Diurnal Variation of Cerebrospinal Fluid Hypocretin-1 (Orexin-A) Levels in Control and Depressed Subjects

Ronald M. Salomon, Beth Ripley, John S. Kennedy, Benjamin Johnson, Dennis Schmidt, Jamie M. Zeitzer, Seiji Nishino, and Emmanuel Mignot

Background: Hypocretins, excitatory neuropeptides at monoaminergic synapses, appear to regulate human sleep-wake cycles. Undetectable cerebrospinal fluid hypocretin-1 levels are seen in narcolepsy, which is frequently associated with secondary depression. Shortened rapid eye movement latency is observed in both narcolepsy and depression. Cerebrospinal fluid hypocretin-1 levels have not been reported in mood disorders.

Methods: We examined hypocretin-1 levels in 14 control and 15 depressed subjects. Cerebrospinal fluid was drawn continuously in supine subjects for 24 hours with an indwelling intrathecal catheter under entrained light-dark conditions. Depressed subjects were studied before and after 5 weeks of sertraline (n = 10, three nonresponders) or bupropion (n = 5, two nonresponders).

Results: Hypocretin-1 levels varied slightly (amplitude 10%) but significantly across the diurnal cycle in control subjects, with amplitude significantly reduced in depression (3%). Levels were lowest at midday, surprising for a hypothetically wake-promoting peptide. Mean hypocretin levels trended higher in depressive than in control subjects. Hypocretin-1 levels decreased modestly but significantly after sertraline (−14%) but not bupropion.

Conclusions: Our results are consistent with previous physiologic findings in depression indicating dampened diurnal variations in hypocretin-1. The finding that sertraline but not bupropion slightly decreased cerebrospinal fluid hypocretin-1 indicates a serotoninergic influence on hypocretin tone.

Key Words: Depression, hypocretin, sertraline, bupropion, circadian rhythms, sleep

Introduction

Among pathophysiologic findings in depression, relationships with sleep and circadian abnormalities have been emphasized (Detre et al 1972; Nowell and Buyssse 2001; Reynolds et al 1987; Ringel and Szuba 2001; Winokur et al 2001). Clinically, depressive symptoms early in the course of the disease include sleep disturbances (Nowell and Buyssse 2001). Sleep deprivation, and especially rapid eye movement (REM) deprivation have striking antidepressant effects (Borbely and Wirz-Justice 1982; Gillin et al 2001; Ringel and Szuba 2001; Wu and Bunney 1990). REM sleep is suppressed by almost all antidepressant medications (Duncan 1998, Winokur et al 2001), and decreased REM latency in depression is highly replicable (Bence et al 1992; Kupfer et al 1991). Decreased amplitudes of behavioral, physiologic, and neuroendocrine circadian measures and disrupted responses of the circadian pacemaker to the light-dark cycle are observed in depression (Beersma et al 1983; Healy 1987; Kleitman 1939; Kripke et al 1987; Steiner et al 1987; Van den Hoofdakker et al 1994; Wirz-Justice 1995). Depression is associated with diurnal changes in hypothalamic-pituitary-adenal axis activity and other endocrine abnormalities (Arborelius 1999; Holsboer 2001; Shelton et al 1993; Young et al 1991). The hypocretin (orexin) neuropeptides (Beuckman and Yanagisawa 2002; Mignot 2001; Willie et al 2001), hypocretin-1 and hypocretin-2, are processed from a prepropeptide encoded by a single gene (de Lecea et al 1998; Sakurai et al 1998, 1999). Hypocretin neurons in the tuberal region of the hypothalamus (Peyron et al 1998, 2000) project to the entire neuraxis, including the spinal cord (van den Pol 1999). Extremely dense and almost invariably excitatory projections are noted to aminergic cell groups (e.g., adrenergic locus coeruleus, serotonergic raphe nuclei, histaminergic tuberomammillary nucleus, dopaminergic substantia nigra, and ventral tegmental area; Hungs and Mignot 2001; Peyron et al 1998) and also to cholinergic cell groups (Beuckman and Yanagisawa 2002; Taheri et al 2002). Three decades of hypothesis development links transmitter systems of each of the respective nuclei to the pathophysiology of depression (Janowsky...

