Similar Effects of an Orexin Receptor Antagonist and Benzodiazepine Receptor Agonist on Cerebral Blood Flow Regulation

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Orexin receptor antagonists have a different sleep-inducing mechanism from common hypnotic benzodiazepine receptor agonists. Thus, orexin receptor antagonists may affect cerebral circulation differently, although their effects have rarely been investigated. In this study, the effects of an orexin receptor antagonist on cerebral blood flow regulation were compared with those of a benzodiazepine, including dynamic cerebral autoregulation. Fifteen healthy males received suvorexant (1 tablet, 20 mg), an orexin receptor antagonist, and brotizolam (1 tablet, 0.25 mg), a benzodiazepine receptor agonist, in a randomized order at least 7 days apart. Before and 1.5 h after drug administration, arterial blood pressure and cerebral blood flow velocity were measured by transcranial Doppler in a supine position. Dynamic cerebral autoregulation was evaluated by transfer function analysis between mean arterial blood pressure variability and mean cerebral blood flow velocity variability. The steady-state mean cerebral blood flow velocity decreased significantly with both suvorexant and brotizolam (significant main effect of time, $P = 0.003$) and was associated with unchanged steady-state mean arterial blood pressure. Moreover, transfer function gain in the low- and high-frequency ranges were decreased significantly by both suvorexant and brotizolam (significant main effect of time, $P < 0.001$), suggesting a decrease in the magnitude of transfer from arterial blood pressure oscillations to cerebral blood flow fluctuations. These changes were not significantly different between suvorexant and brotizolam (no significant interaction effect). The present results indicate that suvorexant and brotizolam, two different types of hypnotic drug, have similar effects on cerebral blood flow regulation, including possible improvements in dynamic cerebral autoregulation with decreased steady-state cerebral blood flow.

Key words: suvorexant, dual orexin receptor antagonist, cerebral circulation, dynamic cerebral autoregulation

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ical examination that included electrocardiography and blood pressure measurements. Volunteers were excluded if cerebral blood flow velocity signals in the right middle cerebral artery (MCA) could not be obtained by transcranial Doppler ultrasonography or if they had various allergies or a history of major medical illness. A total of 15 healthy males with a mean age (± standard deviation [SD]) of 28 (± 4) years, mean height of 170.2 (± 5.9) cm, and mean weight of 70.2 (± 10.8) kg were enrolled.

All participants fasted for at least 2 h before the experiments, and they refrained from heavy exercise and the consumption of caffeinated or alcoholic beverages for at least 24 h before the experiments. All participants were familiarized with the measurement techniques before the data collection day.

Participants reclined in a supine position on a comfortable bed in an environmentally controlled room at an ambient temperature of 23–25°C in the hospital. An electrocardiograph, pulse oximeter (Life Scope BSM-5132; Nihon Kohden, Tokyo, Japan), infrared CO2 sensor for respiration (CO2 monitor OLG-2800; Nihon Kohden), and bispectral (BIS) index monitor for assessment of the depth of sleep (BIS XP®; Aspect Medical Systems, Inc., Norwood, MA, U.S.A) were applied. Continuous arterial blood pressure waveform was obtained using finger photoplethysmography (Finometer MID; Finapres Medical Systems, Amsterdam, The Netherlands). The cerebral blood flow velocity in the right middle cerebral artery was continuously measured by transcranial Doppler ultrasonography (EZ-Dop; Compumedics Germany GmbH, Singen, Germany). A 2-MHz probe was placed over the temporal window by an experienced technician and fixed at a constant angle with a probe holder customized to fit individual facial bone structure and the ear⁵. Each waveform of continuous arterial blood pressure, cerebral blood flow velocity, and electrocardiography was recorded at a sampling rate of 1 kHz using commercial software (Notocord-hem 3.3; Notocord, Paris, France) on a personal computer.

Blood flow velocity in MCA by transcranial Doppler ultrasonography was used for the index of cerebral blood flow, since the MCAs are the largest cerebral arteries and supply blood to a larger territory (~80% of the cerebral hemispheres) than the other cerebral arteries⁶,⁷. Moreover, the previous studies reported that changes in blood flow velocity in MCA are highly correlated with global cerebral blood flow measured by SPECT and Fick’s principle⁸, two magnetic resonance imaging techniques with the tracer gadolinium and arterial spin labeling⁹, or Xenon¹⁰ clearance techniques¹¹.

