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REVIEW ARTICLE

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Molecular, Biological, Physiological and Clinical Aspects of Melatonin

Melatonin and inflammation—Story of a double-edged blade

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Abstract

Melatonin is an immune modulator that displays both pro- and anti-inflammatory properties. Proinflammatory actions, which are well documented by many studies in isolated cells or leukocyte-derived cell lines, can be assumed to enhance the resistance against pathogens. However, they can be detrimental in autoimmune diseases. Anti-inflammatory actions are of particular medicinal interest, because they are observed in high-grade inflammation such as sepsis, ischemia/reperfusion, and brain injury, and also in low-grade inflammation during aging and in neurodegenerative diseases. The mechanisms contributing to anti-inflammatory effects are manifold and comprise various pathways of secondary signaling. These include numerous antioxidant effects, downregulation of inducible and inhibition of neuronal NO synthases, downregulation of cyclooxygenase-2, inhibition of high-mobility group box-1 signaling and toll-like receptor-4 activation, prevention of inflammasome NLRP3 activation, inhibition of NF-κB activation and upregulation of nuclear factor erythroid 2-related factor 2 (Nrf2). These effects are also reflected by downregulation of proinflammatory and upregulation of anti-inflammatory cytokines. Proinflammatory actions of amyloid-β peptides are reduced by enhancing α-secretase and inhibition of β- and γ-secretases. A particular role in melatonin's actions seems to be associated with the upregulation of sirtuin-1 (SIRT1), which shares various effects known from melatonin and additionally interferes with the signaling by the mechanistic target of rapamycin (mTOR) and Notch, and reduces the expression of the proinflammatory *lncRNA-CCL2*. The conclusion on a partial mediation by SIRT1 is supported by repeatedly observed inhibitions of melatonin effects by sirtuin inhibitors or knockdown.

KEYWORDS

aging, circadian, cytokines, immune system, inflammaging, melatonin, SIRT1

1 | INTRODUCTION

After the discovery of melatonin's chronobiological role, numerous additional functions have been identified.[1,2] Although the circadian system is intertwined with many of these other actions, they exceed, in both physiological terms and practical relevance, the aspect of rhythmicity. This statement is also applicable to the immunological effects of melatonin. This field of action had not been foreseeable and many researchers were surprised by the first reports on that topic.[3-6] Since then, a large body of information has accumulated, which has been repeatedly reviewed.[2,7-13] A complete overview of the immunological role of melatonin would exceed the scope of this article. Instead, the focus will be laid on its specific actions in inflammation.

Inflammation is of particular importance to health. This should not only be seen in the context of local or systemic high-grade inflammation, but gains remarkably high

relevance, as soon as the role of low-grade inflammation is considered, especially in the areas of aging, disturbances of brain function including mood disorders and neurodegeneration.[14-19] As summarized in these publications, inflammation can become chronic and, by time, aggravating because of several vicious cycles by which cross talks between immune cells and other cell types, including neurons and astrocytes in the brain, lead to mutual stimulations. In a gerontological context, aging-specific alterations caused by processes of immunosenescence and, in non-immune cells, by SASP (senescence-associated secretory phenotype) and garb-aging can lead to proinflammatory conditions fueled by cytokines, chemokines, nitric oxide, and aberrant proteins.[14,15,18] The contribution of inflammation to aging has become known under the term of inflammaging. Additional effects are observed in Alzheimer's disease (AD), in which amyloid-β (Aβ) peptides and oligomers, to a certain extent also amyloid plaques, enhance inflammatory responses and in which brain insulin resistance may initiate and aggravate inflammation.[16]

As already indicated by the title, the immunological actions of melatonin are not uniform, but rather conditional and can comprise both pro- and anti-inflammatory effects.[10,12] This duality represents a challenge to investigators and clinicians, since the conditions under which the respective response takes place are only partially known and require further clarification. Nevertheless, these entirely opposite actions should not be perceived as contradictory or even controversial. They are not a matter of experimentation, but rather depending on conditions and participating cell types, as will be discussed in this article.

