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Decreased Melatonin Levels in Postmortem Cerebrospinal Fluid in Relation to Aging, Alzheimer's Disease, and Apolipoprotein E-ϵ4/4 Genotype*

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ABSTRACT

Sleep disruption, nightly restlessness, sundowning, and other circadian disturbances are frequently seen in Alzheimer's disease (AD) patients. Changes in the suprachiasmatic nucleus and pineal gland are thought to be the biological basis for these behavioral disturbances. Melatonin is the main endocrine message for circadian rhythmicity from the pineal. To determine whether melatonin production was affected in AD, melatonin levels were determined in the cerebrospinal fluid (CSF) of 85 patients with AD (mean age, 75 ± 1.1 yr) and in 82 age-matched controls (mean age, 76 ± 1.4 yr). Ventricular postmortem CSF was collected from clinically and neuropathologically well defined AD patients and from control subjects without primary neurological or psychiatric disease. In old control subjects (>80 yr of age), CSF melatonin levels were half of those in control subjects of 41–80 yr of age [176 ± 58 (n = 29) and 330 ± 66 (n = 53) pg/mL, respectively; $P = 0.016$]. We did not find a diurnal rhythm in CSF melatonin levels in control subjects. In AD patients the CSF melatonin levels were only one fifth (55 ± 7 pg/mL) of those in control subjects (273 ± 47 pg/mL; $P = 0.0001$). There was no difference in the CSF melatonin levels between the presenile (42 ± 11 pg/mL; n = 21) and the senile (59 ± 8 pg/mL; n = 64; $P = 0.35$) AD patients. The melatonin level in AD patients expressing apolipoprotein E-ϵ3/4 (71 ± 11 pg/mL) was significantly higher than that in patients expressing apolipoprotein E-ϵ4/4 (32 ± 8 pg/ml; $P = 0.02$). In the AD patients no significant correlation was observed between age of onset or duration of AD and CSF melatonin levels. In the present study, a dramatic decrease in the CSF melatonin levels was found in old control subjects and even more so in AD patients. Whether supplementation of melatonin may indeed improve behavioral disturbances in AD patients should be investigated. (*J Clin Endocrinol Metab* **84:** 323–327, 1999)

THE CIRCADIAN rhythm of melatonin secretion is generated in the suprachiasmatic nucleus (SCN) (1). In previous studies, a decreased number of arginine vasopressin and vasoactive intestinal polypeptide neurons in the SCN was found during aging and even more dramatically so in Alzheimer's disease (AD) (2, 3). In addition, an impaired daily variation in the concentration of melatonin in the human pineal gland was found in the older subjects and even more so in AD patients (4). Changes in the SCN and pineal gland are considered to be responsible not only for the disturbed circadian rhythms in hormones, body temperature, and sleep-wake behavior, but also for behavioral disorders in elderly people and AD patients. Demented patients often suffer from sleep disruption, nightly restlessness, and sundowning (5). Disruption of sleep of the care giver due to nocturnal restlessness of the patient is a more important reason for placement of a demented patient in a nursing home than cognitive impairment (6). Moreover, disturbed circadian rhythms are considered to be related to the cognitive performance of elderly people and AD patients (7, 8). In addition, it was reported that melatonin inhibits the progressive formation of β-sheets and amyloid fibrils *in vitro* (9). Although various studies indicate that the circadian rhythm of melatonin is disturbed during aging (10–14), only limited information on serum melatonin in dementia is available (8, 15), and information on melatonin levels in cerebrospinal fluid (CSF) is totally lacking. As the brain is presumed to be the main target for melatonin action, we determined in the present study melatonin levels in postmortem CSF during aging and in neuropathologically confirmed AD patients.