The study was a randomized, double-blind, crossover comparison between suvorexant (1 tablet, 20 mg) as an orexin receptor antagonist and brotizolam (1 tablet, 0.25 mg) as a benzodiazepine receptor agonist. In order to ensure blinding, a double-dummy design was used. Participants were assigned to treatment using a computer-generated randomized allocation schedule. Study investigators, site staff, and participants remained blinded to treatment allocation throughout the study. The individual who administered the drugs carried out allocation of participants in the study. At least 7 days were allowed between the two data collection periods (two drug administrations). Prior to drug administration, the data for “before drug administration” were recorded for 6 min after 15 min of quiet rest (Fig. 1). At approximately 21:00, the drug was administered with 50 mL of water in the presence of an investigator. Afterward, the investigator turned off the lights, and the participant laid down on the bed. Both drugs reach their maximum concentration in the blood in 1.5 h, as described in the manufacturers’ materials (suvorexant [Belsomra®]; MSD, Tokyo, Japan and brotizolam [Lendormin®]; Boehringer Ingelheim, Tokyo, Japan). Therefore, at approximately 22:30, the data for “after drug administration” were recorded for 6 min. Afterward, participants slept naturally through the night. The participants got up the following morning after a physician confirmed that they had no adverse effects from the drugs.

Averaged values of steady-state MAP, MCBFV, and heart rate (HR) were obtained by averaging the 6-min data segments. Values for respiration rate, end-tidal carbon dioxide pressure (EtCO2), arterial oxygen saturation (SpO2), and the BIS index were manually recorded every minute as one-off measures. The values at 7 time points (0, 1, 2, 3, 4, 5, and 6 min) during this period were averaged and used as each participant’s individual data. Also, a numeric rating scale (NRS) in which 0 represents “not sleepy at all” and 10 represents “very sleepy” was used to assess sleepiness immediately before each data measurement.

To assess dynamic cerebral autoregulation, 6 min of continuous arterial blood pressures and cerebral blood flow velocity waveforms were used for spectral and transfer function analysis during spontaneous respiration on room air. Beat-to-beat values of MAP and MCBFV were obtained by integrating signals within each cardiac cycle using personal computer-based software (Notocord-hem 3.3) for spectral and transfer function analysis. Beat-to-beat data for MAP and MCBFV were resampled at 4 Hz. Fast Fourier transform and transfer function analyses were performed using a Hanning window on 512-point segments with 50% overlap, with this process resulting in five segments of over 6 min of data recordings. This data analysis was based on international cerebral autoregulation research network–recommended algorithms¹² and performed using DADiSP software (DSP Development, Cambridge, MA, U.S.A). The spectral power of beat-to-beat MAP and MCBFV variabilities (Fig. 2) and the mean
coherence function, phase, and transfer function gain (Fig. 3) values were calculated in the very-low-frequency (0.02–0.07 Hz), low-frequency (0.07–0.20 Hz), and high-frequency (0.20–0.35 Hz) ranges. These ranges were specifically selected to reflect different patterns of the dynamic pressure-flow relationship. A coherence function between 0 and 1 reflects a linear relationship between arterial blood pressure oscillation and cerebral blood flow velocity fluctuation. When coherence is > 0.5, phase and transfer function gain can be commonly used as interpretable indices. However, the group-averaged coherence value in the suvorexant protocol was < 0.5 in the very-low-frequency range. Therefore, average values in this very-low-frequency range were not calculated for all the other indices. Phase reflects the temporal relationship between arterial blood pressure oscillation and cerebral blood flow velocity fluctuation. Transfer function gain reflects the magnitude of transfer from arterial blood pressure oscillation to cerebral blood flow velocity fluctuation. A small gain implies that the buffering ability of cerebral vessels to respond to transient changes in arterial pressure is augmented.

Prior to drug administration, the data for “before drug administration” were recorded for 6 min after 15 min of quiet rest. At approximately 21:00, the drug was administered with 50 mL of water in the presence of an investigator. Afterward, the investigator turned off the light, and the participant laid down on the bed. At approximately 22:30, the data for “after drug administration” are recorded for 6 min. The participants then sleep naturally through the night.

