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Brain Inflammaging: Roles of Melatonin, Circadian Clocks and Sirtuins

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Abstract

Inflammaging denotes the contribution of low-grade inflammation to aging and is of particular importance in the brain as it is relevant to development and progression of neurodegeneration and mental disorders resulting thereof. Several processes are involved, such as changes by immunosenescence, release of proinflammatory cytokines by DNA-damaged cells that have developed the senescence-associated secretory phenotype, microglia activation and astrogliosis because of neuronal overexcitation, brain insulin resistance, and increased levels of amyloid-β peptides and oligomers. Melatonin and sirtuin1, which are both part of the circadian oscillator system share neuroprotective and anti-inflammatory properties. In the course of aging, the functioning of the circadian system deteriorates and levels of melatonin and sirtuin1 progressively decline. Protective effects of melatonin and sirtuin1 are outlined and emphasis is given to possibilities of upregulating sirtuin1 by melatonin and circadian amplitude-enhancing actions of sirtuin1.

Keywords: Amyloid-β; Circadian; Cytokines; Inflammaging; Melatonin; Oxidative stress; Peroxynitrite; Sirtuins

Introduction

Low-grade inflammation is a characteristic of many aging processes. This can be related to immunosenescence, in the course of which an immune risk profile (IRP) may develop [1,2]. Aging also implies an increasing number of DNA-damaged cells, which are mitotically arrested by epigenetic processes, but still respond in multiple ways, including the development of a senescence-associated secretory phenotype (SASP) [3-6]. SASP was originally discovered in peripheral tissues, but has meanwhile been observed in astrocytes [7,8], which can, in fact, turn them into neurotoxic cells [9]. SASP enables non-immune cells to release proinflammatory cytokines and chemokines and, thereby, to spread inflammatory responses within the affected tissue. Additional sources of low-grade inflammation emerge within the brain, such as increased release of nitric oxide (•NO) in the course of neuronal overexcitation, with consequences to the activation of microglia and astrocytes, which results in a proinflammatory crosstalk between these cell types [10,11]. Microglia and astrocytes can further contribute to enhanced •NO levels by upregulating iNOS (inducible NO synthase), effects that not only enhance inflammation, but also generate oxidative and nitrosative stress as well as mitochondrial malfunction [10,11]. Moreover, progressing inflammation is observed in many, perhaps all neurodegenerative diseases [10]. Some pathological alterations such as imbalance between amyloid-β (Aβ) release and clearance, typically observed in Alzheimer's disease (AD), are also detected at lower severity in the course of normal aging. These processes have even been shown to result from sleep disturbances in apparently healthy subjects [12,13] and, with regard to the increasing frequency of insomnia during senescence, they are of substantial gerontological interest. Notably, Aβ peptides and oligomers stimulate inflammatory responses, as shown by microglia activation [14,15] and upregulation of NADP oxidase in microglia and astrocytes [16]. In AD, enhanced levels of proinflammatory cytokines and markers are typically observed, such as IL-1β, IL-6, IL-15, IL-18, TNFα, and C-reactive protein (CRP) [10]. Beyond formation and clearance of Aβ and the development of AD, sleep disturbances can also promote inflammatory processes in the brain, to which neuronal overexcitation, microglia activation, astrocytic coactivation, SASP and crosstalk between these three cell types via •NO, inflammatory cytokines and reduced astrocytic glutamate uptake seem to contribute. However, these details are rarely addressed in mechanistic terms, whereas upregulation and/or release of proinflammatory cytokines has been repeatedly demonstrated, in particular, IL-1β, IL-6, IL-8, and TNFα in the cortex, basal forebrain, hippocampus and hypothalamus of rodents [17-21]. Additionally, anti-inflammatory cytokines such as IL-4 and IL-10 were downregulated in the hippocampus [20,21]. Induction of low-grade inflammation by sleep deprivation was also shown in various peripheral organs and in rodent and human blood, details that would exceed the scope of this article.

With regard to the multiple associations of inflammation with aging and its progression, the term inflammaging had been coined [22-25]. Although inflammatory responses and processes of aging occur in every organ, brain inflammaging is of particular interest, because dysfunction of this organ changes – often profoundly–the personality of a subject and, in this sense, exceeds pathologies of peripheral organs.