2 | A REMARK ON THE CONDITIONALITY AND VARIABILITY OF IMMUNE RESPONSES

The statement that melatonin's actions in the immune system are conditional should not be regarded as being surprising. Neither melatonin nor the immune system responds uniformly under different conditions and in different cells. Melatonin, otherwise known as an antioxidant and anti-apoptotic agent in the majority of nontumor cells and as an upregulator of sirtuin-1 (SIRT1) under conditions of aging, displays prooxidant, proapoptotic and SIRT1-downregulating properties in other cases, especially in cancer cells.[12,20,21] Concerning the immune system, a researcher who dives deep enough will be impressed by its complexity, which becomes evident in the multitude of participating cell types and subtypes as well as humoral factors, the flexible regulatory network, the participation of non-immune cells in the classic sense, and, even more, in the temporal changes of both short and long duration. With regard to acute inflammation, the immune system has to activate the required cellular and humoral processes, but later to actively terminate them and initiate healing. Moreover, it has to discriminate between different degrees of severity. The behavior of the immune system also differs between acute and chronic inflammation.[10] Low-grade chronic inflammation is a basis of numerous diseases and a major contributor to aging.[14-18] Because of vicious cycles, the immune system is frequently incapable of terminating the inflammatory processes rapidly enough or not at all. A particular aspect of temporal variability concerns immunosenescence. Especially, the age-dependent thymic involution causes a shift in the balance between T-lymphocyte-mediated processes of adaptive immunity and the less affected innate immune system, although both of them are changing in the course of aging.[22,23] Moreover, the lifelong exposure to foreign antigens causes reductions of certain cell types. Additionally, thymic involution leads to partial exhaustion of subpopulations of other immune cells, not surprisingly observed especially in the loss of $CD95^-$, virgin T lymphocytes, mostly in the $CD8^+$ subpopulation.[24] To remain capable of sufficiently counteracting infectious challenges, the immune system has to be remodeled. For instance, the losses of $CD95^-$ $CD8^+$ T lymphocytes are tried to be compensated by clonal expansion of $CD28^-$ cells, which display, however, a reduced proliferative activity.[24] Another typical feature of immunosenescence is a shift toward a proinflammatory phenotype, which is observed by increased proinflammatory cytokines. Interestingly, centenarians also exhibit these increases, however, in conjunction with elevated anti-inflammatory cytokines.[25] Additionally, numerous changes are observed in composition of B lymphocytes and antibody subtypes.[14] This short outline indicates that identical responses to the immune modulator melatonin cannot be expected under conditions that vary with regard to the inflammatory grade and chronification, the cause and progression of the pathological or aging-related changes.

3 | PROINFLAMMATORY ACTIONS AS REVEALED BY STUDIES ON IMMUNE CELLS

Melatonin can be classified as an immune stimulatory agent.[7-10] This comprises, of course, proinflammatory actions. Notably, many of these effects have been observed in cells collected from blood or other body fluids or in leukocyte-derived cell lines. One of the earliest findings of this type was obtained in monocytes, which responded to melatonin by enhanced formation of reactive oxygen species (ROS).[26] This can be interpreted as a defense reaction normally associated

with inflammation, apart from the fact that oxidative stress can elicit attraction and activation of other immune cells, with inflammatory consequences. The prooxidant response was later confirmed in the myeloid cell line U937,[27,28] cells that have the potential of differentiating into macrophage-like cells and release chemokines and cytokines such as interleukin-1β (IL-1β). Moreover, the frequently cited down-regulation of 5-lipoxygenase (5-LOX) by melatonin, usually interpreted as an antioxidant action,[29,30] was not confirmed in U937 cells, which instead increased ROS formation via this enzyme, a response that was inhibited by 5-LOX inhibitors.[31] Additionally, the original interpretation of a mediation of the melatonin effect by the retinoic acid receptor-related orphan receptor RORα, which was believed to represent a nuclear melatonin receptor,[29,30] should be dropped, since RORα does not bind melatonin, as has now been convincingly shown.[32,33] Finally, it should be briefly noted that U937 cells and, especially, their macrophage-like derivatives are also capable of releasing enhanced levels of the anti-inflammatory IL-10,[34] an effect that is only occasionally observed in normal macrophages,[35] but remains to be studied under the influence of melatonin. Nevertheless, the capacity of the same cell type to respond in an either pro- or anti-inflammatory way sheds light on the complexity and conditionality of immunological processes.

