Plasma Orexin-A Levels in Obstructive Sleep Apnea-Hypopnea Syndrome*

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Study objectives: Orexin and orexin receptors are present in the CNS. The effects of orexin peptides have been uniformly reported as excitatory, and the posterior hypothalamus containing orexin neurons has been implicated in arousal state control. Therefore, it is probable that the orexin system may have a neuromodulatory effect on arousal states. The aim of the present study was to investigate the relationship between plasma orexin-A levels and arousals from sleep in patients with obstructive sleep apnea-hypopnea syndrome (OSAHS).

Design: An analysis was conducted in 30 male patients with OSAHS, which had been diagnosed by polysomnography by the presence of an apnea-hypopnea index (AHI) of > 5, and 20 male age-matched and body mass index (BMI)-matched control subjects.

Results: Plasma orexin-A levels were higher in patients with OSAHS compared with those in control subjects (p < 0.05). Plasma orexin-A levels correlated positively, but weakly, with the arousal index (r = 0.51; p < 0.05) and the AHI (r = 0.52; p < 0.05). However, plasma orexin-A levels did not relate to age, BMI, Epworth sleepiness scale, PaO2, PaCO2, minimum arterial oxygen saturation (SaO2) during sleep, or mean SaO2 during sleep. Plasma orexin-A levels can be a measure of both AHI and arousal index.

Conclusion: These results suggested that the orexin system may be involved in arousal mechanisms in patients with OSAHS.

Key words: arousal index; arousal mechanisms; Epworth sleepiness scale

Abbreviations: AHI = apnea-hypopnea index; BMI = body mass index; CSF = cerebrospinal fluid; EMG = electromyography; ESS = Epworth sleepiness scale; nCPAP = nasal continuous positive airway pressure; OSAHS = obstructive sleep apnea-hypopnea syndrome; PSG = polysomnography; REM = rapid eye movement; SaO2 = arterial oxygen saturation

The hypocretins, subsequently described as orexins, were initially implicated in the control of food intake. Appetite and feeding behavior are regulated by many neurotransmitters, and the orexins have been identified as a class of neuropeptides that stimulate food intake.1,2 Immunohistochemical studies indicated that orexin-immunoreactive nerve fibers project widely in the CNS, suggesting that orexins are multifunctional.3,4 Apart from participating in the regulation of food intake, orexins have been implicated in other biological systems, such as CNS regulation of cardiovascular and autonomic systems.5,6 Research7,8 has shown that the orexin system participates in the sleep-wake cycle and in the sleep disorder narcolepsy. Chemelli et al9 reported that orexin knockout mice could serve as a model of human narcolepsy, and that orexin neurons play a role in controlling arousal in the cortical activation system. Hagan et al10 demonstrated that orexin neurons activate locus ceruleus neurons and increase arousal in rats. These studies suggested that orexin stimulated the arousal response, although the precise mechanisms remain undefined.

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apnea-hypopnea syndrome (OSAHS). We tested the hypothesis that the amount of sleep fragmentation was significantly associated with plasma orexin-A levels.

**Materials and Methods**

The study population consisted of 50 male consecutive subjects who had been examined by polysomnography (PSG) from October 2001 to February 2002. All subjects had snoring and were suspected of having OSAHS. No patients were suspected of having narcolepsy, which is an incurable disorder characterized by excessive sleepiness that typically is associated with episodes of cataplexy. All subjects were free from respiratory infection, heart failure, other respiratory problems (including COPD), or known diabetes mellitus at the time of undergoing PSG. They were asked to complete a questionnaire on sleep symptoms, medical history, and medications. Objective sleepiness was assessed using the Epworth sleepiness scale (ESS). None of the subjects had experienced any cardiovascular disease, except for hypertension, in the past. A diagnosis of OSAHS was established on the basis of clinical and polysomnographic criteria. The average number of episodes of apnea plus hypopnea per hour of sleep (ie, the apnea-hypopnea index [AHI]) was calculated as the summary measurement of sleep-disordered breathing. In addition to clinical symptoms, an AHI of more than five events per hour also was used as a selection criterion. The study protocol was approved by the Research Ethics Committee of Chiba University School of Medicine, and all patients gave their informed consent prior to the study.

Overnight PSG (Compumedics; Melbourne, Australia) was performed between 9:00 pm and 6:00 am. The PSG consisted of continuous polygraphic recording from surface leads for EEG, electrooculography, electromyography (EMG), electrocardiography, thermistors for nasal and oral airflow, thoracic and abdominal impedance belts for respiratory effort, pulse oximetry for oxygen saturation, tracheal microphone for snoring, and a sensor for sleep position. PSG records were staged manually according to standard criteria. Arousals were scored according to American Sleep Disorders Association criteria. Arousal non-rapid eye movement (REM) sleep may occur without concurrent changes in submental EMG amplitude, and arousals are scores in REM sleep only when accompanied by concurrent increases in submental EMG amplitude. Respiratory events were scored according to American Academy of Sleep Medicine criteria, as follows: apnea was defined as a complete cessation of airflow lasting ≥ 10 s; hypopnea was defined as either a ≥ 50% reduction in airflow for ≥ 10 s or a 0% to 50% decrease in airflow accompanied either by a decrease in oxygen saturation of > 3% or an arousal. Severity of OSA was measured by AHI, minimum oxygen saturation (SaO2), during sleep, mean SaO2 during sleep, and arousal index.

