Reduced orexin levels in the cerebrospinal fluid of suicidal patients with major depressive disorder

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Abstract Orexins are neuropeptides selectively expressed in a small number of neurons in the lateral–posterior hypothalamus. We measured orexin-A in the cerebrospinal fluid (CSF) of 66 patients with major depressive disorder (MDD), dysthymia and adjustment disorder after a suicide attempt. Blood samples confirmed that the patients were free from antidepressive and neuroleptic medication at the time of the lumbar punctures. CSF levels of orexin-A were significantly lower in patients with MDD than in patients with adjustment disorder and dysthymia. Orexin correlated significantly with CSF levels of somatostatin, delta sleep inducing peptide-like immunoreactivity (DSIP-LI) and corticotrophin releasing factor (CRF), but not with leptin or vasopressin. Plasma levels of thyroid-stimulating hormone (TSH) were not reduced in MDD patients, and did not correlate with CSF-orexin. Our results suggest that suicidal patients with MDD have distinct neurobiological features, involving compromised levels of hypothalamic peptides regulating the state of arousal.

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1. Introduction

Suicide is a major cause of death worldwide (Bertolote et al., 2004). Disturbances of sleep, vigilance and appetite are hallmarks of severe depression and suicidality (Singareddy and Balon, 2001). Hypothalamic peptides are likely to significantly influence these behavioural symptoms. Subgroups of patients with major depressive disorder (MDD) exhibit disturbed hypothalamic–pituitary–adrenal (HPA)-axis and there is comorbidity between chronic stress and certain types of depression (Swaab et al., 2005).

Dogs with the neurological condition narcolepsy have been found to have mutations in the orexin receptor gene (Lin et al., 1999), and orexin knockout mice display similar symptoms (Chemelli et al., 1999). Narcolepsy is a disabling condition where the affected individuals among other symptoms suffer from excessive daytime sleepiness and severe disturbances of the sleep pattern. Human patients with narcolepsy have low to undetectable levels of orexin in the cerebrospinal fluid (CSF) (Peyron et al., 2000; Nishino et al., 2001). Taken together, these findings demonstrated that orexins have an important role for regulation of sleep and state of arousal.

Neurons containing orexin have widespread projections to many CNS regions, including hormonal centres in the hypothalamus. Several interactions with hormones in the HPA-axis have been demonstrated (Spinazzi et al., 2006). Levels of CSF-orexin have been measured in different neurological...
conditions. In addition to narcolepsy, orexin can be affected in subarachnoid haemorrhage and other conditions with a trauma to the hypothalamus (Baumann et al., 2005). There are also reports of low levels of orexin in patients with neurodegenerative diseases affecting the hypothalamus, such as Niemann–Pick's disease (Vankova et al., 2003) and some cases of Parkinson's disease (Drouot et al., 2003).

Although disturbances of vigilance and appetite are common in psychiatric disorders, only few studies to date have investigated orexin in psychiatric patients. We have recently shown that low CSF-orexin levels correlate with more pronounced symptoms of inertia and slowness of movement, as investigated in a cohort of 100 patients with different psychiatric diagnoses (Brundin et al., 2006). In the same study, we found that patients with low CSF-orexin levels were rated as more globally ill, using the Comprehensive Psychopathological Rating Scale (Asberg et al., 1978). In another study, CSF-orexin was measured in 15 patients with unipolar and bipolar disorder (Salomon et al., 2003). The patients were found to exhibit a reduced diurnal variation of orexin. Nishino et al. (2002) have reported an association between orexin levels and sleep latency in 13 schizophrenic patients.

The aim of the present study was to investigate CSF-orexin A in suicidal patients with different psychiatric diagnoses. We were also interested in examining whether orexin levels were correlated with other factors involved in regulation of sleep and appetite in suicidal patients.

2. Experimental procedures

2.1. Overall design of the study

This study was approved by the Lund University Medical Ethics Committee. Patients gave a written informed consent to participate in the research program. Seventy-six patients were admitted to the University Hospital after a suicide attempt, between 1987 and 2001. They were recruited to the study from the emergency room, the medical intensive care unit, or from a general psychiatric ward. The patients did not receive any antidepressive or neuroleptic medication during a wash-out period consisting of 13 ± 6 (mean ± SD) days. Some of the patients received occasional doses of benzodiazepine tranquillizers during this period. No patient received any tranquilizer for at least 9 h before the spinal tap, which was performed after a night of fasting and bed rest. Twenty-one patients received some kind of benzodiazepine during the wash-out period, and 43 did not. For two patients, the information is incomplete regarding this subject. The benzodiazepines administered included flunitrazepam (n=7), oxazepam (n=6), lorazepam (n=5), nitrazepam (n=4), diazepam (n=1) and alprazolam (n=1). Two patients received zopiclone and one patient received chloral hydrate. At the end of the wash-out, a lumbar puncture was performed as described below.

2.2. Patients

Ten patients with traces of neuroleptic or antidepressive medication in their blood samples were excluded, leaving 66 patients in the study. On the day of the lumbar puncture, the patients were rated for psychiatric symptoms using the Comprehensive Psychopathological Rating Scale

![Figure 1](image-url) Mean orexin-A levels in the cerebrospinal fluid of patients with major depressive disorder (MDD) (n=32), dysthymia (n=23) and adjustment disorder (n=11), respectively. Bars indicate 95% confidence interval. Orexin levels were significantly lower in patients with MDD than in patients with dysthymia, or adjustment disorder (one-way analysis of variance followed by Bonferroni–Dunn's post-hoc test). The mean orexin level was 195 ± 24 pg/ml (mean ± SD) in patients with MDD, 219 ± 21 pg/ml in patients with dysthymia and 213 ± 20 pg/ml in patients with adjustment disorder.
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2.6. Statistical analysis

The Statistical Package for the Social Sciences (SPSS) program version 13.00 for Windows was used for all statistical analyses. For comparisons between two groups, Student’s t-test was used for parametric data, and Mann–Whitney’s U-test for nonparametric data. For multiple parametric comparisons, a one-way analysis of variance (ANOVA) was used, followed by Bonferroni–Dunn’s post-hoc test to adjust for multiple comparisons. For multiple non-parametric comparisons, Kruskall–Wallis test was used. Tests of non-parametric correlations were performed using Spearman’s rho and parametric correlations using Pearson’s r.

3. Results

3.1. CSF-orexin and mood disorders

Orexin levels in CSF were significantly lower in patients with MDD than in patients with dysthymia or adjustment disorder (one-way ANOVA, p = 0.001, followed by Bonferroni–Dunn’s post-hoc test). Figure 3 shows a significant positive correlation between orexin and DSIP-LI (p < 0.005).

Figure 2

Individual CSF-orexin levels in each of the 66 patients in the study, distributed over the months when the suicide attempts took place. There was no significant difference in the orexin levels between different months. Filled circles represent the five patients who later committed suicide. There was no significant difference between these patients and those who did not commit suicide on a later attempt with regards to orexin levels.

Figure 3

Correlation between orexin and delta sleep inducing peptide-like immunoreactivity (DSIP-LI) levels in the CSF of the patients. There was a significant positive correlation between orexin and DSIP-LI (p < 0.005).
hoc-test, which yielded \( p < 0.01 \) for comparison with adjustment disorder and \( p < 0.01 \) for comparison with dysthymia. The mean orexin level was \( 195 \pm 24 \text{ pg/ml (mean } \pm 1 \text{ SD)} \) in patients with MDD, \( 219 \pm 21 \text{ pg/ml in patients with dysthymia and } 213 \pm 20 \text{ pg/ml in patients with adjustment disorder (Fig. 1).} \)

### 3.2. Orexin and suicidality

There was no difference in the CSF-orexin levels between non-violent \( (n=50) \) and violent \( (n=16) \) suicide attempters (Student’s \( t \)-test, \( p > 0.05 \)). Furthermore, we found no difference between repeaters \( (n=21) \) and non-repeaters \( (n=45) \) \( (t\text{-test, } p > 0.05) \).

The suicide attempts that led to the patients being included in this study took place during all months of the year. Fifteen of the attempts occurred in the month of March. Only one suicide attempt took place in each of the months July and August.

There was no significant difference in the orexin levels related to the month when the suicide attempts took place (Kruskall–Wallis analysis, \( p > 0.05 \)) \( (\text{Fig. 2).} \)

Five of the patients later committed suicide \( (\text{Fig. 2).} \) CSF-orexin did not differ significantly between patients who committed suicide, and those who did not \( (n=61) \) (Mann–Whitney’s \( U \)-test, \( p > 0.05 \)). Two patients committed suicide within 1 year of the initial attempt. One patient had the diagnosis MDD and an orexin value of 164 pg/ml at the first attempt. The second patient had the diagnosis adjustment disorder and had an orexin value of 197 pg/ml at the first attempt.

One patient in the group with MDD had 117 pg orexin/ml in CSF, which is almost 3 SD lower than the mean value for the whole group. This patient was a woman, first-time suicide attempter and the attempt was violent. She did not have any somatic illness. When the orexin values were analysed with this patient excluded, the statistically significant differences between the groups still remained.

### 3.3. Impact of age, gender and storing-time

Orexin levels in all groups did not differ significantly between male and female suicide attempters (Student’s \( t \)-test, \( p > 0.05 \)). Neither was there any correlation between the age of the suicide attempter and the orexin levels (Pearson’s \( r = -0.08, p > 0.05 \)).

The storage time of the samples ranged from 5 to 19 years. The CSF samples were maintained at \(-80{\,}^\circ\text{C} \) and were never thawed prior to the analyses. We examined if there was a correlation between orexin levels and storage time, but found no such correlation \( (p > 0.05 \text{ for all groups. Spearman’s } r = 0.05) \). It has previously been demonstrated that orexin is very stable in both aged frozen samples and in repeatedly thawed and refrozen samples \( (\text{Nishino et al., 2001) .} \)

### 3.4. CSF-orexin and other neuropeptides

Orexin levels correlated significantly with CSF levels of three other peptides involved in the regulation of sleep and appetite: DSIP-LI (Pearson’s \( r = 0.57, p < 0.005 \)), somatostatin \( (\text{Pearson’s } r = 0.52, p < 0.01) \) and CRF \( (\text{Spearman’s } r = 0.47, p < 0.05) \), \( (\text{Figs. 3, 4 and 5).} \) However, we found no significant correlation between CSF-leptin and CSF-orexin \( (\text{Spearman’s } r = -0.1, p > 0.05) \) \( (\text{data not shown).} \)

In order to test the hypothesis that suicidal subjects with MDD may have a compromised function of the hypothalamo-pituitary system as a whole, we also analyzed orexin in relation to the levels of TSH in plasma and vasopressin in the CSF. The plasma TSH levels did not differ between the three groups of patients \( (\text{one-way ANOVA, } p > 0.05) \), and all samples were within the normal range of a standard population. Mean TSH plasma levels were \( 1.9 \pm 1.0 \text{ mU/l (mean } \pm 1 \text{ SD)} \) in the MDD group, \( 1.4 \pm 0.6 \text{ mU/l in the patients with adjustment disorder and } 1.5 \pm 0.6 \text{ mU/l in the patients with dysthymia. There was no correlation between CSF-orexin and plasma-TSH (Pearson’s } r = -0.37, p > 0.05 \). For vasopressin, levels have previously been shown not to differ between the same groups of patients \( (\text{Traskman-Bendz et al., 1992).} \) We did not find any correlation between CSF-orexin and CSF-vasopressin \( (\text{Pearson’s } r = 0.16, p > 0.05) \).
3.5. Anxiolytic treatment and CSF-orexin levels

There was no significant difference in CSF-orexin between patients who received any kind of benzodiazepine treatment (including the related substance zopiclone), 202±32 pg/ml (±1 SD), and patients who did not, 208±20 pg/ml (Student’s t-test). The patient who received chloral hydrate had an orexin level of 199 pg/ml. One patient had an extremely low orexin level, 117 pg/ml, and this patient tested positive for diazepam in the wash-out blood sample. The patient who had the highest CSF orexin level, 258 pg/ml, also tested positive for a benzodiazepine, lorazepam, in the wash-out blood sample.

4. Discussion

We analysed CSF orexin-A in 66 patients who recently attempted to commit suicide. The orexin level was significantly lower in the group of patients with MDD than in patients with adjustment disorder and dysthymia. Orexin correlated significantly with CSF-levels of somatostatin, DSIP-LI and CRF, three other hypothalamic peptides involved in the regulation of sleep and appetite. We have previously shown that these three peptides are significantly reduced in suicidal MDD patients (Traskman-Bendz et al., 1992). In contrast, CSF-vasopressin, CSF-leptin and plasma-TSH did not correlate with CSF-orexin. TSH plasma levels were normal in all patients, and did not differ between the three groups.