The release of proinflammatory cytokines, such as IL-1β (in earlier studies, IL-1), IL-2, IL-6, IL-12, TNFα, and IFNγ in response to melatonin has been repeatedly observed in monocytes, monocyte-derived cells and T-helper cells type 1,[8-10,12,36-48] of IL-17 in non-preactivated Th17 cells,[49] and of IL-1β, TNFα and IFNγ in splenocytes, too.[50] Although most studies clearly point into this direction, deviating findings have also been published. In isolated human PBMCs, neither TNFα nor IFNγ were found to be increased by melatonin, but rather, upon stimulation by phytohemagglutinin, to be reduced. However, this effect was only observed in 22% of donors.[51] In addition to the stimulation of IL-2 formation, some regulatory aspects concerning this cytokine have to be mentioned. When the repeatedly observed increase of IL-2 and IL-6 was tested for an involvement of the MT_1 receptor, the MT_1-selective agonist S0098 failed to reproduce this effect.[8] Therefore, further research was directed toward the ROR subfamily of the retinoid receptors,[8,37] in those years a reasonable concept, which has now to be abandoned. More recently, the involvement of MT_1 in IL-2 release has been reconsidered.[10] In a transfected cell line overexpressing *MT1* antisense RNA, IL-2 production was decreased. Additionally, melatonin was shown to upregulate the expression of the IL-2 receptor (IL-2R) via MT_1.[10,44,45] Melatonin was also found to counteract the inhibition of IL-2 production by prostaglandin E_2 (PGE_2).[44,45] Moreover, it seems important to consider an effect of IL-2 on IL-10 secretion by Th-lymphocytes.[52] The action on this anti-inflammatory cytokine indicates a dampening of inflammation by an otherwise proinflammatory agent, underlining the complexity of the immune system, which does not generally act in an unidirectional way. However, the effects of melatonin on IL-10 are, again, difficult to interpret. An early study reported that melatonin does not directly influence the release of IL-10 by human Th2-lymphocytes, but it was found to reduce the IL-2-induced secretion of IL-10.[52] A decreased IL-10 production by melatonin was also observed in Th-lymphocytes in primary culture.[53] Although the suppression of an anti-inflammatory cytokine may be interpreted as a shift toward the proinflammatory side, it has remained unclear to what extent these results obtained in vitro really favor inflammation in vivo.

Although direct upregulations of proinflammatory cytokines, as far as they have been demonstrated, may be understood as a role of melatonin in favoring the inflammatory limb of the immune system, several reservations have to be addressed. As most of the respective effects have been obtained in isolated immune cells or transformed leukocyte-derived cell lines, the relevance in vivo is not similarly certain. The complexity of the immune system comprises numerous regulatory connections that may negatively or positively feed back and complicate an a priori judgment, which cannot replace measurements of in vivo samples. Moreover, many of the effects obtained are associated with developmental aspects of clonal or subpopulational expansion, which is also indicated by concomitant enhancements of factors such as M-CSF, TGFβ, IL-5, or IL-9 as well as by shifts in size of leukocyte subpopulations.[2,7-11] The role of melatonin in Th17 cells that secrete the strongly proinflammatory IL-17 will require further attention. However, this has been mainly studied under already inflammatory pathological conditions.[54] In the context of aging, in otherwise unchallenged mice, changes of IL-17 and the Th17-stimulating IL-23 remained rather moderate.[55] The action of melatonin as a broad-spectrum immune modulator may also create countless secondary effects on both the pro- and anti-inflammatory sides that are not easily foreseeable. It should also be noted that the extent of melatonin-stimulated increases in several proinflammatory cytokines has strongly varied between studies. Nevertheless, proinflammatory effects of melatonin are a matter of fact and have to be considered in humans, especially under conditions of autoimmune diseases, as will be discussed below in section 9.