With regard to its frequently documented neuroprotective properties that extend into the field of anti-inflammatory actions, melatonin is of particular interest to the maintenance of the health state in the central nervous system [10,11]. Melatonin is mostly known as the hormone of the pineal gland, but is, in fact, also produced in extrapineal sites including parts of the CNS. In total, the quantities synthesized and stored outside the pineal are by orders of magnitude higher than those in pineal and circulation [26,27]. For instance, the gastrointestinal tract (GIT) contains about 400-500 times more melatonin than the pineal gland at night [28,29]. In humans of about 20-30 years, the maximal nocturnal quantities attain values of about 100 ng in the pineal gland and 500 ng in the circulation, while the total amount in the GIT, which has not been determined in the human, may

be estimated to be in the range of 40-50 µg. However, melatonin from the GIT contributes rather poorly to circulating levels, e.g., in a postprandial response [30], but can be massively released under exceptional conditions such as tryptophan load and exceed the nocturnal levels secreted by the pineal [31]. With regard to the immunological importance of the GIT, it the immune modulatory role of local melatonin would be of interest, but has not been sufficiently studied. In addition to its secretion into the blood, pineal-derived melatonin is released in substantial amounts via the pineal recess into the third ventricle of the brain [32-34]. Melatonin regulates countless functions in the body, many of which are relevant to the brain [26]. These include modulatory actions in the immune system [35,36], antiexcitatory effects [10,11,26], antioxidative protection [37,38], support of mitochondrial integrity and function [39-41], control of circadian rhythms [42-44], sleep induction [43,45], and various effects that have been considered as contributions to healthy aging [10,11,46]. In the gerontological context, melatonin has been repeatedly shown to upregulate sirtuin1 (SIRT1) [47], an agent that also enhances circadian amplitudes by interacting with core oscillator components [47-49] and seems to possess neuroprotective and anti-inflammatory properties [50,51]. In the context of brain inflammaging, it is a remarkable fact that the three interrelated players, namely, melatonin, the circadian system and SIRT1, share the property of functionally declining with age [10,11,46,47].

Neuroprotective and immunological effects of melatonin in the context of aging

Neuroprotection by melatonin has been reported in models of stroke, traumatic brain injury and hypoxia, findings that would exceed the scope of this article. Additionally, antiexcitatory and antiexcitotoxic effects of melatonin were reported. These are insofar of importance to inflammatory responses, as they have the potential for microglia activation, e.g., via •NO release. Inhibition of neuronal NO synthase (nNOS) by melatonin has been documented [52,53] and may be of particular importance under conditions of strongly activated nNOS under the influence of high cytosolic Ca^{2+} [10]. As summarized elsewhere [10], the antiexcitatory actions also comprise various different mechanisms, such as decreases in cytosolic Ca^{2+} via GABAc or metabotropic glutamate mGlu3 receptors, GABAergic facilitation, inhibitory effects on high voltage-activated Ca^{2+} channels, changes in K^+ currents, modulation of the opioid system, and potentiation of glycine receptor-mediated inhibitory post-synaptic currents. The connection between neuronal overexcitation and microglia activation is not a unidirectional one, but extends to excitatory effects elicited by microglia [54], to the roles of coactivated astrocytes that release •NO and upregulate NADPH oxidase, to astrocytes damaged by locally overshooting inflammation, with the result of impaired glutamate uptake [55], and to consequences resulting from dying cells that release histone H1, which acts as additional proinflammatory signal and chemoattractant [57]. Moreover, release of proinflammatory cytokines such as IL-1β and IL-18 and induction of apoptotic or pyroptotic cell death are possible under the control by inflammasomes present in neurons (NLRP1 and AIM2), astrocytes (NLRP2) and microglia (NLRP3) [57].

A specific neuroprotective role of melatonin is based on the support of mitochondrial electron flux and integrity [10,58,59]. This is of particular importance as mitochondrial dysfunction further results in oxidative stress, apoptosis or mitophagy. Peripheral mitochondrial depletion that causes losses in neuronal connectivity represents a severe consequence of overexcitation and/or neuroinflammation. Again, several protective mechanisms of melatonin are jointly acting. One of them concerns the avoidance of bottlenecks in the electron transport chain (ETC) by reducing damage of proteins and membrane lipids by free radicals [39-41,60], processes that comprise upregulation of subunits of ETC complexes, enhanced formation of reduced glutathione, upregulation of glutathione peroxidase (GPx), inhibition of cardiolipin peroxidation, limitation of •NO synthesis, a compound that interacts with sulfurs in ETC proteins, further leads to transnitrosation reactions, and causes formation of peroxynitrite ($ONOO^-$) by combining with superoxide ($O_2•^-$). Melatonin also scavenges the most dangerous free radicals deriving from the decay of peroxynitrite adducts, such as ONOOH and $ONOOCO_2^-$, which yield either a hydroxyl radical (•OH) and $•NO_2$, or a carbonate radical ($CO_3•^-$) and $•NO_2$, respectively. The latter pathway may be of particular relevance to mitochondria, because of the high levels of CO_2 generated in the citric acid cycle [61].

Aβ peptides and oligomers represent pathologically relevant proinflammatory compounds to which neurons, astrocytes and microglia respond by upregulation of NADPH oxidase (NOX) and cyclooxygenase 2, release of proinflammatory cytokines, in the case of neurons at least TNFα and IL-1β [10], and of the T-cell and monocyte attracting chemokine CX3CL1 [62]. Melatonin has been shown to antagonize several Aβ-related effects, including release of IL-6, IL-1β and TNFα, which, in turn, promote Aβ peptide formation by neurons and astrocytes [10]. Moreover, it inhibits the activation of NADPH oxidase in microglia, by reducing the translocation of the NOX subunit p47phox to the plasma membrane, thereby preventing its phosphorylation in the phosphatidylinositol 3-kinase (PI3K)/Akt cascade, which is required for assembly with the subunits gp91phox and p67phox [63]. A full record of all anti-amyloidogenic effects of melatonin [10] would exceed the scope of this short communication. However, it should be mentioned that, in cell lines, melatonin reduced the activities of β- and γ-secretases and, instead, upregulated α-secretase, which cleaves the β-amyloid precursor protein (βAPP) differently to form the nonamyloidogenic and neuroprotective fragment sAPPα [64], as discussed elsewhere [11].

The effects of melatonin in the immune system are manifold [26,35,37,65,66]. A major difficulty in judging the precise immunological role of melatonin consists in the fact that it can behave both in an anti- and a proinflammatory way [35,36]. It either down- or upregulates proinflammatory cytokines and, correspondingly, either up- or downregulates anti-inflammatory cytokines, in a conditional way. Proinflammatory actions have been especially observed in cell cultures and, in humans, occur in arthritis and presumably other autoimmune diseases. However, anti-inflammatory actions were particularly observed under two conditions, high-grade inflammation and aging. In various aging organs including the brain, melatonin reduced TNFα, IL-1β and IL-6 [10,67]. Moreover, microglia activation, neutrophil and macrophage/monocyte infiltration were strongly attenuated under conditions of ischemia-reperfusion or hypoxia-ischemia [68,69]. Similar results on suppression of TNFα, IL-1β and IL-6 by melatonin were observed in various cell lines, along with downregulation of iNOS and NADPH oxidase [10,70-72]. The general impression is that melatonin mostly acts in an anti-inflammatory way with regard to brain inflammaging, despite its potential of also behaving as a proinflammatory agent.

The nexus between melatonin, circadian oscillators and sirtuins

Melatonin is mutually connected to the circadian system [26,42,47]. On the one hand, pineal melatonin synthesis is steered by the circadian master clock, the hypothalamic suprachiasmatic nucleus (SCN). On the other hand, melatonin feeds back to the SCN, where it acts a synchronizing or resetting signal. Additionally, it exerts synchronizing effects on other central or peripheral oscillators, some of which are partially or almost fully autonomous with regard to the SCN. Circadian oscillators are also mutually intertwined with sirtuins, which are regulatory factors with protein deacetylase activities. Most of the pertinent information concerns SIRT1. This protein interacts with the cellular circadian core oscillator, perhaps, in two ways [47-49]. In either case, a decisive feature is the presence of an E-box in the promoter of the *Nampt* gene. NAMPT (nicotinamide phosphoribosyltransferase) is the rate-limiting enzyme of the NAD salvage pathway, in which the sirtuin substrate NAD^+ is formed. E-box-containing genes are activated by binding of two core oscillator proteins, the BMAL1/CLOCK heterodimer (BMAL1: brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1; alias ARNTL, ARNTL1; CLOCK: circadian locomotor output cycles kaput). Therefore, *Nampt* expression and NAD^+ concentration are rhythmic and the NAD^+ level determines the activities of SIRT proteins [49,73,74]. The NAD^+ cycle drives numerous activities of all sirtuin subforms (SIRTs1-7), which comprise among others metabolic sensing, mitochondrial activities and proliferation, chromatin remodeling and anti-inflammatory actions. At least some of the sirtuin subforms also support healthy aging and may extend life span [75]. SIRT1 also displays properties of enhancing circadian rhythm amplitudes. One of the mechanisms seems to be based on the interaction of SIRT1 with the BMAL1/CLOCK heterodimer [49]. Another one involves deacetylation of PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α) by SIRT1. In its deacetylated form, PGC-1α binds to RORα (retinoic acid receptor-related orphan receptor-α), an activator at the RORE (ROR response element) in the promoters of *Bmal1* and *Clock* (Figure 1) [48].

A feature of aging is the gradual reduction of physiological capacities. This concerns also the circadian oscillator system, which exhibits various deviations in the course of aging, with considerable differences between local oscillators. Some of them, including the SCN, display reductions in amplitude, often in association with phase advances, whereas others appear to be more or less unaffected by aging. Other oscillators entirely lose their rhythmicity, which can, however, be re-initiated by suitable stimuli [76]. The deterioration of the SCN has consequences to the secretion of melatonin, which is additionally affected by various age-related diseases. As a consequence, melatonin levels are typically decreasing by age [77]. The reductions of melatonin concentrations are interindividually highly variable. In typical cases of more or less healthy persons, young subjects of 21-5 years may have nocturnal maxima between 35 and 87 pg/mL, adults of 51-55 years 17-42 pg/mL, and elderly individuals of 82-86 years 8-30 pg/mL [78]. Occasionally, much higher levels are found. The variability due to diseases is even larger. Lowest values are usually found in AD, along with a loss of circadian rhythmicity. In post-mortem pineals, the amounts of nocturnal melatonin were reduced in AD to about 20%, compared to age-matched controls [79]. The consequence of age- or disease-related losses of melatonin signifies that an important pleiotropic regulator and multiply acting protector of cellular functions is, often progressively, reduced. The functional decline is also evident at the level of SIRT1. The interrelation with the circadian system may indicate that this might be the consequence of circadian malfunction, but an additional connection exists with the decrease of melatonin. Recently, the evidence for melatonin's capacity of increasing SIRT1 expression has been summarized in the context of aging and inflammation [47]. These effects were observed in various brain regions, but also in peripheral organs or cells, such as liver, pancreas, heart, aorta and leukocytes. Notably, these actions of SIRT1 upregulation should not be perceived as being controversial to downregulations observed in cancer cells, in which the circadian oscillators are strongly dysregulated and presumably epigenetically fixed in proliferation-promoting phase positions [47]. The effects of melatonin may have the dual potential of stimulating the expression of the aging suppressor SIRT1 and of enhancing circadian amplitudes. Moreover, administration of the neuroprotective and partially anti-inflammatory melatonin has the potential of enhancing its efficacy by upregulating the likewise neuroprotective and anti-inflammatory SIRT1 [50,51].