A comparison between patients suffering from MDD and those afflicted by adjustment disorder and dysthymia is very interesting. Based on the differences in clinical presentations, we hypothesize that the underlying neurobiological features also differ between suicide attempters given these diagnoses. Patients with MDD differ from each of the other patient groups in important aspects. In adjustment disorder, the patients have been exposed to clearly identifiable external events or stressors, which precipitated the disorder. The duration of adjustment disorder is also by definition relatively short (less than 6 months). Typically, these patients have been completely healthy as recently as a few weeks before the suicide attempt. They can be argued to most closely resemble healthy subjects, and were is a condition that is relatively stable over at least 2 years in order for the patient to obtain the diagnosis. Symptoms are by definition milder than those in MDD (American Psychiatric Association, 1987).

We found that orexin levels were significantly lower in suicide attempters with MDD than in those with dysthymia and adjustment disorder. An important limitation to our study is the lack of a group of healthy controls. We cannot, therefore, make any claims regarding if orexin levels differ between suicide attempters and normal healthy individuals. The range of orexin levels in normal individuals is large. In one study comparing CSF-orexin between different patient groups, orexin levels ranged between 224–653 pg/ml in control subjects, and were <100 pg/ml in narcoleptic cases (Ripley et al., 2001). Similar values have been reported in other studies (Grady et al., 2006; Baumann et al., 2004). The values reported for all three groups of patients in our study are thus somewhat lower than those reported in normal individuals, but substantially higher than those observed in narcolepsy. Direct comparisons of radioimmunoassay orexin levels between studies should be done with caution though, because of the possibility of intra-assay variability. In narcolepsy, there is an almost complete absence of orexin and orexin neurons, and this leads to severe disturbances in the transitions between sleep and wakefulness (Saper et al., 2005). We suggest that smaller changes in the orexin levels lead to different and subtler symptoms in MDD patients. Our previous results show that low CSF-orexin correlate with lassitude and slowness of movement in psychiatric patients (Brundin et al., 2006).

To our knowledge, CSF-orexin in depressed patients has been investigated in only one earlier study (Salomon et al., 2003). The authors found reduced diurnal variation of orexin levels in 15 depressed subjects, suggestive of an altered orexinergic function in this patient group. However, the mean orexin levels were not different between the depressed patients and controls (Salomon et al., 2003). Importantly, in contrast to our study, suicidal patients were actively excluded. A small number of studies have examined orexin in animal models of depression. The Wistar–Kyoto rat has been proposed to be a model of depression, since it is characterised by decreased locomotion and disturbances of sleep pattern (Dugovic et al., 2000). This particular rat strain exhibits decreased levels of prepro-orexin mRNA as well as reduced size and number of orexin neurons in the hypothalamus (Taheri et al., 2001, Allard et al., 2004). This supports the concept that changes in orexin neurotransmission may be involved in the generation of depressive symptoms.

Most of the patients who made suicide attempts by intoxication used high amounts of benzodiazepines. This does not seem to have affected orexin levels, at least not in the time perspective investigated in our study, since there was no significant difference in CSF-orexin between patients who made a violent and non-violent (intoxication) suicide attempt. Several patients also received tranquillizers during the wash-out period, when they were free from neuroleptic- and antidepressive medication. In an ideal "research situation", the patients should have been completely free of medication, but this would not have been ethically justifiable from a clinical treatment standpoint. It was considered of crucial importance that these suicidal patients had the possibility of anxiety relief during the wash-out period. We did not find any difference in CSF-orexin levels between patients who received tranquilizers and those who did not. Altogether, our findings do not point in the direction that the benzodiazepine treatment, or intoxication, would have affected the orexin levels in any significant way. However, the study design does not allow us to draw any precise conclusions about this matter, which could be the target for future studies.