4 | ANTI-INFLAMMATORY ACTIONS IN MEDIUM- AND HIGH-GRADE INFLAMMATION

It is a remarkable fact that a compound like melatonin, which has the potential of acting in a proinflammatory way, can also efficiently suppress inflammatory responses, especially when

these are strong and, in the extreme, life-threatening. One type of experiments in which these observations have been made, concerns the use of bacterial lipopolysaccharide (LPS) or experimental sepsis, for example, by cecal ligation and puncture. In the majority of cases, melatonin turned out to be highly protective. These effects comprised both immunological and non-immunological actions. In the latter category, antioxidative protection and preservation of mitochondrial function have to be foremost mentioned, as they counteract consequences of inflammation and reduce inflammation itself via promoting antioxidative processes, decreasing free radical formation, and excessive NO formation. As these details exceed the immunological topic of this article, most of them should be followed via the references provided here.[56-78] However, it should be emphasized that an important nexus exists between mitochondrial dysfunction and inflammation that concerns elevated levels of NO. This includes the consequences induced by NO metabolites, in particular, other nitrosating derivatives (NO^+, HNO, N_2O_3, *S*-nitrosoglutathione), peroxynitrite ($ONOO^-$), and free radicals formed from this compound, such as the hydroxyl radical ($^{\bullet}OH$), the carbonate radical ($CO_3^{\bullet-}$), and nitrogen dioxide ($^{\bullet}NO_2$). The reactive nitrogen species (RNS) and the two oxidizing radicals mentioned can partially or almost completely block the electron transport chain (ETC) by binding to irons and iron-sulfur clusters, by nitrosation or nitration and by oxidizing ETC components.[14,79-82] The crucial role of NO levels, with regard to both proinflammatory signaling and induction of mitochondrial dysfunction has been convincingly elaborated, studies in which the downregulation of inducible NO synthase (iNOS) including its mitochondrially targeted variant (mt-iNOS) has been shown to be a key effect of melatonin in suppressing sepsis-induced damage.[56,57,59,61-64,71,77,83-86]

Anti-inflammatory actions of melatonin can be also traced by following various different parameters and mechanisms. Inhibition of activation of inflammatory cells by melatonin has been demonstrated by reduced myeloperoxidase activity.[58,60,87] Another line of evidence results from the studies of inflammasomes, as recently reviewed.[88] These can be activated by various means and are to a certain degree cell type-specific. Apart from activations by foreign double-stranded DNA, nuclear envelope disruption, thioredoxin-interacting protein (TXNIP), and signaling by surface receptors, a particularly important activation pathway consists in the intramitochondrial association with caspase-1, sometimes also with caspase-5. In the context of melatonin, only one type of inflammasome has been studied, the NLRP3 (nucleotide-binding oligomerization domain, leucine-rich-containing family, pyrin domain-containing-3). In the future, it might be important to also study other inflammasomes, such as NLRP1 and AIM2 (absent in melanoma-2) present, for example, in neurons, NLRP2 in astrocytes, NLRC4, which is of particular relevance to autoimmune diseases, NLRP6 and NLRP12, which have become of interest in cancer research. Suppression of NLRP3 activation by melatonin has been observed under various conditions. This was shown in radiation-induced damage by radiotherapy of oral mucositis,[89] and small intestine toxicity,[90] in the septic heart,[91-93] pyroptosis induction in adipose tissue,[94] subarachnoid hemorrhage,[95,96] acute lung injury,[97] and cadmium hepatotoxicity.[98] In cultured keratinocytes, exposure to elevated glucose concentrations caused NLRP3 activation, which was inhibited by melatonin, findings that were interpreted as a means for promoting wound healing in diabetics.[99] In mice, osteoporosis induced by ovariectomy was shown to involve NLRP3 activation.[100] This inflammatory facet of osteoporosis might not appear that much surprising with regard to the development of osteoclasts from monocytes, but instead effects mainly concerned osteogenesis. NLRP3 knockdown was reported to attenuate the inhibition of osteogenesis and similar results were obtained with melatonin, findings that were interpreted in terms of Wnt/β-catenin signaling. A further argument for the involvement of NLRP3 inhibition in the osteogenic action of melatonin was deduced from the counteraction by the NLRP3 activator, monosodium urate.[100] As the role of melatonin in the balance of osteogenic differentiation and osteoclastic activity seems to be much more complex and may also require the consideration of SIRT1,[101-104] further studies may be necessary to clarify the relative contributions of the melatonin effects. Concerning signaling, the well-known inhibition of NF-κB activation by melatonin[105] has also been implicated in the suppression of NLRP3.[89-92,94] Some of the actions may also be tissue-specific. In adipose tissue, NF-κB initiated pyroptosis via gasdermin D (GSDMD).[94] Another, redox-related pathway inhibited by melatonin concerned the activation of NLRP3 by thioredoxin-interacting protein (TXNIP).[98] The role of RORα had been also addressed in the context of NLRP3 inhibition.[91] However, contrary to a rather frequently communicated earlier belief, RORα should no longer be discussed as a nuclear melatonin receptor, since it demonstrably does not bind melatonin.[32,33] However, this does not exclude indirect actions of melatonin via this transcription factor. Since melatonin influences circadian oscillators including the peripheral clocks, effects on the accessory oscillator component RORα are possible and have been discussed earlier as actions beyond direct binding.[106] Meanwhile, a connection between melatonin and another accessory component, SIRT1, has become evident and various effects of melatonin may be mediated by this sirtuin.[21,107,108] In fact, the assumption of indirect effects of melatonin on RORα via the circadian system has received recent support. SIRT1 has been shown to deacetylate PGC-1α, which facilitates the binding of RORα to RORE sequences and, thus, increases the transcription of the core oscillator genes *Bmal1* and *Clock*. This effect that has been interpreted as a means for enhancing the rhythm amplitude,[109] but may also influence