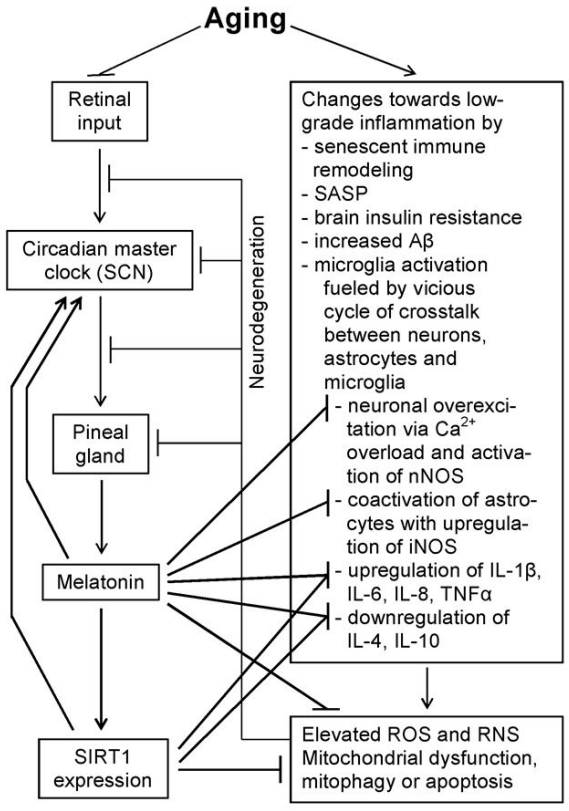

Figure 1: Simplified scheme of the relationships between the circadian master clock, melatonin secreted by the pineal gland and sirtuin1 (SIRT1), in the context of aging, brain inflammaging and neurodegeneration. Arrows indicate stimulatory actions, whereas other lines indicate inhibitions. Abbreviations: IL, interleukin; iNOS, inducible NO synthase; nNOS, neuronal NO synthase; RNA, reactive nitrogen species; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; TNFα, tumor necrosis factor-α.

Conclusion

Melatonin, circadian oscillators and SIRT1 share the property of declining with age, despite some interindividual variability. These functionally intertwined alterations seem to be disfavorable in terms of allowing tendencies towards low-grade inflammation and especially brain inflammaging. These changes are overlaid by other aging-dependent processes, in particular immunosenescence, SASP, moderate or stronger rises in Aβ release, and inflammatory consequences of insulin resistance, especially in the brain. Age-related increases in the inflammatory state have numerous consequences to exposed cells, such as oxidative and nitrosative stress with resulting mitochondrial dysfunction, depletion by mitophagy, loss of neuronal connectivity or cell death [10,11,26,41,58]. Other consequences of neurodegeneration, which are summarized in Fig.1, concern the SCN, the pineal gland and the cellular expression of SIRT1. The aging-associated decrease of melatonin depends largely on degenerative processes at different levels. Increasing turbidity of the lens reduces the retinal input to the SCN and, thereby, weakens the oscillatory capacity [11,80]. This reduction is partially responsible for lower amplitudes and levels of the melatonin rhythm. Additionally, neurodegeneration contributes to functional losses in the circadian system and to decreases in melatonin secretion. This can concern the entire path of neuronal transmission to the pineal gland, i.e., the retinohypothalamic tract, the SCN, the connections to the pineal via paraventricular nucleus, upper thoracic cord and upper cervical ganglion, and in the pineal gland itself. Sometimes, melatonin secretion may be also reduced by pineal calcification [26]. While these age-related changes can be interpreted with sufficient plausibility, the disease-induced reductions have to be differently judged. In AD or frontotemporal dementia, dysfunction of SCN and pineal are of mainly neurodegenerative nature, however, more strongly and rapidly progressing than in normal aging. Reductions of melatonin because of pain-associated diseases [77] have to be differently interpreted, but would require experimental clarification. Age-related reductions in SIRT1 expression may be caused by decreased melatonin levels, since exogenous melatonin elevated SIRT1 levels in various entirely different studies, as far as they were done in the context of aging but not that of cancer [47]. Notably, both melatonin and SIRT1 represent compounds that act in the SCN and in peripheral oscillators as amplitude-enhancing factors [47,80]. Melatonin displays numerous properties that attenuate or even prevent cellular malfunction, including protection against oxidative and nitrosative damage and support of mitochondrial function. Moreover, melatonin can reset circadian oscillators and upregulate the neuroprotective, anti-inflammatory and circadian amplitude-enhancing SIRT1. With regard to these beneficial properties, the possibilities of reducing age-related low-grade inflammation and inflammaging by the pineal hormone should be more consequently considered in the future.

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Conflict of Interest

None declared.

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