

Hypocretin (orexin) loss in Alzheimer's disease

Rolf Fronczek^{a,b,*}, Sarita van Geest^{a,b}, Marijke Frölich^c, Sebastiaan Overeem^d,
Freek W. C. Roelandse^c, Gert Jan Lammers^b, Dick F. Swaab^a

^a Netherlands Institute for Neurosciences, an Institute of the Royal Netherlands Academy of Arts and Sciences, Meibergdreef, Amsterdam, The Netherlands

^b Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands

^c Department of Clinical Chemistry, Leiden University Medical Center, Leiden, The Netherlands

^d Donders Institute for Neuroscience, Department of Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

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Abstract

Sleep disturbances in Alzheimer's disease (AD) patients are associated with the severity of dementia and are often the primary reason for institutionalization. These sleep problems partly resemble core symptoms of narcolepsy, a sleep disorder caused by a general loss of the neurotransmitter hypocretin. AD is a neurodegenerative disorder targeting different brain areas and types of neurons. In this study, we assessed whether the neurodegenerative process of AD also affects hypothalamic hypocretin/orexin neurons. The total number of hypocretin-1 immunoreactive neurons was quantified in postmortem hypothalami of AD patients ($n = 10$) and matched controls ($n = 10$). In addition, the hypocretin-1 concentration was measured in postmortem ventricular cerebrospinal fluid of 24 AD patients and 25 controls (including the patients and controls in which the hypothalamic cell counts were performed). The number of hypocretin-1 immunoreactive neurons was significantly decreased by 40% in AD patients (median [25th–75th percentiles]); AD 12,935 neurons (9972–19,051); controls 21,002 neurons (16,439–25,765); $p = 0.049$). Lower cerebrospinal fluid (CSF) hypocretin-1 levels were found in AD patients compared with controls (AD: 275 pg/mL [197–317]; controls: 320 pg/mL [262–363]; $p = 0.038$). Two AD patients with documented excessive daytime sleepiness showed the lowest CSF hypocretin-1 concentrations (55 pg/mL and 76 pg/mL). We conclude that the hypocretin system is affected in advanced AD. This is reflected in a 40% decreased cell number, and 14% lower CSF hypocretin-1 levels.

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1. Introduction

Alzheimer's disease (AD) is primarily characterized by cognitive decline. However, sleep disturbances and a disturbed sleep-wake pattern are also a major feature of AD (Samanta et al., 2006). These sleep disturbances are correlated with the severity of dementia (Mirmiran et al., 1992; Witting et al., 1990) and are often the primary reason for institutionalization (Pollak and Perlick, 1991). Up to 82% of

AD patients get up during the night (Tractenberg et al., 2003). Other characteristics of the sleep problems of AD patients are excessive daytime sleepiness, napping during daytime, rapid eye movement (REM) sleep dysregulation and circadian rhythm disturbances (Bliwise et al., 1989, 2004; McCurry et al., 1999). These features partly bear a resemblance to the symptoms of narcolepsy.

Narcolepsy is a sleep-wake disorder characterized by excessive daytime sleepiness and cataplexy. Other symptoms are REM sleep dysregulation, resulting in premature transitions to REM sleep, sleep paralysis, and hypnagogic hallucinations (Overeem et al., 2001; Rechtschaffen et al., 1963). The disorder is caused by a general loss of hypocretin (orexin), reflected in undetectable hypocretin-1 cerebrospinal fluid (CSF) levels. The hypocretins are neuropeptides

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* Corresponding author at: Leiden University Medical Centre, Department of Neurology, Albinusdreef 2, 2300 RC Leiden, The Netherlands. Tel.: +31 71 5263902.

E-mail address: r.fronczek@lumc.nl (R. Fronczek).

produced only in the lateral hypothalamus by a restricted group of cells that project widely throughout the brain (Peyron et al., 1998). The most densely innervated regions are sleep-wake areas involved in sleep regulation and attention such as the locus coeruleus and raphe nuclei of the brainstem, the cholinergic neurons of the brainstem and forebrain, and histaminergic neurons of the posterior hypothalamus (Fung et al., 2001; Nambu et al., 1999; Peyron et al., 1998; Siegel, 2004; van den Pol, 1999). Hypocretin neurons thus play a vital role in sleep-wake regulation.