Hypocretin deficiency causes human narcolepsy (Nishino et al 2000; Peyron et al 2000; Thannickal 2000), a disabling disorder characterized by daytime sleepiness, cataplexy, and extremely short REM sleep latency (Mignot 2001). Hypocretin release is higher during the active phase in rats (Fujiki et al 2001; Yoshida et al 2001) and may consolidate wakefulness and reduce sleep. Monoaminergic tone, which is high during wakefulness, decreases during sleep and REM sleep and may be driven by hypocretins (Hungs and Mignot 2001; Kilduff and Peyron 2000; Mignot 2001). In narcolepsy, low hypocretin would reduce monoaminergic activity, leading to daytime sleepiness and abnormally short REM sleep latency (Mignot 2001).

Narcolepsy is frequently associated with depression (Daniels et al 2001), and both are treated with agents that enhance monoaminergic activity and affect sleep physiology. The hypocretin system is activated by sleep (or REM) deprivation (Yoshida et al 2001) and may mediate its antidepressant effects (Mignot 2001). Finally, hypocretins activate the hypothalamic-pituitary-adrenal axis (Jaszberenyi et al 2000; Kuru et al 2000; Russell et al 2001). These relationships led us to study the role of hypocretins in depression. To do so we examined lumbar cerebrospinal fluid (CSF) hypocretin-1 levels in 14 control and 15 depressed subjects (before and after antidepressant therapy) during a 24-hour period.

**Methods and Materials**

**Subjects**

Fourteen control subjects (six of them male, 41 ± 4 years old [all expressions are mean ± SD]) and 15 depressive subjects (five of them male, 39 ± 3 years old) were studied with continuous CSF sampling. Diagnosis was determined by psychiatric interview and confirmed by the *Structured Clinical Interview for DSM-IV Axis I Disorders* and *Structured Clinical Interview for DSM-IV Axis II Disorders* (First et al 1996a, 1996b) according to DSM-IV criteria (American Psychiatric Association 1994). Baseline 17-item Hamilton Rating Scale for Depression (HRSD) score was 17.7 ± 1.1 (Mazure et al 1986). The sample included three subjects with bipolar type I, four with bipolar type II, and others with unipolar illness or a first depressive episode (one patient). On average, patients described 2.5 discrete depressive episodes, although this may be a low estimate because many episodes were described as prolonged and thus may have represented several in series without full interepisode recovery, and most patients described onset early in life with only recent diagnosis. None had illness considered refractory to treatment in previous episodes. The studies were approved by the Vanderbilt University institutional review board and the committee of the National Institutes of Health–supported Vanderbilt General Clinical Research Center (GCRC) from 1990 to present. Patients showing any suggestion of less than optimal cognition and thought processes were excluded because of inability to provide consent. Additionally, seven healthy subjects (four of them male, 27.7 ± 4.86 years old) were studied repeatedly on separate days at different hours across the day and night with standard lumbar puncture procedures with the approval of the Stanford University institutional review board.

At the Vanderbilt University site, subjects were recruited by newspaper advertisement. Depressed patients were in medically stable condition, were 18 to 65 years old, had a DSM-IV major depressive episode, and had been free of antidepressant drugs for at least 2 months. Exclusion criteria included high suicidal risk (e.g., previous severe suicide attempt), past or present psychosis, current tobacco use, and the existence of any other primary psychiatric diagnosis. Subjects were also free of abnormalities on physical examination, electrocardiogram, or extensive laboratory evaluation (including hepatitis screens and pregnancy testing). Eligibility required agreement from the subject to comply with all study requirements. All subjects were reimbursed for inconvenience and expenses and income lost, and depressed patients received free treatment for 8 study weeks. In the case of the limited number of healthy volunteers studied at Stanford University, subjects were recruited by word of mouth, compensated for their effort, and included only if they were free of any medical illness and psychotropic treatment.

