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Decreased MT1 melatonin receptor expression in the suprachiasmatic nucleus in aging and Alzheimer's disease

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Abstract

The pineal hormone melatonin is involved in the regulation of circadian rhythms and feeds back to the central biological clock, the hypothalamic suprachiasmatic nucleus (SCN) via melatonin receptors. Supplementary melatonin is considered to be a potential treatment for aging and Alzheimer's disease (AD)-related circadian disorders. Here we investigated by immunocytochemistry the alterations of the MT1 melatonin receptor, the neuropeptides vasopressin (AVP) and vasoactive intestinal peptide (VIP) in the SCN during aging and AD. We found that the number and density of AVP/VIP-expressing neurons in the SCN did not change, but the number and density of MT1-expressing neurons in the SCN were decreased in aged controls compared to young controls. Furthermore, both MT1-expressing neurons and AVP/VIP-expressing neurons were strongly diminished in the last neuropathological stages of AD (Braak stages V–VI), but not in the earliest stages (Braak stages I–II), compared to aged controls (Braak stage 0). Our study suggests that the MT1-mediated effects of melatonin on the SCN are disturbed during aging and even more so in late stage AD, which may contribute to the clinical circadian disorders and to the efficacy of therapeutic melatonin administration under these conditions.

Keywords: Melatonin receptor; MT1; Suprachiasmatic nucleus; Aging; Alzheimer's disease; AVP; VIP; Immunocytochemistry

1. Introduction

The suprachiasmatic nucleus (SCN) in the anterior hypothalamus is considered to be the self-sustained master circadian pacemaker. Via neuronal and/or hormonal pathways the SCN coordinates circadian rhythms in a variety of biochemical, physiological, and behavioral processes, including the rhythm of pineal melatonin production [6]. Melatonin is involved in the regulation of circadian rhythms and appears to feedback to the SCN via two specific, high-affinity G protein-coupled melatonin receptors, i.e. MT1 (also called Mel_{1a}) and MT2 (or Mel_{1b}) (reviewed in Ref. [21]). Physiologically, the MT1 receptor mediates the acute inhibitory action of melatonin on the SCN [13], which may be important for defining SCN sensitivity to phase-shifting stimuli, and may contribute to the regulation of sleep. The MT2 receptor is reported to mediate the phase shift effect of melatonin on the SCN of mammals [7,12]. The circadian effects of melatonin have led to substantial therapeutic applications for jet lag, shift

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work, blindness, and some circadian-based sleep disorders [2,31].

Specific ^{125}I-melatonin binding sites have been revealed in the SCN area of mammals, including human [20]. The MT1 receptor is the primary subtype of melatonin receptors as targeted disruption of the MT1 receptor eliminates detectable ^{125}I-melatonin binding from the mouse brain [13]. In addition, MT1 receptor mRNA has been detected in the post-mortem human SCN in an overlapping pattern with the distribution of ^{125}I-melatonin binding, while MT2 subtype was not detected, probably due to a very low expression level in the human hypothalamus [32,37].

Sleep disruptions, nightly restlessness and other circadian rhythm disturbances are frequently seen in elderly and even more severely so in Alzheimer's disease (AD) patients [17,35]. These disturbances actually are the main reasons for hospitalization of AD patients [19]. Our previous studies showed degeneration of the SCN during aging, and particularly in AD, reflected by the decrease of the neuropeptide vasopressin at both the protein and the mRNA level [14,30]. Moreover, melatonin levels are decreased during aging and predominantly in AD with flattened circadian rhythm [29,40], in fact, from the earliest AD neuropathological stages onwards [39,42]. Such changes in the SCN and pineal gland are considered to be the neurobiological basis for the circadian disturbances in aging and AD [29,40]. However, so far no data are available concerning the possible involvement of the MT1 receptor in the SCN in these circadian disorders. In addition, as supplementary melatonin is considered to be a potential treatment for aging and AD-related circadian disturbances, it is a prerequisite to investigate the alterations of the MT1 receptor in the SCN in these processes.

In the present study, we studied the alterations of the MT1 receptor in the SCN during aging and the progression of AD by using immunocytochemistry. We found that the MT1 receptor expression in the SCN was decreased in aging. Moreover, MT1 in the SCN was even more strongly diminished in the late neuropathological stages of AD (Braak stages V–VI), but not in the early stages (Braak stages I–II). Our study thus suggests that effects of melatonin on the SCN, mediated by the MT1 receptor, may be disturbed during aging and even more so in late stage AD, which may contribute to the clinical circadian disorders and to the efficacy of therapeutic melatonin administration under these conditions.

2. Subjects and methods

2.1. Subjects

Brain material was obtained via the rapid autopsy system of the Netherlands Brain Bank (NBB) at the Netherlands Institute for Neuroscience, in accordance with the formal permissions for a brain autopsy and the use of human brain material and clinical information for research purposes. We studied the hypothalamus of 46 subjects, subdivided into four groups: 13 young controls, 11 old controls, 11 preclinical "AD" subjects and 11 late clinical AD patients. Young controls (aged 19–40 years old) and aged controls (61–85 year old) were free of any psychiatric and neurological diseases, and without any AD neuropathological change in the brain (Braak stage 0). Preclinical "AD" subjects (64–87 year old) were cognitively intact subjects but appeared to have minor AD neuropathology (Braak stages I–II). Late clinical AD patients (59–86 year old) fulfilled the NINCDS-ADRDA criteria [16], i.e. they had a clinical diagnosis of probable AD, excluding other causes of dementia by means of history, physical examination and laboratory tests, and were neuropathologically confirmed with systematic and extensive AD neuropathology (Braak stages V–VI) [4,33]. All the groups were matched for sex, season of death (according to their month of death, Summer: June–August, Winter: December–February), day/night distribution (according to their clock time of death, day: 10:00–22:00 h, night: 22:00–10:00 h) ($p = 0.79$, 0.22 and 0.46, respectively)(for diurnal and seasonal fluctuations in the human SCN see Refs. [10,11]). In addition, aged controls, preclinical "AD" subjects and late clinical AD patients were matched for age ($p = 0.78$). Clinico-pathological data are presented in Table 1 . There were significant differences in brain weight, post-mortem delay, and fixation time between groups ($p = 0.02$, 0.01, 0.01, respectively). However, later linear regression analysis indicated that these parameters did not seem to affect our data comparison (all $p > 0.05$).

2.2. Histology

Hypothalamic samples were fixed in formalin and embedded in paraffin. Serial coronal sections (6 μm) were made from the level of the lamina terminalis to the mamillary bodies. Depending on availability, either the left or right hemi-hypothalamus was used. For anatomical orientation every 100th section was collected and mounted on superfrost plus slides (Menzel GmbH & Co. KG, Baunschweig, Germany) and subsequently dried for at least 2 days at 37 °C, followed by Nissl staining (0.5% thionine in distilled water). Additionally, in order to delineate the SCN, double immunocytochemical staining for vasopressin (AVP) and vasoactive intestinal polypeptide (VIP) was performed on every 50th section taken along the rostrocaudal axis throughout the complete SCN region. The rostral and caudal border of the SCN was defined as the most rostral and most caudal section that contained one or more AVP or VIP positive cells in the area. The central cross-section containing the maximal SCN was defined as the central section containing the most AVP and VIP positive cells, to which adjacent central SCN sections were taken for the MT1 receptor staining.

2.3. AVP and VIP immunocytochemical double staining

The combined immunocytochemical staining for AVP and VIP followed the protocols as previously described with some minor modifications [30,41], which are as follows: