Diurnal Variation in CSF Orexin-A in Healthy Male Subjects

Scott P. Grady, MD1; Seiji Nishino, MD, PhD2; Charles A. Czeisler, PhD, MD3; David Hepner, MD4; Thomas E. Scammell, MD5

1Portland Diabetes and Endocrinology Center, Portland, OR; 2Department of Psychiatry & Behavioral Science, Stanford University, Palo Alto, CA; 3Division of Sleep Medicine, Department of Medicine, Brigham and Women’s Hospital and Division of Sleep Medicine, Harvard Medical School, Boston, MA; 4Department Of Anesthesia, Perioperative and Pain Medicine, Brigham and Women’s Hospital, Harvard Medical, Boston, MA; 5Department of Neurology, Beth Israel Deaconess Medical Center, Division of Sleep Medicine, Harvard Medical School, Boston, MA

Study Objective: Orexin-A is hypothesized to promote wakefulness, and we examined whether cerebrospinal fluid (CSF) orexin-A levels are higher during the waking period in man.

Design: Within-subjects, repeated-measures design with balanced ordering of sampling at approximately 5 AM and 5 PM.

Participants: Eight healthy young males.

Measurements: CSF orexin-A levels and standard polysomnography.

Results: Orexin-A levels during the sleep period were 4% higher than during the waking period (314.9 pg/ml versus 302.8 pg/ml, p < 0.03). Sleep period orexin-A levels were negatively correlated with REM sleep as a percentage of total sleep time (p < 0.05). The day and night levels of orexin-A were strongly correlated within subjects (r = 0.97; p < 0.0001) even though the samples were collected 1-2 weeks apart.

Conclusions: Orexin-A levels in lumbar CSF are slightly higher at 5 AM than at 5 PM. Because orexin release is thought to be highest during the waking period, this observation was unexpected and may reflect a long delay between the release of orexin and its appearance in lumbar CSF. Orexin-A levels vary moderately between subjects, but are quite consistent within the same subject. Thus, for the diagnostic evaluation of narcolepsy, the time of CSF collection should have little impact.

Keywords: Orexin-A, hypocretin-1, diurnal variation, REM-sleep, cerebrospinal fluid, diagnostic, narcolepsy, hypothalamus, sleep

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INTRODUCTION

OREXIN-A IS A PEPTIDE NEUROTRANSMITTER ESSENTIAL FOR THE CONTROL OF BEHAVIORAL STATE. OREXIN-A IS PRODUCED BY NEURONS IN THE LATERAL hypothalamus and has strong excitatory effects on many wake-promoting brain regions. In diurnal squirrel monkeys, orexin levels in cerebrospinal fluid (CSF) from the cisterna magna are highest near the end of the wake period and lowest during the sleep period.1 In contrast, Salomon and colleagues examined lumbar CSF in humans and found higher orexin-A concentrations at night, with peak values between 1 and 2 am, though they did not control for sleep.2 We sought to confirm whether a diurnal variation in CSF levels of orexin-A occurs in healthy human volunteers with the hypothesis that orexin-A levels would be highest during waking and lowest during the latter half of the sleep period.

METHODS

Eight healthy men of mean age 24 ± 4 years completed this within-subjects, repeated-measures design study. The protocol was approved by the Human Subjects Committee of Brigham and Women’s Hospital, and all subjects gave informed consent. Subjects were screened for medical and psychiatric suitability. Each subject participated in 1 week of ambulatory, in-home, actigraphic recording with an 8-hour sleep period with self-selected, though fixed, sleep onset. After this run-in, subjects were admitted to the General Clinical Research Center of the Brigham and Women’s Hospital for the first of two 68-hour admissions. On the first admission, subjects were randomly assigned to receive either of 2 possible conditions: Condition 1 consisted of a standard lumbar puncture (LP) 9 hours after their habitual waking time on day 3 (about 5 PM), while condition 2 consisted of an LP 5 hours into the sleep period on day 3 (about 5 AM). Subjects were blinded to the timing of the LPs and were not informed of the specific hypothesis of the study. Subjects were scheduled to 16-hour wake-periods and 8-hour sleep periods identical to the sleep-wake schedule maintained at home. After the first admission, subjects were discharged for 7 to 14 days before returning for the second half of the protocol. During this period, the same sleep-wake schedule was maintained and verified by actigraphy. The second half of the study was identical to the first, with the exception that they received the other condition (i.e., timing of LP).

Standard polysomnographic recordings were performed each night during each admission. Sleep recordings for the 5-hour period preceding the morning LP condition were scored visually in 30-second epochs using standard criteria.1 Polysomnogram data were unusable in 1 subject.

Disclosure Statement

This was not an industry supported study. Dr. Czeisler has received consulting fees from or served as a paid member of scientific advisory boards for Cephalon, Inc., Hynpion, Inc., Lifetrac, Inc., Respririonics, Inc., Sanofi-Aventis, Takeda Global Research & Development Center, Inc., Uniliever, and Vanda Pharmaceuticals, Inc.; owns an equity interest/options in Axon, Inc., Hynpion, Inc., and Lifetrac, Inc.; has received lecture fees from Cephalon, Inc., Sanofi-Aventis, Neurocine, Inc., the Society for Neurological Sur- geons, and Takeda Pharmaceuticals, Inc.; has received research support from Cephalon, Inc., Pfizer, Inc., Merck, ResMed, Sepracor, Respririonics, Jordari’s Furniture, George Kidder, Herbert Lee, Simmons, and SpringAire. Dr. Scammell has received consulting fees and honoraria from Cephalon, Inc., Respririonics, Inc., and Orphan Medical/Jazz Pharmaceuticals, Inc., and he has received research support from Cephalon, Inc. Orphan Medical/Jazz Pharmaceuticals, Inc., and deCode Genetics, Inc. Drs. Grady, Nishino, and Hepner have indicated no financial conflicts of interest.

