Cerebrovascular melatonin MT1-receptor alterations in patients with Alzheimer's disease

Egemen Savaskan[a,*], Gianfranco Olivieri[a], Lena Brydon[b], Ralf Jockers[b], Kurt Kräuchi[c], Anna Wirz-Justice[c], Franz Müller-Spahn[a]

[a]*Department of Psychiatry, University of Basel, Wilhelm Klein-Str. 27, CH-4025 Basel, Switzerland*
[b]*Institut Cochin de Génétique Moléculaire, Laboratoire d'Immuno-Pharmacologie Moléculaire, Paris, France*
[c]*Center for Chronobiology, Department of Psychiatry, University of Basel, Switzerland*

Abstract

The pineal hormone melatonin has two major functions: as a transducer of the circadian day-night signal across the seasons, and as a vasoactive substance regulating cerebral circulation. The vasoconstrictive effects of melatonin have been postulated to be mediated by the melatonin 1a-receptor (MT1). The objective of this study was to provide the first immunohistochemical evidence for the localization of vascular MT1 in human control hippocampus compared to Alzheimer's disease (AD) patients, since regional blood flow impairments contribute to the neurodegenerative course of the disease. Both superficial and intrahippocampal arteries revealed MT1 immunoreactivity in adventitia in controls, which was distinctly increased in AD patients. The increased MT1 in AD may indicate a regulatory response to impaired melatonin levels in those patients, contributing to the regulation of cerebral circulation. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Besides contributing to the regulation of circadian rhythms, the pineal hormone melatonin has the ability to attenuate or to enhance vascular responsiveness, and contributes decisively to the regulation of cerebral blood flow [14,17]. Nocturnal melatonin secretion is impaired in stroke and migraine patients [3,9]. Melatonin intake lowers blood pressure in healthy humans [1]. Its administration induces cerebral vasoconstriction followed by an improvement in the vasodilatory response to hypercapnia, resulting in increased cerebrovascular dilatory capacity and an improved cerebrovascular security margin [14]. Therefore, melatonin may have a distinct cerebrovascular protective role.

Melatonin is considered to mediate its specific effects through two receptor subtypes in humans, melatonin 1a-receptor (MT1) and melatonin 1b-receptor (MT2), both belonging to a novel subfamily of G-protein coupled receptors [7]. The cDNAs of both subtypes have been cloned from humans [15], and, recently, an antibody against MT1

has been developed [4]. Whereas the MT2 receptor mediates relaxation of vascular smooth muscle, the MT1 receptor has been proposed to be responsible for constriction of the rodent caudal artery [6]. The vasoconstrictor role of MT1-mediated effects is well established in large cerebral arteries of rats [10], but information is lacking concerning the effect of melatonin on cerebral microvessels, especially in humans [14].

A lowered cerebral perfusion as a consequence of hemodynamic microcirculatory insufficiency underlies Alzheimer's disease (AD), which is a neurodegenerative disorder leading to progressive cognitive impairment [5]. In order to determine the localization of MT1 in human cerebral vessels and to investigate its possible role in vascular regulation in AD, we studied with immunohistochemistry postmortem tissue, kindly provided by the Netherlands Brain Bank, including hippocampus and entorhinal cortex of 11 control cases (mean age: 79.2 years ± SD: 8.3) and 11 AD cases (79.5 years ± 8.7). The hippocampus was chosen as a paradigm of a severely affected brain region in AD. The mean postmortem delay was 415 min (± 154) for controls and 258 min (± 49) for AD cases. In addition to the histopathological diagnosis of AD, Braak staging, which differ-

* Corresponding author. Tel.: +41-61-3255111; fax: +41-61-3255594.
E-mail address: esavaskan@datacomm.ch (E. Savaskan).

entiates six neuropathological stages in AD [2], and apolipoprotein E (ApoE) allele frequency had been determined for each case. The ε4 allele of ApoE is a major risk factor for sporadic AD [11]. According to Braak stageing, pathological involvement of the hippocampus marks stage III, and disease severity proceeds until stage VI [2]. Therefore, all AD cases in our series correspond to Braak stages III to