**Fig. 1** Experimental protocol.

**Fig. 2** Group-averaged spectral power density of MAP and MCBFV with suvorexant and brotizolam.

PSD, power spectral density; MAP, mean arterial blood pressure; MCBFV, mean cerebral blood flow velocity; VLF, very-low-frequency range (0.02–0.07 Hz); LF, low-frequency range (0.07–0.2 Hz); HF, high-frequency range (0.2–0.35 Hz). Thin lines = before drug administration; Thick lines = 1.5 hours after drug administration.
Statistical analysis

Data are presented as the mean ± SD. Normality was evaluated by the Kolmogorov-Smirnov test. Variables were compared using two-way repeated measures analysis of variance with Time (before and after drug administration) × Drug (suvorexant and brotizolam). To determine where significant differences occurred, a Student-Newman-Keuls post hoc test was used for all pairwise comparisons. A P-value of < 0.05 was considered significant. The analyses were performed using PC-based software (SigmaStat; Systat Software, Inc., Chicago, IL, U.S.A).

Correlation analyses were performed using R software (The R Foundation for Statistical Computing, Vienna, Austria). To evaluate the relationship between BIS value and cerebral blood flow velocity, repeated measures correlation analysis was applied using the rmcorr R package developed by Bakdash and Marusich (https://cran.r-project.org/web/packages/rmcorr/)⁵. This correlation analysis is a statistical technique for determining the common within-individual association for paired measures on two or more occasions for multiple individuals⁶. A P-value of < 0.05 was considered significant.

Results

All 15 participants completed the study protocol without adverse events or side effects. Table 1 shows the average values of steady-state hemodynamic and respiratory conditions. Steady-state HR decreased significantly with suvorexant and brotizolam administration (significant main effect of Time, P < 0.001). Steady-state MAP, respiratory rate, and EtCO₂ did not change. SpO₂ decreased slightly but significantly with both suvorexant and brotizolam administration (significant main effect of Time, P = 0.003).

The BIS index decreased, and the NRS increased significantly with suvorexant and brotizolam administration (significant main effect of Time, P = 0.001). Associated with this result, steady-state MCBFV decreased significantly with suvorexant and brotizolam administration (significant main effect of Time, P = 0.003). The repeated measures correlation analysis showed a significant correlation between BIS value for sleep depth and MCBFV with suvorexant (r = 0.877, df = 14, 95% CI [0.649, 0.9607], P < 0.001) and with brotizolam (r = 0.597, df = 14, 95% CI [0.0977, 0.8565], P = 0.014) (Fig. 4A, B).
For the group-averaged power spectral density, only the spectral power of MAP variability in the low-frequency range increased significantly with brotizolam administration (significant main effect of Time, $P = 0.005$) (Table 2, Fig. 2).

For the transfer function analysis of beat-to-beat changes in MAP and MCBFV (Table 2, Fig. 5), coherence in the low- and high-frequency ranges was > 0.5, and phase in these ranges did not change. In contrast, the transfer function gain in the low- and high-frequency ranges decreased significantly with suvorexant and brotizolam administration (low-frequency: significant main effect of Time, $P < 0.001$; high-frequency: significant main effect of Time, $P = 0.01$) (Fig. 5). These values and changes after administration were not significantly different between suvorexant and brotizolam, except for coherence in the low-frequency range.

### Discussion

In the present study, two main findings showed primarily similar effects on cerebral blood flow regulation between the two different types of hypnotics. First, steady-state cerebral blood flow velocity decreased after both suvorexant and brotizolam administration, associated with sleep-inducing effects indicated by decreased BIS and increased NRS. Second, the transfer function gain in the low- and high-frequency ranges decreased significantly, and the degrees did not differ between suvorexant and brotizolam, suggesting that both suvorexant and brotizolam augment the buffering ability of cerebral vessels to respond to transient changes in arterial pressure.

Suvorexant and brotizolam have different mechanisms...
of sleep induction. Suvorexant is a dual orexin receptor antagonist that selectively binds to orexin receptor 1 (OX1R) or orexin receptor 2 (OX2R). OX1R is highly expressed in the locus coeruleus, and OX2R is abundantly expressed in the tuberomammillary nucleus. Both regions are important for the maintenance of arousal. Suvorexant induces a physiologic sleep pattern by blocking OX1R and OX2R. In contrast, brotizolam is a benzodiazepine receptor agonist that enhances the effect of the neurotransmitter gamma-aminobutyric acid (GABA) at the GABA_A receptor. GABA is the major inhibitory neurotransmitter in the central nervous system and acts through the GABA_A and GABA_B receptors. Thus, these two types of hypnotics could also have different effects on cerebral circulation. However, the present study showed that both alter the indices for cerebral circulation similarly.