At both sites the study and potential risks were fully explained and all questions were answered before informed consent was signed, typically after several days of consideration by the subject along with family members and close friends. Discomfort and inconvenience, most notably spinal headache, and potential risk for serious adverse outcomes, such as the potential for paralysis or death as a result of treatment-resistant bacterial meningitis resulting from participating in the CSF collection procedure, were all thoroughly discussed.

**Overall Design**

In the continuous 24-hour CSF sampling studies, healthy subjects were studied on only one occasion, whereas depressed subjects were studied before and after 5 weeks of antidepressant therapy with sertraline (n = 10, three nonresponders) or bupropion (n = 5, two nonresponders). All subjects consumed a controlled, balanced monoamine diet provided by the outpatient GCRC for three daily meals for 3 days before each catheterization. During the entire predmission and inpatient study, subjects were not permitted to consume methylxanthine-containing foods, such as caffeinated beverages, chocolate, and artichokes. Diets were designed by the nutritionist according to individual taste preferences to maximize compliance. Balanced meals contained approximately 50% carbohydrates, 20% protein, and 30% fat. On admission into the GCRC, meals were given only at 6:30 AM and 9:30 AM, yielding the same total daily intake. All subjects remained strictly supine during the precatheterization (12 hours), catheterization (48 hours), and postcatheterization (24 hours) periods and were not allowed to rise for any reason (including being required to toilet in the supine position). Thus the total time that each subject was required to be recumbent in bed was a
Table 1. Depressed Patient Protocol and Rating Scale Results

<table>
<thead>
<tr>
<th>Time</th>
<th>Study Phase</th>
<th>Inpatient Days</th>
<th>CSF Collection</th>
<th>Medication</th>
<th>Physical Symptom Checklist Score</th>
<th>HRSD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Screening and consent</td>
<td>0</td>
<td>No</td>
<td>No</td>
<td>Maximum 2.7, Mean ± SD 1.7</td>
<td>End 18.7</td>
</tr>
<tr>
<td>Week 2</td>
<td>Baseline CSF sampling</td>
<td>4</td>
<td>Catheter, 48 hours</td>
<td>No</td>
<td>Beginning 2.7, End 14.8</td>
<td></td>
</tr>
<tr>
<td>Week 3</td>
<td>Medication treatment</td>
<td>0</td>
<td>No</td>
<td>Sertraline or bupropion</td>
<td>Beginning 2.7, End 8.4</td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>Medicated, CSF sampling</td>
<td>4</td>
<td>Catheter, 48 hours</td>
<td>Sertraline or bupropion</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Week 5</td>
<td>Final assessments</td>
<td>0</td>
<td>No</td>
<td>Sertraline or bupropion</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

For physical symptom checklist, baseline symptoms were rated before first CSF study as 1, none or negligible; 2, mild; 3, moderate; 4, severe. There was no clinically or statistically significant worsening during the study from baseline at any point, although there were frequently significant improvements.

Antidepressant Treatment and Definition of Clinical Trial Response

As shown in Table 1, depressed patients were assigned to 8 weeks of open-label, outpatient treatment with either sertraline (Zoloft, all of the first five patients plus five randomly assigned of the other 10 depressed subjects) or bupropion (Wellbutrin-XR, all five randomly assigned). Antidepressant treatment was initiated in the morning of the third day after the completion of pretreatment lumbar intrathecal catheterization. All patients were free of spinal headaches before beginning therapy and reported taking all their medications. Sertraline therapy began with 50 mg each morning for 7 days and increased to 100 milligrams each day for 7 weeks. Bupropion was given as extended release 150-mg tablets each morning for the first 7 days and then 150 milligrams each morning and each evening at 1:00 PM for 7 weeks.

The HRSD was the primary mood outcome measure. An HRSD decrease of 50% or reaching 8 or less defined remission at 5 weeks. After 8 weeks of treatment, end point remission was defined similarly as meeting both a priori criteria: (1) decrease on the HRSD total score of at least 50% from the mean of HRSD screening and initial GCRC intake scores at week 8 of treatment (study week 10), and (2) an HRSD total score at the final week 8 assessment of 8 or less. According to these criteria, only five of 15 subjects were nonresponders, three with sertraline and two with bupropion.