Materials and Methods

Autopsies were performed within the framework of The Netherlands Brain Bank. Ventricular postmortem CSF was obtained at autopsy, 1–12 h after death, from 85 Alzheimer patients and 82 controls without a primary neurological or psychiatric disease. Before the brain was removed, ventricular CSF was collected, and pH was determined immediately as a measure of agonal state. Individuals who die after a long terminal phase accumulate lactic acid and therefore have a lower pH (16) independent of the postmortem time (17). CSF was immediately centrifuged at 700 × *g*. The supernatant was subdivided into 250- to 1000-μL aliquots that were kept at −80 C until assayed. The following variables were included in the present study for both Alzheimer patients and controls: postmortem interval, CSF pH, brain weight, sex, age, and clock time and month of death (Table 1). All Alzheimer patients had a history of a gradual intellectual deterioration, and the diagnosis of probable Alzheimer's disease was made according to the NINCDS-ADRDA criteria (18), excluding other causes of dementia by means of history, physical examination, and laboratory tests. The severity of dementia was evaluated by the global deterioration scale (GDS) (19). All brains were investigated in a systematic way by neuropathologists (Prof. F. C. Stam,

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TABLE 1. Clinical and pathological data for the controls and Alzheimer's patients studied

	Control group	AD group	*P* values
No. of cases (male/female)	82 (44/38)	85 (33/52)	0.06
Age (yr)	74.71 ± 1.2	76.26 ± 1.4	0.28
Brain wt (g)	1215 ± 15	1056 ± 17	0.001
Postmortem delay (h)	6.91 ± 0.36	4.21 ± 0.12	0.001
pH of CSF	6.71 ± 0.05	6.59 ± 0.03	0.001

Values are the mean ± SEM.

The Netherlands Brain Bank; Dr. W. Kamphorst, Free University Amsterdam; or Dr. D. Troost, Academical Medical Center, University of Amsterdam). The neuropathological diagnosis of Alzheimer's disease was made on the basis of the occurrence of many senile plaques, neurofibrillary tangles, and a disorganized fiber pattern and the presence of dystrophic neuritis and neuropile threads in Bodian and Congo stainings of the hippocampus and five cortical areas in formalin-fixed tissue (20). To exclude the presence of Parkinson's disease, the substantia nigra was also examined. To determine whether diurnal variations were present in levels of melatonin, subjects were grouped into two diurnal periods based on the clock time of death, 1000–2200 and 2200–1000 h, as these times are known to be associated with circadian differences in the level of melatonin in human plasma (21–24). We also checked whether there was any correlation between the season of death and CSF melatonin levels in controls and AD patients. Subjects were divided into four seasonal groups based on the date of the death: *i.e.* spring (March 21 through June 21), summer (June 21 through September 21), autumn (September 21 through December 21), and winter (December 21 through March 21).

Melatonin assay

Melatonin in postmortem CSF was measured by a direct RIA. The assay was run in a 0.1 mol/L tricine buffer (Sigma Chemical Co., St. Louis, MO) containing sodium chloride (0.15 mol/L; Merck, Rahway, NJ) and 0.1% gelatin (Merck) adjusted to pH 7.5. Iodinated melatonin (2-[^{125}I]iodomelatonin; Amersham, Roosendaal, The Netherlands) was diluted in tricine buffer at a final concentration of 25,000 cpm/mL. The melatonin antibody (AB/R/O3, Stockgrand, Guildford, UK) that was raised in rabbits was shown to be sufficiently specific for clinical application in CSF without preassay treatment. The antibody cross-reacted with 6-hydroxymelatonin at 5.3% and less than 0.2% with 6-sulfatoxymelatonin (25). Standards were diluted in tricine buffer to give a range of dilutions from 1–1000 pg/mL. Samples of CSF (100 μL) were aliquoted in tubes with 100 μL tricine buffer and 250 μL antimelatonin (final dilution, 1:200,000). They were vortexed, capped, and incubated for 3 nights at 4 C. Bound melatonin was separated by 50 μL donkey antirabbit antiserum coupled to cellulose (SAC-CEL, IOS, Boldon, UK). Precipitate was counted in a γ-counter (Cobra 500s, Packard, Groningen, The Netherlands). The intraassay coefficient was 8.7%.

Apolipoprotein E (ApoE) assay

ApoE genotyping was performed on frozen tissue from the cerebellum of the AD patients. The genotype of each extracted DNA sample was determined by PCR amplification using the primers 5′-TCCAAGGAGCTGCAGGCGGCGCA-3′ and 5′-ACAGAATTCGCCCCGGCCTGGTACACTGCCA-3′. Then, the PCR product was digested by Cfol (Boehringer, Mannheim, Germany), and fragments were separated by electrophoresis in a 5% agarose gel (26).

Statistics

Differences in melatonin levels between groups were tested using the Mann-Whitney U test. The difference in the number of males and females between controls and AD patients was tested by χ^2 analysis. The effects of sex and postmortem time on CSF melatonin levels were evaluated statistically by a two-factor ANOVA. Correlations of postmortem interval, brain weight, and pH of CSF *vs.* melatonin levels were analyzed by the Spearman correlation test. Differences among the three groups were tested by Kruskal-Wallis ANOVA. All results were expressed as the mean ± SEM. Differences were considered statistically significant at the $P < 0.05$ (two-tailed) level.

Results

A larger brain weight was found in the controls than in the AD patients (1215 ± 15 *vs.* 1056 ± 17 g; $P = 0.001$). In AD patients the melatonin levels (56 ± 7 pg/mL) were 5 times lower than those in controls (273 ± 47 pg/mL; $P = 0.0001$; Fig. 1). Presenile AD patients (<65 yr of age; n = 21) had decreased CSF melatonin levels (42 ± 11 pg/mL), which were 5 times lower than those in young controls (254 ± 75 pg/mL; n = 12; $P = 0.01$). The melatonin levels of presenile AD patients (42 ± 11 pg/mL) did not differ from those of senile AD patients (59 ± 8 pg/mL; $P = 0.35$). The difference between senile AD patients (n = 64) and controls older than 65 yr of age (270 ± 54 pg/mL; n = 70) was significant ($P = 0.0001$). There was no difference in CSF melatonin according to the severity of dementia; the CSF melatonin levels in AD patients with a GDS score of 7 (57 ± 9 pg/mL, n = 50) did not differ from those with a GDS score of 6 (53 ± 11 pg/mL; n = 18) or from those with a GDS score less than 6 (33 ± 11 pg/mL; n = 9; $P = 0.82$). No significant correlation was found between age at onset of dementia and CSF melatonin levels in AD patients (r = 0.07; $P = 0.52$). In addition, no correlation was observed between duration of AD and CSF melatonin levels (r = −0.10; $P = 0.37$).

In controls, a significant decrease in ventricular CSF melatonin was found with age. Melatonin levels in controls older than 80 yr of age (176 ± 58 pg/mL; n = 29) were 50% lower than those in controls who were of 41–80 yr old (330 ± 66 pg/mL; n = 53; $P = 0.016$; Fig. 2). No significant daily rhythm in CSF melatonin levels was detected in AD patients ($P = 0.58$) or controls ($P = 0.66$). In controls, the nighttime level of CSF melatonin (269 ± 52 pg/mL; n = 44) was similar to that during the day (1000–2200 h; 276 ± 84 pg/mL; n = 38; Fig. 3). Two-way ANOVA revealed that postmortem delay and sex had no effect on CSF melatonin levels in control subjects ($P = 0.18$ and $P = 0.89$, respectively). There was no difference in CSF melatonin levels between the different

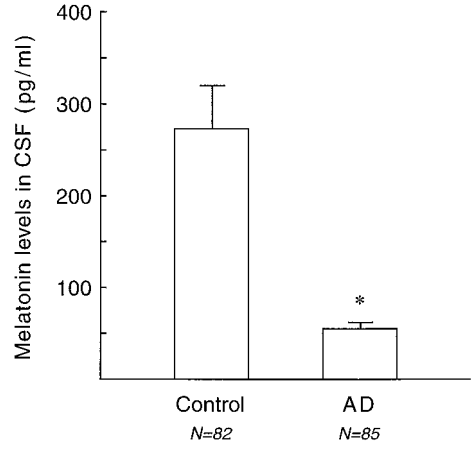

FIG. 1. Melatonin levels in CSF of control subjects (n = 82) and AD patients (n = 85). *, $P < 0.0001$.

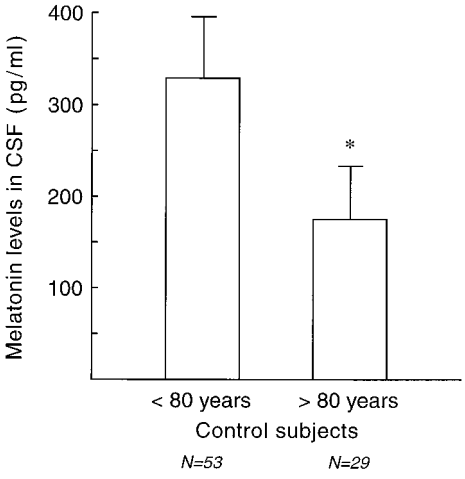

FIG. 2. Melatonin levels in CSF of control subjects, 41–80 yr of age (n = 53) and older than 80 yr (n = 29). *, $P < 0.01$.

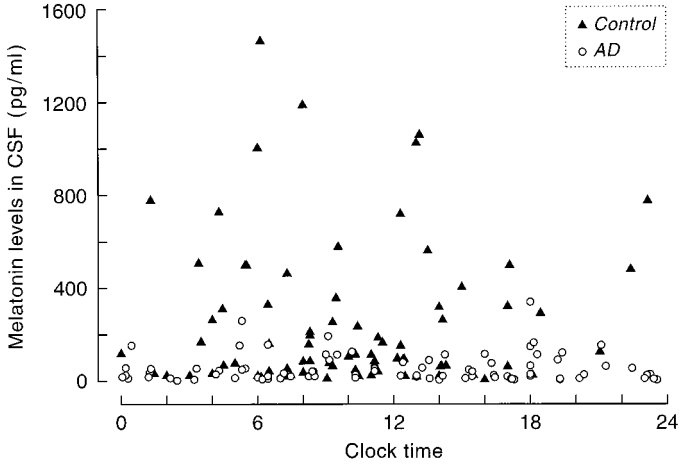

FIG. 3. Melatonin levels in CSF of control subjects and AD in relation to the clock time of death. Note that the overall levels of melatonin in AD were significantly lower than those in controls and that there was no obvious day/night rhythm in CSF melatonin levels in either controls or AD patients.

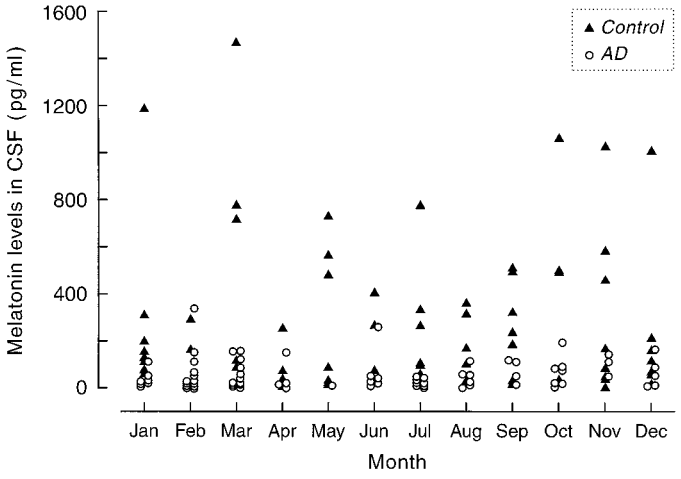

FIG. 4. Melatonin levels in CSF in control subjects and AD in relation to the month of death. Note that there was no significant seasonal rhythm in CSF melatonin levels in either controls or AD patients.

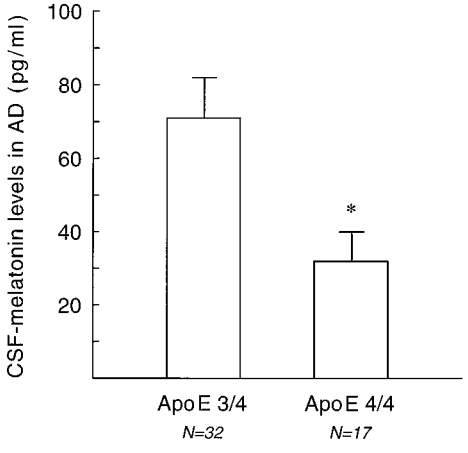

FIG. 5. Melatonin levels in CSF of AD patients who expressed ApoE-∈3/4 (n = 32) and ApoE-∈4/4 (n = 17). *, $P < 0.02$.

seasons in control subjects [spring, 157 ± 54 pg/mL (n = 18); summer, 234 ± 46 pg/mL (n = 21); autumn, 262 ± 70 (n = 24); winter, 321 ± 99 (n = 19); $P = 0.82$, by ANOVA; Fig. 4).

An interesting finding of the present study was that there was a significant difference between AD patients expressing ApoE-ϵ3/4 (n = 32) and those expressing ApoE-ϵ4/4 (n = 17) in CSF melatonin levels (71 ± 11 and 32 ± 8 pg/mL, respectively; $P = 0.02$; Fig. 5). There was only one control subject expressing the ApoE-ϵ4/4 genotype.

No significant correlation was found between ventricular CSF melatonin levels in controls or AD patients, on the one hand, and brain weight (r = −0.11; $P = 0.32$ and r = 0.04; $P = 0.72$, respectively), postmortem delay (r = −0.0002; $P = 0.99$ and r = −0.16; $P = 0.15$, respectively), or pH (r = 0.19; $P = 0.08$ and r = −0.08; $P = 0.47$, respectively), on the other. There is, consequently, no reason to presume that the differences in brain weight, postmortem delay, and pH of the CSF between controls and AD patients (Table 1) influenced the results in any way.

Discussion

The present study shows markedly lower melatonin levels in ventricular CSF of AD patients. Melatonin levels were 5-fold lower in AD patients than in age-matched controls. Interestingly, the level of decreased nocturnal melatonin was reported to be related to the severity of the mental impairment in demented patients (8, 27). The data in the literature concerning melatonin levels in dementia are discordant, however. No differences in plasma or pineal melatonin levels between demented and elderly subjects were reported in earlier studies (4, 10, 27). Magri *et al.* (8) found also no difference in plasma melatonin levels in six demented patients compared with those in normal elderly subjects. However, more recent studies showed a decrease in nocturnal plasma melatonin levels in senile AD patients (8, 28). In addition, decreased pineal melatonin levels were found in aging and AD (4). The discrepancies between the studies on melatonin levels may be attributed to differences in the age of subjects, to the use of in- or out-patients, or to the severity and type of dementia, which also varied across studies. The subjects used in the present study were neuropathologically con-

firmed AD and control subjects. Our finding of the decreased CSF melatonin levels suggests that melatonin may indeed be involved in the symptoms of AD. We did not find a relationship between the postmortem CSF melatonin levels and the onset, duration, or severity of dementia. Others also found no relationship between the duration of dementia and the flattening of the melatonin rhythm in living demented patients (28). The decreased CSF melatonin levels observed by us coincide with the general disturbance of circadian rhythms in AD, *e.g.* in sleep-wake cycle, body temperature, and rest-activity cycle (7) and with the degeneration of the SCN in aging and AD (2, 29, 30). Furthermore, demented patients tend to be exposed to less environmental light than healthy people (31). It has been reported that bright light therapy, an interference presumed to stimulate the SCN directly, was effective for sleep and behavior disorders in elderly patients with dementia (28, 32). These observations support the idea that degeneration of the SCN in AD is the central phenomenon in these changes.