We have previously shown that CSF levels of somatostatin, DSIP-LI and CRF are reduced in suicidal patients with MDD compared to other suicidal patients (Traskman-Bendz et al., 1992). We now demonstrate that these peptides correlate significantly with measurements of CSF-orexin. Somatostatin is known to regulate secretion of growth hormone (GH), and can also influence sleep and appetite (Steiger, 2006). The DSIP was first isolated from cerebral venous blood in rabbits (Schoenenberger, 1984). Under experimental conditions, DSIP has been shown to have a multitude of biological effects, and is postulated to be a hypothalamic hormone (Kovalzon and Strekalova, 2006). DSIP and analogues have been shown to have certain sleep-promoting effects when...
infused in normal human subjects. Immunohistochemistry has revealed the existence of a DSIP-like protein (or proteins) primarily in the hypothalamus and limbic system (Kovalzon and Strekalova, 2006). However, despite substantial effort, it has not been possible to isolate the DSIP protein or mRNA from humans, nor to localize any specific receptors. Consequently, the term DSIP-like immunoreactivity (DSIP-LI) is used for the reactivity measured. We used a radioimmunoassay to monitor DSIP-LI and observed a strong correlation between CSF-orexin and CSF-DSIP-LI. This finding should be viewed in light of the fact that the existence of DSIP in humans has not yet been fully verified. The significant correlation with orexin, a peptide known to affect arousal, tentatively supports a role for endogenous DSIP (or analogues) in sleep also in humans.

CRF is the prime regulator of the HPA-axis and has been shown to affect sleep as well as the metabolic stress response. CRF has previously been shown to influence the levels of orexin in CSF. In turn, CRF release may also be affected by orexin (Winsky-Sommerer et al., 2004; Sakamoto et al., 2004). Most studies showing interactions between orexin and cortisol secretion have been performed in animals (Spinazzi et al., 2006). Regarding humans, narcoleptic patients display a decreased basal ACTH secretion (Kok et al., 2002). In the present study, we show a correlation between CSF-orexin and -CRF levels, which further supports that orexin and the HPA-axis hormones interact in humans.

Depressed patients often exhibit an activation of the HPA-axis, with elevated cortisol in serum and urine. Cortisol levels have been shown to normalize with antidepressive treatment (Swaab et al., 2005; Westrin, 2000). The HPA-axis may be dysregulated already before the onset of symptoms of depression (Holsboer et al., 1995). Thus, HPA-axis dysfunction may be a cause rather than a result of depression. Although a majority of studies show an activation of the HPA-axis in depression, we and others have previously shown a reduction of CRF in suicidal patients with MDD (Traskman-Bendz et al., 1992; Geracioti et al., 1992). One explanation could be that the HPA-axis becomes exhausted and the peptide secretion reduced in severe and chronic depression. Suicidality in MDD may represent such an end-stage scenario. Interestingly, a recent post-mortem study of severely ill depressed and bipolar patients revealed a 50% reduction of neurons in the hypothalamic paraventricular nucleus (PVN) compared to controls (Manaye et al., 2005). The PVN is known to have a role in the regulation of sleep and appetite and is a major site for production of CRF.

4.1. Conclusion

We found that suicidal patients with MDD have significantly lower CSF-orexin levels than other suicidal patients. This speaks in favour of the hypothesis that suicidal MDD patients exhibit specific neurobiological features. We have shown that CSF-levels of somatostatin, CRF and DSIP-LI are reduced in the same group of patients. Plasma-TSH levels were normal in our study and did not differ between the groups, indicating that there is no generalized hypothalamic hypofunction in the suicidal patients with MDD. This indicates a compromised hypothalamic function foremost regarding peptides regulating the state of arousal, appetite and vigilance. Severe MDD with suicidality may represent the end-stage of a disease involving the hypothalamus, beginning with a hyperfunction of the HPA-axis and progressing into hypofunction of specific hypothalamic areas.

Our results also raise the question of whether CSF-orexin levels could be measured in order to identify individuals with high risk for suicide, among patients with MDD. While this is an exciting idea, the variations of CSF-orexin in a normal population are probably too great for the peptide to be used as a sensitive and specific biomarker. The changes in orexin that we have observed, however, provide important insight into the role of the hypothalamus in the pathogenesis of major depression and suggest that orexin neurotransmission may be a therapeutic target in this disorder.

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Conflict of interest

The authors have no financial relationships that might bias their work.

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References

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Low cerebrospinal fluid hypocretin (orexin) and altered energy homeostasis in human narcolepsy. Ann. Neurol. 50, 381–388.