Monitoring of Side Effects and Adverse Events

Weekly outpatient and twice-daily inpatient ratings for study-related adverse events were recorded and analyzed. There was no statistically or clinically significant worsening relative to baseline physical symptom checklist, which included headache, constipation, poor memory, nausea, drowsiness, blurred vision, increased appetite, difficulty starting urination, trouble concentrating, nightmares, difficulty sitting still, irregular heartbeat, diarrhea, frequent urination, dry mouth, decreased appetite, tremors or shakiness, skin rash, ringing in ears, sweating, fainting or light-headedness, poor muscle coordination, and muscle stiffness. A total physical symptom checklist score was also generated and did not vary across the study (Table 1), although a small improvement was noted in treated depressed subjects (data not shown). Importantly, there were also no time points with group differences for headache. Three days after withdrawal of the CSF catheter, all depressed subjects were free of headache. Most subjects had extremely limited symptom changes through the course of the study. Severe headaches were unusual, any headache was infrequent, and all postspinal headaches resolved with local blood patch administered between 24 and 72 hours after the catheter removal. A blood patch procedure was offered and performed after seven of the 30 catheterizations that were performed during this study period.

CSF Hypocretin-1 Assays

CSF hypocretin-1 levels were measured in duplicate with an established direct radioimmunoassay in 10-min samples (50 μL × 2; Ripley et al 2001). The CSF hypocretin-1 assay was highly reliable, showing stability in samples measured repeatedly from samples kept frozen (−80°C) for as long as 10 years, after 72 hours at room temperature, or repeatedly thawed and frozen (Nishino et al 2001). Specificity was shown (r = .99, p < .001) by linear correlation of exogenous hypocretin-1 (0–1200 pg/mL) in 1 mL CSF to the obtained level measurements (Nishino et al 2001). Measured levels were at least 15 times greater than...
minimal detectable levels (Nishino et al. 2001). In 11 subjects (six control subjects and five depressed subjects before and after treatment), all samples were assayed. In the remaining eight control subjects and 10 depressed (before and after) subjects, one 10-min sample was assayed per hour.

Data Analysis

To study overall diurnal variation, data smoothing was performed by averaging 10-min hypocretin-1 values across 2 hour periods. Data were normalized within subjects by dividing each data point by the mean 24-hour hypocretin value of the subject and then averaging across subjects within treatment groups. Group data were fitted with a 24-hour cosine-wave function (\( \frac{a}{1 + b \cos(2\pi/24 \times x + c)} \)) with a nonlinear, least-squares fitting method (Levenberg-Marquardt algorithm, Microcal Origin v.6.0; Microcal Software, Northampton, MA). The phases of hypocretin maxima and hypocretin fluctuation amplitude (half peak to trough) were estimated for each group.

Daytime and nighttime values were also compared. In each individual subject mean hypocretin levels during the day (11 AM–6:00 PM) and night (11 PM–6:00 AM) were calculated, together with mean 24-hour individual hypocretin-1 levels. The 7-hour day and night windows were selected according to light-dark schedule (lights were off from 11 PM–6:00 AM). Day-night differences in mean levels (11 PM–6:00 AM minus 11AM–6:00 PM) for each subject were considered an approximation of the amplitude on each experimental night. We compared daytime and nighttime hypocretin levels within each of the three subject groups (two-tailed, paired Student t tests), daytime and nighttime levels, and day-night differences between groups (single-factor ANOVA) and the effect of antidepressant treatment (two-factor ANOVA). Statistical analyses were performed on a personal computer with Excel 2000 (v. 9.0.2720; Microsoft Corporation, Redmond WA), with the exception of the two-factor ANOVAs that were performed with the Java script found on http://faculty.vassar.edu/lowry/anova2u.html (Richard Lowry, Vassar College, 2001).

