Olfactory dysfunction in patients with narcolepsy with cataplexy is restored by intranasal Orexin A (Hypocretin-1)

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Until recently, olfactory dysfunction was an unknown feature of narcolepsy. Orexin A, also called hypocretin-1, is abnormally decreased or undetectable in the cerebrospinal fluid of narcoleptic patients with cataplexies. As hypothalamic orexin-containing neurons project throughout the entire olfactory pathway, from the olfactory mucosa to the olfactory cortex, disturbed orexinergic transmission may crucially be involved in impaired olfactory performance of narcolepsy patients. In our study we analysed the olfactory performance (threshold, discrimination, identification and sum score of these measurements, the TDI score) of narcoleptic patients with cataplexies (n = 10) and of age-, gender-, BMI- and smoker/non-smoker-matched healthy controls (n = 10). We then in a double-blind, randomized, placebo-controlled cross-over design applied orexin A intranasally to seven of the patients and measured 2-phenyl-ethyl alcohol (PEA) single-staircase odour detection thresholds. Compared to the controls, patients showed significantly lower scores for olfactory threshold (patients: median 8.0, range 4.0–10.5; controls: median 9.4, range 7.5–13.3; P < 0.05), discrimination (patients: median 12.5, range 10–15; controls: median 15.0, range 12–16; P < 0.005), identification (patients: median 13.0, range 10–16; controls: median 14.0, range 13–16; P < 0.05) and TDI score (patients: median 33.4, range 30–36; controls: median 38.4, range 35–43; P < 0.0001). In all patients, the PEA olfactory threshold score increased after administration of orexin A (median 11.5, range 6.5–13.25) compared to placebo (median 7.75, range 6.25–11.25; P < 0.05). Our results support the hypothesis that mild olfactory dysfunction is an intrinsic symptom of narcolepsy with cataplexies. The observation that intranasal orexin A restores olfactory function is in favour of this hypothesis. Furthermore, our data support that the pathophysiological mechanism underlying olfactory dysfunction in narcolepsy is the lack of CNS orexin.

Keywords: narcolepsy; cataplexy; orexin A; hypocretin-1; olfactory dysfunction

Abbreviations: HLA = human leucocyte antigen; OD = olfactory dysfunction; PEA = 2-phenyl-ethyl alcohol; RBD = REM sleep behaviour disorder; TDI score = threshold discrimination identification score; UNS = Ullanlinna Narcolepsy Scale


Introduction

Narcolepsy is an intrinsic sleep disorder characterized by excessive daytime sleepiness, disturbed nocturnal sleep and abnormalities in rapid eye movement (REM) sleep regulation. Although excessive daytime sleepiness usually is the predominant symptom, there are some specific symptoms in narcolepsy that clearly differentiate it from other sleep disorders: cataplexy, sleep paralysis and hypnagogic hallucinations (for review, see Nishino, 2007). Recently in this journal, Stiasny-Kolster and colleagues (2005, 2007) reported on impaired olfaction as an unknown additional symptom in narcoleptic patients. In their initial study, they tested the hypothesis that idiopathic REM sleep behaviour disorder (RBD) and olfactory dysfunction (OD) are early predictors of Parkinson’s disease—hyposomnia often preceding the motor symptoms (literature reviewed in
Hawkes, 2003). Their experimental group consisted of patients with idiopathic RBD or with narcolepsy and concurrent RBD—so-called 'symptomatic' RBD. There is no known association between narcolepsy and Parkinson's disease. Hence, it was somewhat surprising that the subgroup analysis between patients with idiopathic and symptomatic RBD did not reveal any differences with regard to their olfactory performance (Stiasny-Kolster et al., 2005). In a subsequent study, comparing narcoleptic patients with and without RBD to healthy controls, Stiasny-Kolster et al. (2007) strengthened the hypothesis that OD is a feature of narcolepsy, independent of concomitant RBD.