AD is a neurodegenerative disorder that affects multiple brain areas and types of neurons, very likely also including the primary sleep-regulating systems. The hypocretin system is affected in various neurodegenerative diseases, such as Parkinson's disease and Huntington's disease (Aziz et al., 2008; Fronczek et al., 2007; Thannickal et al., 2007). We hypothesized that the neurodegenerative process in AD might also affect the hypocretin system, which could contribute to the sleep disturbances of AD patients. The observation of Friedman and others who have shown an inverse relation between lumbar CSF hypocretin-1 concentration and the amount of daytime naps of AD patients supports this possibility (Friedman et al., 2007).

The aim of the current study was to examine whether the hypocretin system is affected in AD. Two approaches were used: the total number of hypocretin-1 producing neurons was determined in the lateral hypothalamus of 10 AD patients and 10 matched controls. Furthermore, the concentration of hypocretin-1 was measured in postmortem ventricular CSF of 24 AD patients and 25 controls (including the patients and controls in which the hypothalamic cell counts were performed).

2. Methods

2.1. Postmortem material

Postmortem hypothalami and ventricular CSF were provided by the Netherlands Brain Bank (NBB). Permission was obtained for brain autopsy and for the use of human material and clinical information for research purposes. Ten patients with AD (Braak 5 or 6) and 10 nondemented controls (Braak 0 or 1) were matched for sex, age, postmortem delay (PMD), and fixation time (hypothalamus group). Clinicopathological information of these subjects is shown in Table 1. Ventricular CSF was not available for 2 controls (NBB # 97-130 and NBB # 97-045). Furthermore, CSF hypocretin-1 was measured in 25 AD patients and 24 controls (including the 10 patients and 8 controls in which the hypothalamic cell counts were performed), matched for sex, age, and PMD. For all cases, the clinical diagnosis was verified by a systematic neuropathological examination according to van de Nes et al. and Braak et al. (Braak et al., 1993; van de Nes et al., 1998). Exclusion criteria were other primary neurological or psychiatric disease and the use of corticosteroids during the last month prior to death. How-

ever, 2 control subjects (NBB # 97-045 and NBB # 97-130) had suffered from vascular event (intracerebral bleedings and aortic disruption respectively). Due to the limitations of the postmortem sample documentation of this brain bank study, there was no detailed clinical, systematic information available regarding sleep disturbances and patterns. Only for a few cases in the hypothalamus group there was some information available, which can be found in Table 1.

2.2. Hypocretin-1 immunocytochemistry

Hypothalami were fixed in 10% phosphate buffered saline (PBS) (pH 7.4) formalin at room temperature. Thereafter, the hypothalami were paraffin-embedded and serially sectioned at 6 μm in rostral-caudal direction. The expected hypocretin-1 immunoreactive (IR) neuron area starts at the level where the fornix touches the paraventricular nucleus and ends behind the level where the fornix reaches the mamillary bodies. Every 100th section in this area was stained for hypocretin-1. If needed, additional sections were stained until the whole area was covered and no more hypocretin-1 IR neurons were present. Neurons were stained with hypocretin-1 antibody (dilution 1:1250; Phoenix Pharmaceuticals, Inc., Belmont, CA, USA; Catalog no. H-003-30, lot no. 00653) of which the specificity was confirmed in a previous study (Fronczek et al., 2005). Antibody binding was visualized according to the avidin-biotin complex method in combination with 3,3-diaminobenzidine-nickel solution, as described previously (Goldstone et al., 2002).

2.3. Hypocretin-1 neuron quantification

The total amount of hypocretin-1 IR neurons was estimated as described previously (Fronczek et al., 2005). Briefly, an image analysis system (ImagePro version 5.1, Media Cybernetics, Silver Spring, MD, USA) connected to a camera (JVC KY-F55 3CCD, Victor Company of Japan, Tokyo, Japan) and plane objective microscope (Zeiss Axioskop with Plan-NEOFLUAR Zeiss objectives, Carl Zeiss GmbH, Jena, Germany) were used. The hypocretin-1 IR area was outlined manually, after which randomly selected fields were counted, covering a total of 15%. Positively stained cells containing a nucleolus were counted by 1 person, blinded to diagnosis. By counting the nucleolus double counting is prevented and the chance of counting a cell is equal in both large and small cells. This counting procedure is based on the principle that there is only 1 nucleolus per cell and that the nucleolus, due to its size and structure, cannot be divided by the microtome knife used (Fronczek et al., 2007).

A conversion program calculated the total number of hypocretin-1 IR neurons by multiplication of the neuronal counts by sample frequency of the sections, as described previously (Goldstone et al., 2002). The mean (\pm SD) number of sections quantified per subject was 14.6 ± 2.2 and the coefficient of variation of this method was 6.9% (calculated by counting 1 complete subject 4 times). To confirm the

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