Results

Diurnal Variation of Hypocretin-1 Levels in Depressed and Control Subjects

Overall the concentration of hypocretin-1 obtained from human lumbar CSF did not vary significantly with age or gender (not shown), in agreement with previous reports. In control subjects, hypocretin levels varied very slightly but consistently and significantly across the diurnal cycle (\( p < 0.001 \)). Contrary to an expected increasing level gradient across serial samples that is often reported in monoamine metabolite measures, levels of hypocretin declined very gradually during the first few hours of sampling (average 8.8% in 3.5 hours). This differs from the monoamine effect in that our opening fluid samples were reserved for culture and microscopy as part of a safety procedure, and the hypocretin gradient was observed over a longer period. Sinusoid curve-fitting results are indicated in Table 2 and Figure 1. A 24-hour sine wave accurately predicted the form of the data from control subjects (\( r^2 = .92 \)), with an amplitude of 6% and a peak occurring several hours after lights out (1:00–2:00 AM; Table 2 and Figure 1). In the depressed subjects, both before and after treatment, a sine wave poorly predicted the overall oscillation of lumbar hypocretin (\( r^2 = .32 \) before treatment, \( r^2 = .42 \) after treatment), and the amplitude of wave was apparently reduced, with nonoverlapping 95% confidence intervals between control subjects and depressed subjects before or after treatment. There may have been a small recovery of amplitude in the depressed patients after treatment, though such an effect is not statistically evident from the sine-fitting data. In an additional limited set of ambulatory control subjects who underwent multiple lumbar punctures

![Graph showing sinusoidal fitted curves to 2-hour interval measurements of CSF hypocretin-1 levels from lumbar catheterization in control (squares), depressed (triangles), and treated (circles) subjects. Error bars represent SEM.](image)
Basal Levels of Hypocretin-1 in Depressed and Control Subjects

Basal 24-hour, daytime, and nighttime levels did not differ between supine control subjects and untreated patients, but slightly higher levels were observed in depressed patients, especially during the day (daytime concentrations in control subjects 251.7 ± 11.5 pg/mL and in pretreatment depressed subjects 275.8 ± 11.5 pg/mL, nighttime concentrations in control subjects 274.0 ± 10.6 pg/mL and in pretreatment depressed subjects 280.9 ± 12.2 pg/mL, mean-24 hour concentrations in control subjects 265.0 ± 11.0 pg/mL and in pretreatment depressed 281.2 ± 11.8 pg/mL, differences not significant; Table 3). These values were also similar to previously reported data in ambulatory control subjects and markedly higher than levels observed in narcolepsy. As suspected from the sinusoid analysis, there was a highly significant difference between daytime (11:00 AM–6:00 PM) and night time (11:00 PM–6:00 AM) hypocretin-1 concentrations in control subjects ($p < .001$, paired two-tailed t test). The day-night difference was lost in depressive subjects before ($p = .32$, paired two-tailed t test) or after ($p = .24$, paired two-tailed t test) treatment.

Effects of Antidepressant Treatment on Hypocretin-1 Levels

Drug effects were associated with changes in hypocretin levels, whereas mood responses were not. Hypocretin levels decreased modestly but significantly both overall with sertraline treatment: ($-14\%$, $p < .01$), both during the day ($-14\%$, $p < .05$) and during the night ($-14\%$, $p < .005$) but not with bupropion treatment: (overall $-3\%$, $p = .59$; daytime $-4\%$, $p = .35$; nighttime $-1\%$, $p = .91$; all $p$ values from two-tailed, paired $t$ tests; Table 4). Absolute concentrations of hypocretin during the day, night, or 24-hour sampling period, as well as the difference between day and night (a measure of circadian amplitude), were affected neither by treatment nor outcome (two-factor ANOVA $p > .38$ for all comparisons and interactions; Table 4). Pretreatment to posttreatment changes in hypocretin concentrations during the day,

at different times of day, levels did not vary significantly but were generally slightly higher during the nighttime, in agreement with the continuous sampling data set (Figure 2).