Over the past 20 years, the understanding of the pathophysiology underlying narcolepsy has rapidly increased. Mainly based on the tight association of narcolepsy with the human leucocyte antigen (HLA) subtype (DQB1*0602), it has been postulated that the disorder may be autoimmune in nature (Overeem et al., 2008), although no direct evidence for this theory is currently available (Hinze-Selch et al., 1998). However, starting with the discovery of a CNS neuropeptide, orexin A, also called hypocretin-1, by two independent groups (de Lecea et al., 1998; Sakurai et al., 1998), the group of E. Mignot soon discovered that this peptide was abnormally decreased or undetectable in the CSF of a large proportion of patients with narcolepsy (Nishino et al., 2001). Subsequent studies confirmed this observation and found that orexin deficiency in the CSF is highly specific for HLA subtype (DQB1*0602) positive narcolepsy with cataplexy (Mignot et al., 2002; Dauvilliers et al., 2003). It is likely that narcolepsy in those cases is a probably autoimmune-mediated neurodegenerative disease, with selective loss of hypothalamic orexin-containing neurons (Peyron et al., 2000; Thannickal et al., 2000). The fact that hypothalamic orexin-containing neurons project throughout the entire olfactory tract from the nasal mucosa (Gorojankina et al., 2007), via the olfactory bulb (Apelbaum et al., 2005; Hardy et al., 2005; Shibata et al., 2008), to the olfactory cortex (Caillol et al., 2003) can lead to speculation that impaired orexinergic transmission is crucially involved in narcolepsy patients' impaired olfactory performance.

The aim of the present basic research study therefore was to address whether olfactory performance is impaired in the well-defined group of narcolepsy patients with cataplexies compared to healthy matched controls and whether this dysfunction can be linked to the orexinergic deficit and thus restored by intranasal application of orexin A.

Material and Methods

Study design

This study was approved by the local Ethics Committee and the subjects’ informed consent was obtained according to the Declaration of Helsinki. It was conducted in the Department of Psychiatry and Psychotherapy, Christian-Albrechts-University, Kiel, from May 2007 to March 2008. First, we compared the olfactory performance (olfactory threshold, discrimination and identification and sum score of these measurements, the TDI score) and divided attention of patients with narcolepsy to that of carefully age-, gender-, BMI- and smoker/non-smoker-matched controls. All patients and healthy controls were tested in one session between 7:00 and 10:00 p.m. Then, we applied orexin A intranasally in a double-blind, randomized, placebo-controlled cross-over design to narcoleptic patients and measured their olfactory thresholds and divided attention under the two conditions. The patients were tested at approx. 11:00 p.m. in two sessions separated by 2 weeks (±3 days), 15 min after intranasal administration of orexin A or placebo.

Patients and control subjects

Narcolepsy patients were contacted personally through our sleep laboratory and via announcements in a German patient organization’s magazine (DNG e.V. ‘Der Wecker’). The inclusion criteria for patients were narcolepsy with cataplexies according to International classification of sleep disorders, 2nd edition criteria (Billiard, 2007); age >18 years and informed consent. Exclusion criteria were: severe psychiatric or other somatic disorders; acute health conditions affecting the upper respiratory tract (in particular, any flu-like symptoms and nasal congestion); pregnancy or nursing and pathological results in blood testing. All patients were subjected to a physical examination by the first author (P.C.B.) and filled in the Ullanlinna Narcolepsy Score (Hublin et al., 1994). For each narcolepsy patient included, one healthy control meeting the eligibility criteria, matched for demographic parameters (for details see Table 1), was recruited for the study with the help of a healthy subject database of the Department of Psychology at the Christian-Albrechts University.