Cerebral blood flow regulation guarantees an appropriate...
ate balance between cerebral oxygen demand and supply. In general, steady-state cerebral blood flow is maintained at relatively constant and appropriate levels despite changes in arterial blood pressure by that function. In contrast, beat-to-beat cerebral blood flow velocity responds rapidly to transient changes in arterial pressure. Transfer function analysis of these rapid changes yields insights into the dynamic properties of cerebral autoregulation. This function is referred to as dynamic cerebral autoregulation.

In the present study, steady-state MCBFV decreased significantly after suvorexant and brotizolam administration, with no change in MAP. This decrease may be induced by suppressed brain activity, as indicated by decreased BIS and increased NRS. The decreased BIS index and increased NRS indicated light sleep and/or sedation. In the present study, MCBFV and the BIS index were apparently correlated, although the BIS index is an undefined index for sleep depth, and the present results for the degree of correlation might be different between suvorexant and brotizolam. Thus, it is likely that both suvorexant and brotizolam reduced brain activity, cerebral metabolic rate, and oxygen demand, and those effects of both drugs are partly responsible for the decrease in cerebral blood flow.

Both drugs also might affect dynamic cerebral autoregulation similarly. In this condition, in which steady-state cerebral blood flow decreased, the transfer function gain in the low- and high-frequency ranges decreased significantly with suvorexant and brotizolam. A gain in transfer function reflects the magnitude of transfer from arterial blood pressure oscillation to cerebral blood flow velocity fluctuation, as described in the Materials and Methods section. In other words, the present transfer function gain results suggest that the buffering ability of cerebral vessels to respond to transient changes in arterial blood pressure is augmented by both suvorexant and brotizolam, indicating improvement in dynamic cerebral autoregulation.

In a previous study, the transfer function gain in the low-frequency ranges decreased significantly with midazolam administration, consistent with the present results for brotizolam. Thus, these collective results suggest that benzodiazepine receptor antagonists generally improve dynamic cerebral autoregulation.

The mechanisms of the two drugs in terms of the effect on dynamic cerebral autoregulation could also be similar. The enhanced dynamic cerebral autoregulation during benzodiazepine sedation might be induced by multiple factors, including autonomic, myogenic, and endothelial cell mechanisms. Suvorexant alters autonomic nervous activity and endothelial cell function. Therefore, suvorexant could affect dynamic cerebral autoregulation via autonomic and endothelial cell mechanisms as well. However, the detailed mechanisms of altered dynamic cerebral autoregulation could not be clarified in the present study.

The changes in cerebral circulation induced by hypnotics could be related to the occurrence of syncope by orthostatic hypotension. When arterial blood pressure decreases temporarily with postural change (e.g., standing) after taking a hypnotic, there is a risk of syncope by temporal severe decreases in cerebral blood flow. Also, the increases in arterial blood pressure oscillation after taking a hypnotic observed in the present study could increase that risk. However, the present findings of possible enhancement of dynamic cerebral autoregulation by suvorexant and brotizolam may contribute to the prevention of temporal decreases in cerebral blood flow.

The primary limitation of the present protocol was the estimation of sleep depth using the BIS index, which was manually recorded every minute. The BIS index has several limitations in assessing sedative/sleep depth. For example, BIS index values can vary widely among individuals and ages, in addition to drug-specific characteristics. Furthermore, different sedative/sleep depths sometimes show similar BIS values. Thus, it would be difficult to provide clear evidence indicating that sleep depth induced by suvorexant and brotizolam is equivalent. Furthermore, the present experimental protocol could not determine whether natural sleep itself has the same effects on cerebral circulation, including dynamic cerebral autoregulation. Finally, it is unclear if the same results would be obtained in a patient with a sleep disorder. Further studies are needed to address these limitations.

Conclusion

In the present study, the effects of an orexin receptor antagonist on cerebral blood flow regulation were compared with those of a benzodiazepine receptor antagonist. The present results showed that both suvorexant and brotizolam decrease steady-state cerebral blood flow velocity associated with sleep-inducing effects, as indicated by decreased BIS index and increased NRS. Moreover, these drugs decrease the magnitude of transfer from arterial blood pressure oscillation to cerebral blood flow fluctuation, indicating possible improvement of dynamic cerebral autoregulation. Thus, the results suggest that suvorexant and brotizolam have primarily similar effects on cerebral blood flow regulation, although these two types of hypnotics have different mechanisms accounting for their sleep and/or sedative actions.

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