The observed decrease in ventricular CSF melatonin levels with aging in controls supports other reports of plasma melatonin changes (33, 34). The age of the subject had a significant effect on the day/night variation in pineal melatonin levels; the rhythmicity was lost in the older group (4). The decline in the production of melatonin with age agrees with previous reports (4, 12, 35–37), whereas in the older group, SCN changes were also observed (2).

It is proposed that the response of the circadian system to environmental (zeitgeber) signals diminishes with aging, and that when the melatonin rhythm deteriorates during aging, other circadian rhythms likewise weaken and become desynchronized (38). Concerning the changes in plasma melatonin observed in elderly people, the mechanism responsible for the reduction of melatonin secretion in aging is not very well understood. Alterations in SCN (2, 4) may be a major factor. Interestingly, a significant decrease in CSF melatonin was found in the control subjects who were older than 80 yr. A decreased number of arginine vasopressin neurons in the SCN was also found in subjects older than 80 yr (2), suggesting that the changes in the SCN and pineal are related. Structural changes in the pineal, such as the calcifications or the variations in melatonin clearance do not seem to play an important role in the decrease in plasma melatonin levels in elderly subjects (14, 39). Nocturnal melatonin secretion is modulated by noradrenaline through β-receptors (40). Therefore, it may be of importance that an impairment of catacholaminergic pathways occurs with aging in the central nervous system (41). The effect of a decline in the CSF production rate or turnover with aging (42–44) on CSF melatonin levels in aging and in AD is not known.

In the present study a daily rhythm of melatonin levels in postmortem ventricular CSF was not observed in controls or AD patients. This may well be due to the fact that our CSF samples were obtained postmortem from hospitalized patients. It has been reported that hospitalized patients have significantly higher daytime plasma melatonin levels, an earlier nocturnal rise, and a more variable timing of their secretion profiles (15, 45). Possibly artificial and supplementary natural lighting in the hospital may not be sufficient to suppress melatonin secretion adequately during daylight hours or act efficiently to entrain day/night secretion of melatonin in a physiological circadian manner. This problem may exist particularly in humans. Room light of low intensity, which is sufficient to suppress melatonin secretion in other mammals, failed to do so in humans (46). Another reason for the lack of an overall circadian rhythmicity in CSF levels of melatonin may be that despite the reproducible pattern observed from day to day in the same individual, a very large interindividual variation was observed (47, 48). In our study, only one data point per patient was available for obvious reasons. In addition, a great variety of pathological conditions and disease states have been associated with alterations in pineal function and 24-h melatonin profiles (4, 11, 49). Therefore, the normal range for daytime and nighttime plasma and CSF levels is very large, and the day-night difference for melatonin levels can vary widely for various reasons.

Recent studies have indicated a significant association between the ApoE type and AD. ApoE is a 34-kDa protein that plays a key role in regulation of the metabolism of lipids and has three major isoforms (E2, E3, and E4). The ApoE-ϵ4 genotype is a risk factor for AD (50–52), and it is likely that this will to some degree be reflected in the neuropathology and neurochemistry of this disease, Indeed, ApoE immunoreactivity has been found in senile plaques and cerebral vessels and neurofibrillary tangles in AD. An interesting finding of the present study is that CSF melatonin levels from ApoE-$\in$3/4 genotype patients were significantly higher than those from the ApoE-$\in$4/4 genotype, again suggesting a relationship between melatonin levels and signs and symptoms of AD.

The production of melatonin declines with increasing age and age-related diseases. In some patients this is associated with clinical symptoms of rhythm disturbances such as sleep-wake disturbance (7, 32). Whether AD patients with low melatonin levels may indeed benefit from chronic supplementation of melatonin should be investigated.

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