VI. The paraffin embedded tissue blocks were cut in the coronal plane into 10 μm-thick serial sections. The primary antibody for MT1 has been developed against the C terminus of the receptor [4]. After incubation with the primary antibody, MT1 was visualized by peroxidase staining using the substrate 3-amino-9-ethylcarbazole (ACE) which provides a red staining as reported previously [12]. To
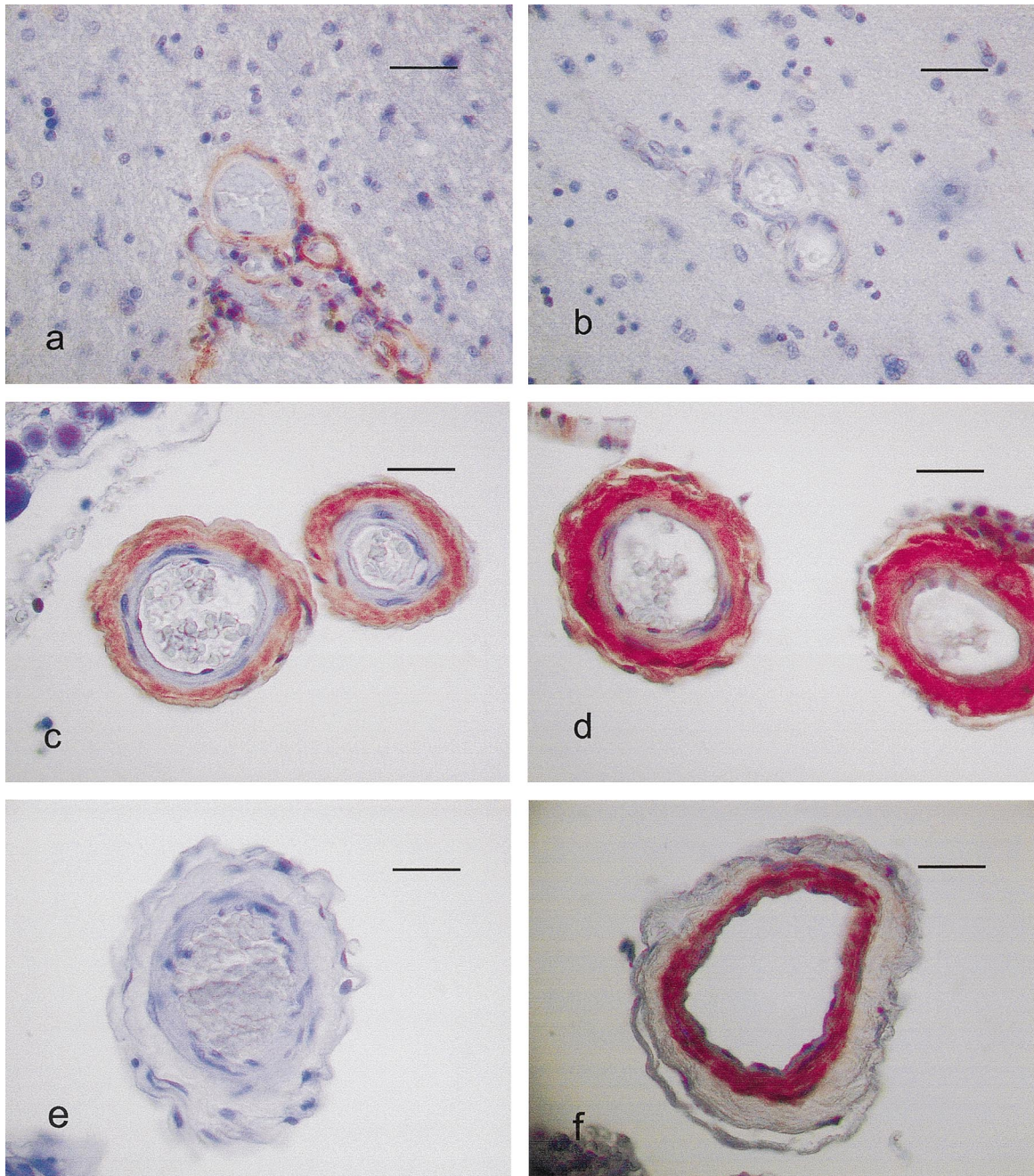