Table 3. Mean ± SEM Hypocretin Levels in Control Subjects and Depressed Patients before and after Treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean 24-Hour Concentration (8:00 AM–7:00 AM)</th>
<th>Daytime Concentration (11:00 AM–6:00 PM)</th>
<th>Nighttime Concentration (11:00 PM–6:00 AM)</th>
<th>Day/Night Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Subjects</td>
<td>264.99 ± 11.00</td>
<td>251.70 ± 11.54</td>
<td>273.98 ± 10.64</td>
<td>22.28 ± 5.03</td>
</tr>
<tr>
<td>All Depressed Patients (pretreatment)</td>
<td>281.22 ± 11.80</td>
<td>275.78 ± 11.45</td>
<td>280.91 ± 12.17</td>
<td>5.12 ± 4.95</td>
</tr>
<tr>
<td>All Depressed Patients (posttreatment)</td>
<td>252.45 ± 8.59</td>
<td>245.04 ± 10.14†</td>
<td>253.04 ± 7.94†</td>
<td>8.00 ± 6.52</td>
</tr>
</tbody>
</table>

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![Figure 2. Seven healthy volunteers had spinal taps at different times of the day, with five of them having at least two taps at different times of the day (singleton values not shown). Symbols connected by solid lines indicate the concentration of hypocretin-1 (Hcrt-1) in the CSF of single spinal taps for each individual. Multiple samplings in the same individual are connected with solid lines. The averages of the samples obtained in the early afternoon, early night, and late night are shown as diamonds, with x and y axis error bars representing SEM, and are connected by a dashed line. The time of day is indicated on the x axis, and the dark period is approximated by the black bar (10:00 PM–7:00 AM).
24-hour cycle. Levels fluctuated moderately but significantly across the continuously sampled (for 24 hours) CSF hypocretin-1 lumbar puncture. Under conditions of constant bed rest, this study presents data from subjects confined to bed rest by treatment or outcome (two-factor ANOVA night, or 24-hour sampling period were also not affected than in the nonhuman mammalian studies. This temporal difference most likely reflects an anatomically delayed and dilution-dampened oscillation of cortical hypocretin, with actual brain fluctuations being more consistent with the monkey data. Consistent with this hypothesis, studies have shown a 90- to 120-minute delay in equilibration between higher CSF compartments and the lumbar sac (Di Chiro et al 1976; Salomon et al 1998, unpublished data). Intense projections from the hypothalamus to the spinal cord have been reported (van den Pol 1999), but even if spinal release occurs, the cell bodies located in the lateral hypothalamic area are likely to be active during the active period. This would not explain the discrepancy between peak and activity.

Hypocretins are uniquely positioned for involvement in depression. Whereas hypocretin cell bodies are all localized within the perifornical area, extremely dense, almost invariably excitatory projections are noted in aminergic cell groups (e.g., adrenergic locus coeruleus, serotonergic raphe nuclei, histaminergic tuberomammillary nucleus, dopaminergic substantia nigra, and ventral tegmental area [Peyron et al 1998; Hungs and Mignot 2001]) and also cholinergic cell groups (Taheri et al 2002). Two receptors have been identified (Sakurai et al 1998) and neuroanatomically mapped (Marcus et al 2001). The role of the hypocretin system in narcolepsy was first demonstrated in animal models (Chemelli et al 1999; Lin et al 1999). Hypocretin deficiency causes human narcolepsy (Nishino et al 2000; Peyron et al 2000; Thannickal 2000), a disabling disorder characterized by daytime sleepiness, part of the active day period, just before the transition to darkness (Zeitzer et al 2002). This has led to the suggestion that hypocretin may be an important wake promoting signal, opposing sleep debt in the second part of the active phase (Mignot 2001; Yoshida et al 2001; Zeitzer et al 2002). In our human lumbar CSF study, highest values were observed around 2:00 AM (3 hours after lights out), which is several hours later than in the squirrel monkey. Furthermore, changes were of much smaller magnitude than in the nonhuman mammalian studies. This temporal difference most likely reflects an anatomically delayed and dilution-dampened oscillation of cortical hypocretin, with actual brain fluctuations being more consistent with the monkey data. Consistent with this hypothesis, studies have shown a 90- to 120-minute delay in equilibration between higher CSF compartments and the lumbar sac (Di Chiro et al 1976; Salomon et al 1998, unpublished data). Intense projections from the hypothalamus to the spinal cord have been reported (van den Pol 1999), but even if spinal release occurs, the cell bodies located in the lateral hypothalamic area are likely to be active during the active period. This would not explain the discrepancy between peak and activity.