Pulmonary function tests were performed to determine the vital capacity and FEV1 using a standard spirometer (Fudac-60; Fukuda Denshi; Tokyo, Japan). Arterial blood gases during room air breathing were drawn with the patient in the supine position and were measured in a blood gas analyzer (model 1312; Instrumental Laboratory; Milano, Italy).

At 7:00 AM on the morning after the sleep study, venous blood was obtained in the fasting state to measure the orexin-A level. Plasma levels of orexin-A were determined using a radioimmunoassay kit (Orexin-A; Peninsula Laboratories; San Carlos, CA) with intraassay and interassay coefficients of variation of 4.8 to approximately 5.6% (10 measurements) and 5.8 to approximately 7.9% (10 measurements), respectively. This assay enables the reliable measurement of plasma orexin-A levels as low as 10 pg/mL. No cross-reactivity was observed between orexin-A and corticotropin-releasing hormone, β-endorphin, vasopressin, met-enkephalin, leu-enkephalin, substance-P, or angiotensin II.

The OSAHS patients with AHI scores of > 20 events per hour were recommended for treatment by nasal continuous positive airway pressure (nCPAP). Titration was performed in the home of the patients (AutoSet T; ResMed; Sydney, Australia). If patients agreed, treatment was continued with a conventional fixed-pressure nCPAP device (derived from the AutoSet-T). Generally, the recommended pressure automatically calculated by the device (95th percentile pressure after excluding periods with a leak > 0.4 L/s) was used. After periods of regular nCPAP use of 3 to approximately 4 months, plasma orexin-A levels and objective sleepiness using the ESS were reassessed.

**Statistical Analysis**

The results are expressed as the mean ± SEM. Since the data were not normally distributed, we used the Spearman rank correlation coefficient to examine the association of two parameters. The Mann-Whitney U test was used to compare the variables between patients with OSAHS and control subjects. Wilcoxon rank-sign analysis was performed for paired samples. A p value of < 0.05 was considered to be statistically significant.

**Results**

Thirty of 50 patients had received a diagnosis of OSAHS. The baseline characteristics of OSAHS and control subjects are shown in Table 1. Pulmonary function tests were normal except in two patients with a restrictive ventilatory disturbance (vital capacity, < 80%). No patients had obstructive airway disease (ie, FEV1/FVC ratio of < 70%). Seven patients were slightly hypoxicemic (PaO2 of < 70 mm Hg), although the PaO2 levels were at > 60 mm Hg in all patients. Eleven patients were hypercapnic (ie, PaCO2 of > 45 mm Hg), although the levels of PaCO2 were < 55 mm Hg in all patients. The body mass

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<th>Table 1—Baseline Characteristics of the Subjects*</th>
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<td>Characteristics</td>
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*Values given as the mean ± SE, unless otherwise indicated. NS = not significant.
index (BMI) of 4 of 11 patients with hypercapnia was > 30, suggesting the presence of obesity-hypoventilation syndrome, although the BMI was < 35 in these four patients. The ESS ranged from 4 to 23, and 24 of the 30 patients were subjectively sleepy and had an ESS of ≥ 10.

AHI positively correlated with the arousal index and ESS (p < 0.05) in patients with OSAHS, while AHI negatively correlated with the minimum and mean SaO₂ during sleep (p < 0.05). The arousal index positively correlated with ESS (p < 0.05) in patients with OSAHS. Plasma orexin-A levels ranged from 24 to 52 pg/mL (mean orexin-A level, 36.2 ± 1.2 pg/mL) in patients with OSAHS. Plasma orexin-A levels were higher in patients with OSAHS compared with those in control subjects (Table 1). There was a significant increase in plasma orexin-A levels with increasing AHI (r = 0.52; p < 0.05) [Fig 1], and also with increasing arousal index (r = 0.51; p < 0.05) [Fig 2] in patients with OSAHS, while plasma orexin-A levels did not correlate with age, BMI, ESS, mean sleep SaO₂, or minimum sleep SaO₂.

Of 30 patients with OSAS, 12 patients had received nCPAP therapy for at least 3 months. Plasma orexin-A levels decreased from 40.8 ± 2.1 to 36.3 ± 1.3 (p < 0.05), and ESS also decreased from 14.7 ± 1.1 to 7.4 ± 0.6 (p < 0.05).

**DISCUSSION**

In the present study, we found that plasma orexin-A levels correlated with the clinical severity of OSAHS, AHI (r = 0.52), and arousal index (r = 0.51). In addition, plasma orexin-A levels decreased in patients who received nCPAP therapy. These results suggest that plasma orexin-A could be used as a biological marker of the severity of OSAHS.