Fig. 1. Vascular MT1 staining shown as a red deposit in the hippocampus. (a) MT1 is localized to adventitia of intrahippocampal vessels in a control case. (b) Probable veins without distinct smooth muscle layer do not reveal MT1 immunoreactivity (same control case as in a). (c) Superficial arterial vessels in a control case showing MT1 staining in adventitia. (d) The MT1 immunoreactivity is distinctly increased in an AD case. (e) A control sample stained simultaneously following the same procedure with the exception that the primary antibody was omitted. The lack of MT1 immunoreactivity confirms the specificity of the reaction. (f) In adjacent sections β-actin staining (arrows) marks the smooth muscle layer excluding the smooth muscle as the localization of MT1.

reveal if MT1 is localized to the smooth muscles, β-actin was stained in adjacent sections as a specific muscular marker using specific monoclonal antibodies raised against the N-terminus of the protein (Sigma, No. A2547).

We observed vascular MT1 immunoreactivity in all control and AD cases (Fig. 1a,c,d). MT1 was localized to the adventitia of the vessel walls, and not to the smooth muscles, both in microvessels and in larger vessels. All arteries revealed a distinct adventitial MT1 staining whereas veins, as identified by missing α-actin staining, did not express the receptor (Fig. 1a,b). Endothelial cells did not show MT1 staining. Both intrahippocampal and superficial vessels were immunoreactive for MT1 (Fig. 1a,c,d). In general, the hippocampus contained only a few vessels. Interestingly, immunoreactive intrahippocampal vessels were mainly found in the molecular layer of the hippocampus, whereas layers rich in neurons were poorly vascularized. The CA1 subfield of the hippocampus revealed less vascularization compared to the CA2–4 subfields. In AD cases, there was a overall increase in MT1 immunoreactivity in all arteries (Fig. 1d). The staining intensity was distinctly enhanced in all observed sections, both in microvessels and in large arteries. The increase in MT1 immunoreactivity was common to all AD cases and was not correlated with increasing Braak staging. The AD patients with ApoE allele frequency $\varepsilon4$ did not show an obvious aberration in increased MT1 compared to those patients without the high risk factor allele.

These results provide the first immunohistochemical evidence for the localization of vascular MT1 in humans. Since a vasoconstrictive role has been postulated for MT1 [6,10] the adventitial localization of MT1 is surprising. The vascular action of melatonin was assumed to be mediated by receptors on smooth muscles [6,16]. The apparent adventitial localization of MT1 in our series suggests two possible explanations. First, other melatonin receptor subtypes may be responsible for direct effects on smooth muscles. Since melatonin causes antagonistic responses in arteries, vasoconstriction via MT1 and relaxation via MT2 [6], the different localizations of the receptor subtypes may be decisive for the resultant action. Second, melatonin may have an indirect effect on smooth muscles. This is evidenced by melatonin's ability to regulate Ca^{2+} influx without involving the primary steps in smooth muscle contraction or relaxation [16].

Our results suggest that, similar to rodents, MT1 mediates melatonin effects on the human arterial wall. The postulated effect of melatonin, not only on large cerebral influx arteries but also on cerebral microvessels [14], has been confirmed by the present data. The distribution pattern of MT1 in hippocampus parallels the vascularization of this region. The hippocampal subfields CA2 and CA3 have been shown to be richly vascularized, whereas CA1 and CA4 contain only a few capillaries [8], consistent with our findings. The intensity of the vascularization in hippocampus has been postulated to parallel the density of synapses and not the number of neurons in certain layers [8].

The finding of increased vascular MT1 immunoreactivity in AD patients suggests an important role for melatonin in regulating regional blood flow. The up-regulation of vascular MT1 may represent a compensatory mechanism to overcome the impaired melatonin levels reported for AD patients [13] and to benefit from melatonin's vasoprotective properties [14]. We have recently found a similar upregulation of MT1 immunoreactivity in hippocampal neurons (unpublished data), indicating that the receptor regulatory response is a more general one. Cerebral hypoperfusion appears to precede neurodegenerative changes in AD, and leads to a vascular endothelialpathy impairing basal nitric oxide release which, in turn, diminishes neuronal metabolism [5]. Acute melatonin infusion, on the other hand, increases cerebral vasodilatory reserve as a result of vasoconstriction diminishing the age-related risk of cerebral ischemia [14]. Therefore, vascular MT1 alterations in AD hippocampus may reflect both regional difficulties in blood flow regulation and adaptation to reduced melatonin levels.

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