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cataplexy, and extremely short REM sleep latency (Mignot 2001). c-Fos studies indicate that hypocretin activity is higher during wakefulness (Estabrook 2001). Hypocretin release is higher during the active phase in rats (Fujiki et al 2001; Yoshida et al 2001) and may consolidate wakefulness and reduce sleep.

Whereas decreased hypocretin tone in narcolepsy is associated with depression, our data indicate no dramatic decrease in baseline CSF hypocretin values in depression. If anything, slightly higher hypocretin values were found in this small number of depressed subjects (Table 2). This finding indicates that hypocretin deficiency is an unlikely cause for depression. Slightly increased hypocretin levels in depression may rather represent disturbed sleep and activity in depression or compensatory mechanisms. Studies with larger numbers are, however, needed to expand on this finding.

In contrast with the lack of striking baseline hypocretin level differences, we found significantly decreased diurnal variation in depressed subjects versus control subjects. These results are consistent with previous findings in depression indicating that diurnal physiologic measures are often dampened in this condition (Van den Hoofdakker and Beersma 1988; Wirz-Justice 1995). The primary source of the observed diminution in signal amplitude cannot be ascribed definitively to any of the involved sites. This observation does, however, provide evidence in depression that diminished circadian rhythms of behaviors, physiologic measures, and peripheral neuroendocrine functions can also be observed centrally. Evidence suggests dampened monoamine metabolite fluctuation in depression (Salomon et al 1998, unpublished data). Sleep fragmentation in depression (Benca et al 1992) may be caused by decreased hypocretin fluctuation. Interestingly, however, relief of depressive symptoms was not correlated with restored diurnal rhythmicity in this small sample. Still, a slight improvement in amplitude was noted in treated subjects. This is highly vulnerable to error because of the small sample size and the relatively mild to moderate depressive episodes in these patients and thus will require replication; however, our results suggest that depressive mood and diurnal variation in hypocretins may be independent characteristics. Chronic treatment studies in depression may lead to more significant changes in hypocretin diurnal variation, especially if the restoration of sleep patterns from direct soporific effects is avoided and the delayed normalization of sleep is related to peptidergic mechanisms.

We also explored whether antidepressant treatment modified CSF hypocretin levels in patients with depression. A possible pharmacologic effect not related to antidepressant response was observed. We found that treatment with sertraline but not bupropion was associated with decreased hypocretin levels, suggesting a small but significant serotonergic influence on hypocretin tone after 5 weeks of treatment. The finding that bupropion, a dopaminergic and adrenergic reuptake blocker with significant wake promoting effect (Nishino et al 1998) did not modify hypocretin levels was rather surprising. The fact that sertraline is a more potent REM-suppressing agent than bupropion (Winokur et al 2001) may be significant in explaining this difference. Alternatively, changes caused by bupropion may be observed either acutely, after initial dosing, or more delayed in recovery. Stimulant medications such as amphetamine-like dopamine releasing agents and reuptake inhibitors have not dramatically modified CSF hypocretin levels in patients with narcolepsy and hypersomnia (Mignot et al, in press), but acute changes in hypocretin are observed in preclinical studies of dopamine releasing agents. Studies including narcoleptic subjects treated with serotonin reuptake inhibitors may be needed to study this effect.

In conclusion, our studies of CSF hypocretin-1 levels in depression indicate that a decrease in mean hypocretin release is not a likely cause of depression. In control subjects, CSF hypocretin-1 levels were found to vary slightly but significantly across the 24-hour study period, with higher levels observed at night. In depression, reduced amplitude of diurnal variation was observed. Additional studies in depression are needed to expand on these findings.

References