Olfactory testing

We performed the olfactory testing in a well-ventilated and odourless room. The subjects did not eat or drink anything other than water and did not smoke 15 min prior to measurements. In order to prevent visual identification of the odorant-containing probes, subjects were blindfolded for the testing. In the baseline comparison between healthy subjects and narcolepsy patients with cataplexies, the olfactory performance from all subjects was characterized with the subtests threshold, discrimination and identification of the commercially available ‘Sniffin’ Sticks’ test (Burghart Elektro- und Feinmechanik GmbH, Wedel, Germany), according to Hummel et al. (2007). This well-established test with normative data from more than 3000 healthy subjects was chosen in order to keep our results comparable to those from previous studies (Stiasny-Kolster et al., 2005, 2007). However, the retest-reliability of the ‘Sniffin’ Sticks’ is not good (test–retest reliability coefficient—threshold: 0.6; discrimination: 0.5; identification 0.7; Doty, 2007). Therefore, we decided to use the 2-phenyl-ethyl alcohol (PEA) single-staircase detection threshold, which has a high test–retest reliability (test–retest reliability coefficient: 0.9; Doty et al., 1995), for the patients in the interventional part of our study.

‘Sniffin’ Sticks’

The ‘Sniffin’ Sticks’ subset threshold consists of 16 triplets of felt-tip pens. Two pens contain odourless solvent, the third a particular concentration of n-butanol (geometric series from dilution step 1 with a 4% n-butanol solution to dilution step 16, 1:2^{16}). Odour thresholds were assessed using the single-staircase, three alternative
## Table 1  Demographic and disease-related information on narcolepsy patients and controls

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age (years)</th>
<th>BMI</th>
<th>Smoker</th>
<th>Duration of symptoms (years)</th>
<th>Disease duration—diagnosis (years)</th>
<th>HLA-type</th>
<th>Cataplexies (UNS subscore)</th>
<th>UNS Threshold score</th>
<th>Discrimination score</th>
<th>Identification score</th>
<th>TDI score</th>
<th>PEA threshold score (placebo)</th>
<th>PEA threshold score (orexin)</th>
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<tr>
<td>N1</td>
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<td>28</td>
<td>29.4</td>
<td>Yes</td>
<td>17</td>
<td>11</td>
<td>HLA-DQB1<em>0603, HLA-DRBI</em>1508</td>
<td>3</td>
<td>19</td>
<td>7.75</td>
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<td>11</td>
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<td>27</td>
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<td>17</td>
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<td>8.25</td>
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<td>16</td>
<td>36</td>
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<td>25.5</td>
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<td>13.3</td>
<td>33.4</td>
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</table>

\*P = 0.016  Wilcoxon Signed Rank test
\*P = 0.16  Mann–Whitney  n.s.  n.s.

P = 0.0001  P < 0.05  P < 0.005  P < 0.05  P < 0.0001

M = male; F = female; UNS = Ullanlinna Narcolepsy Scale; n.a. = not assessed. *Did not give consent to orexin administration; bexclusion criterion occurred.
forced choice procedure. Triplets were presented in 20–30 s intervals in a randomized order and the subject was instructed to identify the stick with odour. Starting with the highest dilution, steps presentation was continued until the dilution step at which the subject correctly identified the n-butanol-containing pen two times in a row at the same dilution, which triggered the reversal of the staircase. Threshold was defined as the mean of the last four of seven staircase reversals and could range from 1 to 16, with low scores indicating a high olfactory threshold.

For measurement of odour discrimination we presented 16 triplets of pens with each two containing the same and one a different odour. Subjects had to identify the pen smelling differently. The subjects’ scores could range from 0 to 16, with low scores indicating impaired odour discrimination.

For odour identification, 16 pens with common fragrances were presented in random order. Using a forced-choice task, identification of individual odours was performed from lists of four descriptors each. The subjects’ scores could range from 0 to 16, with low scores indicating impaired odour identification.

From all three subtests, a composite ‘TDI score’ was calculated as the sum of the results obtained for threshold, discrimination and identification measures. The subjects’ TDI scores could range from 1 to 48.

**PEA single-staircase odour detection threshold**

Because of the higher test–retest reliability, we determined the olfactory threshold in the interventional part of our study with the PEA single-staircase test, as described by Doty and colleagues (1995). The procedure was essentially the same as described for the ‘Sniffin’ Sticks’ threshold subtest. However, instead of felt-tip pens filled with n-butanol, we used glass bottles with PEA or odourless solvent. In narcolepsy patients, PEA odour detection threshold was assessed at baseline, after placebo and after orexin A administration. The patients’ threshold scores could range from 1 to 16, with low scores indicating a high olfactory threshold.

**Assessment of attention**

In order to exclude that attention deficits are the reason for impaired olfactory performance, we did a computer-based dual-task test for divided attention immediately prior to each olfactory testing (Test for Attentional Performance 2.1, Psytest, Herzogenrath, Germany). This test is particularly sensitive to impaired attention due to tiredness (Weess et al., 2000). The study participants were asked to process a visual and acoustic task in parallel. For the visual task, crosses appear in a 4 × 4 matrix on a computer screen, with a random configuration. The subjects were instructed to push a button if the crosses formed the corners of a square. For the acoustic task, a sequence of high and low beeps was presented via the computer’s loudspeakers. Randomly, two consequent high or low beeps occurred. The subjects had to detect this irregularity in the sequence and push the button. Mean reaction time and number of correct responses were calculated for each individual.

**Orexin A administration**

Orexin A (C_{152}H_{243}N_{47}O_{44}S_{4}; Mr: 3561.16; catalogue number H-4172; Bachem Biochemica GmbH, www.bachem.com) was dissolved in sterile water with a final concentration of 774.35 μg/ml. Pairs of orexin A solution and placebo (sterile water)—2.3 ml total volume each—were prepared by a pharmacist and poured into identical containers labelled in random order according to a blinding list. The last author DH-S unblinded this list after final completion of data acquisition and analysis. Orexin A or placebo was applied as a nasal spray by instilling a fine mist into the nostril by the action of a hand-operated pump. Approximately 0.1 ml of fluid was administered to each nostril at 1 min intervals for 10 min, resulting in an approximate total volume of 2 ml.

**Statistics**

Statistical analysis was performed with GraphPad Prism 4.02 for Windows (www.graphpad.com). Results are presented as median and range in the text and as box-and-whisker graphs in the figures. The boxes extend from the 25th to the 75th percentile, with a line indicating the median; whiskers represent the range of values. As groups were matched, but independent, differences between patients and control subjects were analysed with the Mann–Whitney U-test. In the second part of the study, olfactory thresholds were measured repeatedly in individual subjects. Hence, the comparison between placebo and orexin A condition was subjected to an analysis with a Wilcoxon Signed Rank test. The level of significance was set at *P* < 0.05.

**Results**

**Patient characteristics**

Twenty-six patients were assessed for eligibility, 16 were excluded from enrolment (nine not meeting eligibility criteria, seven for other, organizational, reasons). The remaining 10 narcolepsy patients with cataplexies (for demographic details see Table 1) participated in the baseline olfactory measurements. From those 10, seven received orexin A and placebo in randomized order and double blinded (two withdrew consent, one was excluded due to acute rhinitis) and were all included in the analysis. For each narcolepsy patient, one control meeting the eligibility criteria, matched for demographic parameters (for demographic details see Table 1), was recruited for the study with help of a healthy subject database of the Department of Psychology.

Patients were diagnosed according to the diagnostic criteria of the International classification of sleep disorders, 2nd edition (Billiard, 2007), with polysomnography and multiple sleep latency tests performed either in our own (*n* = 5) or in other certified sleep labs (*n* = 5). Nine patients were positive for narcolepsy-associated HLA types. One patient denied HLA typing. In this patient, however, CSF-orexin A was found to be pathologically reduced. Scores from the Ullanlinna Narcolepsy Scale ranged from 19 to 34 (median: 23.5) in patients exceeding the diagnostic cut-off value of 14 (Hublin et al., 1994) and from 4 to 9 in controls (median: 6.5). Neither the patients nor the controls took any medication. By medical history and physical examination we excluded acute medical conditions, other than narcolepsy, in both groups.
Baseline olfactory testing

Figure 1 shows the results from a comparison of the olfactory performance between patients and matched controls. Patients showed significantly lower scores for olfactory threshold (patients: median 8.0, range 4.0–10.5; controls: median 9.4, range 7.5–13.3; \( P < 0.05 \)) discrimination (patients: median 12.5, range 10–15; controls: median 15.0, range 12–16; \( P < 0.005 \)) and identification (patients: median 13.0, range 10–16; controls: median 14.0, range 13–16; \( P < 0.05 \)). This resulted in a significantly lower TDI score in patients (median 33.4, range 30–36) than in controls (median 38.4, range 35–43; \( P < 0.0001 \)).

Neither reaction time (patients: median 677 ms, range 627–813 ms; controls: median 689 ms, range 564–768 ms; Mann–Whitney U-test; \( P = 0.9118 \); NS) nor number of correct answers (patients: median 31, range 26–32; controls: median 31, range 26–33; Mann–Whitney U-test; \( P = 0.6842 \); NS) differed significantly between patients and controls. This indicates that the different performance in olfactory testing between patients and controls was not caused by impaired attention.

Olfactory testing after placebo and orexin A

As shown in Fig. 2A and Table 1, the majority of the patients showed clear increases in their olfactory threshold scores after the administration of orexin A (median 11.5, range 6.5–13.25) compared to placebo (median 7.75, range 6.25–11.25). In the box-and-whisker-plots of the results (Fig. 2B), this observation is reflected in a statistically significant difference between the two treatment conditions (Wilcoxon Signed Rank test; \( P = 0.016 \)).

Neither reaction time (orexin A: median 644 ms, range 548–823 ms; placebo: median 670, range 563–818 ms; Wilcoxon Signed Rank test; \( P = 0.9453 \); NS) nor number of correct answers (orexin A: median 32, range 24–32; placebo: median 32, range 25–32; Wilcoxon Signed Rank test; \( P = 1.00 \); NS) differed between the two treatment conditions. This indicates that the different results in the olfactory testing were not caused by changes in attention.

Although this was not assessed systematically, it is worth mentioning that two patients (Table 1; N2, N5) reported a several days lasting subjective change in olfactory perception after the administration of what turned out to be orexin A after unblinding.

**Discussion**

We found that olfactory performance is significantly impaired in narcolepsy patients with cataplexies. Thus, we confirm the results by Stiasny-Kolster and colleagues (2005, 2007), who were the first to suggest that patients with narcolepsy present with an OD. Furthermore, we are the first group to administer orexin A intranasally to narcolepsy patients and demonstrate that this OD can be restored.

**Olfaction and narcolepsy**

In the first study by Stiasny-Kolster et al. (2005)—focussing on the association of OD with RBD—19 narcolepsy patients were included, all with RBD, but no information was provided on the occurrence of cataplexies in this sample. In a second study (Stiasny-Kolster et al., 2007) with 40 patients (20 with and 20 without RBD), 95% had cataplexies and 67.5% had an HLA-type associated with narcolepsy. This study strengthened the hypothesis that OD is a feature of narcolepsy, independent of concomitant RBD. In our study,
Olfactory dysfunction in narcolepsy

Orexin A and narcolepsy

Orexin A and B (also hypocretin-1 and -2) are two neuropeptides synthesized by a small number of neurons restricted exclusively within and around the lateral hypothalamic area (de Lecea et al., 1998; Sakurai et al., 1998). Shortly after their discovery, several groups found that orexin A is abnormally decreased or undetectable in the CSF of a large proportion of patients with narcolepsy (Nishino et al., 2001; Dalal et al., 2002; Mignot et al., 2002; Dauvilliers et al., 2003). Further evidence for a crucial involvement of the orexins in the pathophysiology of narcolepsy can be derived from animal experiments. Canine narcolepsy was linked to an orexin receptor-2 gene mutation (Lin et al., 1999) and knocking-out the orexin gene led to a narcolepsy phenotype in mice (Chemelli et al., 1999). In only one of the patients in our study was CSF orexin A actually measured and was found to be pathologically decreased (Table 1; patient N10); the other participants did not agree to a lumbar puncture. However, we included a well-defined and homogeneous group of narcolepsy patients, with cataplexies and with a narcolepsy associated HLA-type. For this subgroup of patients, it is known, that >90% have decreased or undetectable CSF orexin (Nishino et al., 2001; Mignot et al., 2002).

Orexin and the olfactory system

There is no doubt that orexin signalling is crucially involved in sleep wake regulation. However, involvement in the regulation of appetite, energy consumption and in addictive behaviour have been discussed (Siegel, 2004; Ganjavi and Shapiro, 2007; Scammell and Saper, 2007). The finding by Schuld and colleagues (2000) that male patients with narcolepsy have significantly increased body mass indices compared to HLA-DR2-matched healthy controls fits well with the concept that orexin signalling is deficient in individuals with narcolepsy. The discussion is facilitated by the fact that hypothalamic orexin containing cell bodies project widely throughout the entire brain. The targets include cerebral cortex, thalamus, hypothalamus, brainstem and the olfactory bulb (Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999). As olfaction is an important input for appetite regulation, food seeking and also motivational behaviour, modulation of the olfactory system through orexin could link those systems. Interestingly, orexin A and B and their receptors are present in the entire olfactory tract, from the nasal mucosa (Gorojankina et al., 2007) via the olfactory bulb (Apelbaum et al., 2005; Hardy et al., 2005; Shibata et al., 2008) to the olfactory cortex (Caillol et al., 2003). Animal experiments suggest a modulation of the olfactory message by centrally synthesized orexins in the olfactory bulb (Apelbaum et al., 2005) and cortex (Caillol et al., 2003) and by locally synthesized peptides in the olfactory mucosa (Gorojankina et al., 2007). In addition, intracerebroventricular administration of orexin A improves olfactory detection performance in rats (Julliard et al., 2007). Therefore, it is a very tempting hypothesis that the lack of orexin A in narcolepsy patients explains their impaired olfactory performance (this study, Stiasny-Kolster et al., 2005, 2007).

Intranasal orexin A administration

Orexin A replacement therapy is, theoretically, a promising avenue for treating the symptoms of narcolepsy that are caused by orexin deficiency. However, it is noteworthy at this point that orexin A has not yet been licensed for any clinical use and our results on the olfactory detection threshold do not necessarily extend to any other causes of olfactory impairment. There are, however, animal studies on the application of orexin A. Whereas in one study in dogs with orexin receptor mutations intravenous orexin A did not effectively penetrate to the CSF and had only little therapeutic effect (Fujiki et al., 2003), in another study intravenous orexin A led to a reduction of cataplexies (John et al., 2000). It seems that central administration of orexin A is required to produce sufficient therapeutic effects (Fujiki et al., 2003; Mieda et al., 2004). It has been shown that various peptides achieve direct access to the CSF, bypassing the bloodstream, when applied to the nasal mucosa of humans (Born et al., 2002; Hallschmid et al., 2003). In a recent study in non-human primates, the intranasal application of orexin A led to increased CSF levels within 10 min of application (Deadwyler et al., 2007). Although this study is limited as we did not have the opportunity to measure CSF orexin A levels, we performed olfactory testing at around this length of time after orexin A administration. Hence, it is reasonable to assume that the effects seen on olfactory threshold in our study could be mediated either by a local effect of orexin A in the nasal mucosa and/or by effects on the central processing of olfactory information.

Conclusions

Our data together with the evidence from the literature cited suggest that: (i) mild OD is a sign of narcolepsy with cataplexies, (ii) the subgroup of narcolepsy patients with...
cataplexies consists largely of individuals in which the disorder is associated with or caused by decreased CSF orexin, (iii) the anatomical distribution of orexin and orexin-receptors within the entire olfactory path constitute an ideal link between orexin deficiency and OD and (iv) intranasal orexin A administration essentially restores olfactory performance. Hence, there is sound evidence for the hypothesis that pathophysiological mechanisms underlying OD are directly caused by a lack of orexin A.

Further studies are warranted on the one hand to find out whether or not the improvement in the olfactory detection threshold after orexin A application in our narcolepsy patients is clinically significant and for daily life needs and on the other to investigate where in the olfactory tract extrinsic orexin A acts. Moreover, one might speculate that other symptoms of narcolepsy, such as disturbed sleep regulation and cataplexies, are modulated by orexin A.

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