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Address correspondence to: Tom Scammell MD, Harvard Institutes of Medicine, Beth Israel Deaconess Medical Center, 77 Ave. Louis Pasteur, Boston, MA 02115; E-mail: tscammel@bidmc.harvard.edu

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Standard LP technique was employed using 25-gauge, noncutting, “pencil-point” spinal needles. Ten milliliters of CSF were collected in 2.5 mL aliquots. Samples used for the orexin-A assay were derived from the fourth aliquot. Samples were frozen at -80°C within a few minutes. CSF orexin-A was measured with a direct radioimmunoassay (i.e., without extraction), as has been described previously. All samples were measured in a single assay, and the intraassay variability was less than 5%. Orexin-A levels were compared using a Wilcoxon matched-pairs test and correlated to sleep parameters using a Pearson correlation coefficient.

Twelve subjects were recruited to this study. Four subjects dropped out after a single LP and were excluded from analysis. Two of these chose not to proceed to the second phase for personal reasons. Another developed a post-LP headache and was disempaneled per protocol. The fourth was excluded because CSF could not be obtained. A post-LP headache occurred in 1 additional subject after his second LP. In total, 2 post-LP headaches occurred out of the 20 LPs performed in 12 subjects; this 10% incidence of headaches is comparable with the rate reported in the literature.

RESULTS

Sleep-period orexin-A levels were 4% higher than waking values (314.9 pg/mL around 5 am versus 302.8 pg/mL around 5 pm, p < .03), and 7 of 8 subjects had higher CSF orexin-A levels during the sleep period than during waking. The average sleep efficiency was 92% (range 87%-96%). Rapid eye movement (REM) sleep averaged 23% of total sleep time (range 19%-32%). While no correlation was observed between orexin-A levels and sleep efficiency, time awake, stage 1, stage 2, or slow-wave sleep, sleep-period orexin-A levels were negatively correlated with REM sleep as a percentage of total sleep time (p < .05). Even though the measurements were separated by 1 to 2 weeks, the day and night levels of orexin-A were strongly correlated (r = 0.97; p < .0001; Figure 1).

CONCLUSIONS

Utilizing within-subjects measurements and polysomnogram recordings, we found that lumbosacral CSF orexin-A levels are slightly higher during the sleep period than during waking. Sleep-period orexin-A levels were negatively correlated with REM sleep. Except for the small day-night variations, orexin-A levels within each individual were quite constant over time.

One limitation of our study is that CSF was obtained using standard LPs. Hypothalamic orexin neurons heavily innervate the brain but also send projections to the spinal cord. We have encountered no data suggesting that orexin has a different temporal profile of release in the spinal cord than in the brain, but the pattern of orexin levels in lumbar CSF might differ from that in state-regulatory brain regions. In addition, radioisotope studies have demonstrated that CSF flows from the third ventricle to the lumbosacral cul de sac in 60 to 90 minutes, but nothing is known about the half life or diffusion of orexin. Thus, we cannot draw firm conclusions about the time lag between orexin-A release and its presence in lumbar CSF.

Another potential limitation of our study relates to sampling times. In squirrel monkeys, cisterna magna orexin-A concentrations rise across the waking period and fall during the sleep period.1 If this same pattern exists in humans, our sampling schedule may have missed the maxima and minima of orexin-A release, thus accounting for the seemingly paradoxical results, especially if there is a significant delay in the movement of orexin to the thecal sac. Nevertheless, the rigorous control of behavioral state in this study reinforces the observations of Salomon and colleagues as they relate to the secretory dynamics of orexin-A in healthy humans.

We found that subjects with higher levels of orexin-A have less REM sleep, and this observation is consistent with several recent animal studies. Infusions of orexin-A near the locus coeruleus suppress REM sleep. Conversely, depriving rats of REM sleep results in higher CSF orexin-A concentrations. Extracellular recordings of the orexin neurons show that these cells fire at their lowest rates during REM sleep. As discussed above, though the level of orexin-A in lumbar CSF may not accurately reflect recent changes in sleep-wake behavior, it still may provide a broad indication of propensity toward REM sleep, so that subjects with low levels of orexin-A generally have more REM sleep.

CSF orexin-A levels are low in most narcoleptic patients with cataplexy, and measuring orexin-A can help in the diagnosis of narcolepsy. We found that orexin-A in CSF samples obtained 1 to 2 weeks apart varied up to 30% between subjects but only 4% within subjects. This within-subject reproducibility suggests that, as a diagnostic test for narcolepsy, lumbar CSF sampling may be performed at any time of day, and a single sample should be sufficient for diagnosis.

Our finding that CSF orexin-A levels are higher at night confirms the observations of Salomon and colleagues but is not consonant with observations in rodents and nonhuman primates. Because we examined only 2 time points, state-dependent variations in orexin-A levels may vary more than is apparent in our experiment. Future clinical studies with continuous sampling of CSF closer to the putative site of orexin-A action on brainstem and hypothalamic sleep-regulating regions should help establish whether orexin-A levels vary substantially with changes in behavioral state.

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