
<td>Wakefulness</td>
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<td>–1.95</td>
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<td>0.12</td>
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<td></td>
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<td>–1.47</td>
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<tr>
<td>Stage 2 sleep</td>
<td>0.30</td>
<td>0.15</td>
<td>2.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.21</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3.53&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>5.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66</td>
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<tr>
<td>Slow wave sleep</td>
<td>0.26</td>
<td>0.52&lt;sup&gt;*&lt;/sup&gt;</td>
<td>–3.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.29</td>
<td>0.20</td>
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<tr>
<td></td>
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<td>1.22</td>
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<td></td>
<td></td>
<td>0.38</td>
<td>–3.99&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Paco₂</td>
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<tr>
<td>Wakefulness</td>
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<td>0.01</td>
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<tr>
<td>Stage 2 sleep</td>
<td>–0.10</td>
<td>–0.27</td>
<td>1.72</td>
<td>0.18</td>
<td>–0.31</td>
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<td>6.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
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<td>0.22</td>
<td>–0.12</td>
<td>3.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40</td>
<td>0.20</td>
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<td></td>
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<td></td>
<td></td>
<td>2.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>3.45&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>z</sup> values are expressed as difference of the correlation coefficient; <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.001, <sup>c</sup>P < 0.005, and <sup>d</sup>P < 0.01. <sup>*</sup>P < 0.05 for r.
Supplementary table 2.  Correlation coefficient of mean arterial blood pressure and Paco₂ to absolute CBF of the frontal and temporooccipital white matter during triazolam-induced sleep and difference of the correlation coefficient during stage 2 and slow wave sleep compared to wakefulness

<table>
<thead>
<tr>
<th></th>
<th>Frontal white matter</th>
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<th>Temporooccipital white matter</th>
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<tbody>
<tr>
<td></td>
<td>Difference versus</td>
<td></td>
<td>Difference versus</td>
<td></td>
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<tr>
<td></td>
<td>r</td>
<td>wakefulness (z)</td>
<td>r</td>
<td>wakefulness (z)</td>
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<tr>
<td>Mean arterial blood pressure</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Wakefulness</td>
<td>0.03</td>
<td></td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Stage 2 sleep</td>
<td>0.44*</td>
<td>6.48a</td>
<td>0.29</td>
<td>3.56a</td>
</tr>
<tr>
<td>Slow wave sleep</td>
<td>0.28</td>
<td>3.13b</td>
<td>0.20</td>
<td>1.87</td>
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<tr>
<td>Paco₂</td>
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<td></td>
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</tr>
<tr>
<td>Wakefulness</td>
<td>0.16</td>
<td></td>
<td>−0.13</td>
<td></td>
</tr>
<tr>
<td>Stage 2 sleep</td>
<td>0.05</td>
<td>−1.46</td>
<td>0.12</td>
<td>3.55a</td>
</tr>
<tr>
<td>Slow wave sleep</td>
<td>0.34</td>
<td>2.67c</td>
<td>0.31</td>
<td>5.66a</td>
</tr>
</tbody>
</table>

*z values are expressed as difference of the correlation coefficient from wakefulness; *P < 0.001, *P < 0.005, and *P < 0.01.  *P < 0.05 for r.
Fig. 3

A. Stage 2 sleep — Wakefulness
B. Slow-wave sleep — Wakefulness
C. Slow-wave sleep — Stage 2 sleep
D. MAP negative correlation
E. Paco2 positive correlation
F. Region of interest
Fig. 4
Fig. 5
Fig. 6
Fig. 7
Supplementary figure 1.