It has been reported that orexin-A is present in the cerebrospinal fluid (CSF) and peripheral blood of healthy individuals and some narcoleptic patients. The origin of plasma orexin-A has not yet been determined. Orexin-A neurons are restricted to the lateral and posterior hypothalamus, and orexin-A has been shown to rapidly cross the blood-brain barrier by simple diffusion. Therefore, circulating orexin-A could originate from the hypothalamus via blood-brain barrier, in which case plasma orexin-A levels at least partially reflect the production of orexin-A in the hypothalamus. However, a consensus regarding the exact functions of the brain orexin system has not yet emerged, although it is reasonable to assume that an elevated plasma orexin level reflects central manifestations of apnea-hypopnea-related arousals.

Alternatively, plasma orexin-A might stem from cells that express orexin-like immunoreactivity, together with functional orexin receptors in human gut cells. There are peripheral manifestations of arousal, particularly arousal from obstructive respiratory events (ie, changes in BP and heart rate, sympathetic activation, intrathoracic pressure swings, and elevated muscle activity) that could conceivably activate peripheral cells containing orexin or orexin-like immunoreactivity.

**FIGURE 1.** Association between plasma orexin-A levels and AHI in 30 patients with OSAHS.
Higuchi et al. measured plasma orexin-A, using the same radioimmunoassay method that we have used, in Japanese patients with narcolepsy, and they found that plasma orexin-A levels in patients with narcolepsy (range, 11 to 25 pg/mL; mean, 20.8 ± 4.3 pg/mL) were lower than those in control subjects (range, 20 to 33 pg/mL; mean, 26.7 ± 3.2 pg/mL). Compared with those measurements, the orexin-A levels were higher in the present study, partly because obesity may influence the plasma levels of orexin-A. The plasma levels of orexin-A were higher in patients with OSAHS than in an age-matched, BMI-matched, and gender-matched group of control subjects, suggesting that the production of orexin-A is augmented in patients with OSAHS.

It has yet to be determined whether plasma and CSF levels of orexin-A correlate with each other, and whether plasma orexin-A levels are regulated by a negative feedback system of the arousal response. Reduced levels of orexin-A in the CSF and a substantial reduction in the number of orexin neurons, specifically in the hypothalamus, have been reported in narcoleptic patients. Combined with the lower levels of plasma orexin-A observed in narcoleptic patients, plasma levels of orexin-A may represent changes in the number or activity of orexin neurons in the CNS. It is possible that the regulation of plasma orexin-A levels differs between narcoleptic patients and patients with OSAHS. In the present study, we acknowledge one important limitation, namely, we did not obtain CSF samples from our patients to measure the levels of orexin-A. However, the correlation of plasma orexin-A levels and the severity of OSAHS, and the simplicity of specimen collection may support the usefulness of plasma orexin-A as a biological marker of OSAHS.

No significant correlation was observed between plasma levels of orexin-A and BMI in the present study. However, Adam et al. reported that plasma orexin-A levels correlated negatively with BMI and that lower levels of plasma orexin-A are present in obese individuals, suggesting that orexin is involved in the regulation of human energy metabolism. In addition to their potent effects on appetite, orexins may interact with the CNS system, controlling sympathetic outflow and cardiovascular function. Orexin-A, when injected into the lateral cerebroventricle, induced an increase of mean arterial pressure and heart rate in conscious rats. The effects of orexin peptides have been uniformly reported as excitatory, and orexin neurons project to monoaminergic cell groups. These findings may explain the relation between underlying narcolepsy symptomatology and orexin deficiency. The posterior hypothalamus containing orexin neurons has been implicated in arousal state control. The projection from orexin neurons to monoaminergic cell groups, which include histaminergic, serotonergic, and noradrenergic cells, could be related to arousal-state regulation, while monoaminergic neurons inhibit the REM-activated neurons in the cholinergic nucleus. Therefore, it is probable that the orexin system may have a neuromodulatory effect on arousal states. Given the putative role of orexin in sleep-wakeful-
ness function, increased orexin transmission, reflected as increased plasma orexin-A levels, may affect the arousal response in patients with OSAHS.

Periodic leg movement disorders are frequently accompanied by full awakenings or by signs of EEG arousals, and leg movements are not primary but, rather, are a phenomenon associated with an underlying arousal disorder. In addition, the intracerebroventricular injection of orexin A into rats increases arousal and locomoter activity. The notion of increased plasma orexin levels in patients with OSAHS being a consequence of multiple arousals would be strengthened if multiple arousals from sleep in patients with periodic limb movement disorders would be accompanied by increased plasma orexin levels.

In summary, the fact that plasma orexin-A levels correlated with AHI and the arousal index suggested that orexin-A may play a crucial role in the regulation of the sleep-arousal system. However, the elucidation of the clinical implications of plasma orexin-A concentrations would require a larger sample study and a further examination of the relationship between plasma and CSF orexin-A levels in patients with OSAHS.

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REFERENCES