the phase position of a rhythm. The role of SIRT1-mediated effects on core oscillator expression in inflammation and inflammasome expression has been discussed in the context of aging[92] and sepsis[93] and recently reviewed in relation to mitochondrial function.[78] In NLRP3$^{-/-}$ knockout mice, the *Bmal1* gene repressor, Rev-Erbα, was not upregulated. Correspondingly, the BMAL1/CLOCK-dependent expression of *Nampt*, the gene of the rate-limiting enzyme of SIRT1 activity, remained unresponsive.[93] Moreover, melatonin was shown to suppress the inflammasome-dependent production of IL-1β[77,92,95] and IL-6.[95]

Additional evidence has been obtained for an anti-inflammatory action of melatonin under various conditions of high- or medium-grade inflammation. In human blood cells, LPS-induced TNFα was reported to be reduced by melatonin.[110-112] Corresponding effects were obtained in neutrophils and also observed in the level of IL-8.[111] Interestingly, the melatonin metabolite AFMK (N^1-acetyl-N^2-formyl-5-methoxykynuramine) was reported to be also effective and to exceed in this regard the parent compound, melatonin.[111] In cultured human lymphocytes and monocytes stimulated by IL-2, melatonin also reduced IL-6.[52] IFNγ was shown to induce melatonin synthesis in lymphocytes, in which melatonin inhibited, however, the phytohemagglutinin-induced synthesis of IFNγ.[113] Reductions of IL-1β and TNFα by melatonin were also observed in cadmium-induced hepatotoxicity.[98] Downregulation of IL-1β, IL-6, IL-8 was reported in a diabetic wound healing model using keratinocytes.[99] Infection by rabbit hemorrhagic disease virus (RHDV) caused fulminant hepatotoxicity and rises in IL-1β, IL-6, TNFα, and C-reactive protein (CRP), changes that were reduced by melatonin.[114] In experimental sepsis by cecal ligation and incision, splenocytes of $MT_1^{-/-}/MT_2^{-/-}$ double-knockout mice responded more strongly than controls showing higher levels of IL-1β, IL-2, TNFα, the chemokine CXCL2 and its receptor, CXCR2, as well as those of the adhesion molecules ICAM-1 and L-selectin.[115]

While proinflammatory cytokines were typically reduced by melatonin in various models of high-grade inflammation, the responses were different under several other conditions. In three murine models of hepatic ischemia/reperfusion which also included partial hepatectomy and partial liver transplantation, melatonin was shown to be protective, however, while upregulating IL-6, IL-10, and TNFα.[116] Notably, this response comprised a concomitant increase in both the proinflammatory agents IL-6 and TNFα and the anti-inflammatory IL-10. However, the upregulation of IL-6 was reported to be crucial for the protective action of melatonin, because IL-6$^{-/-}$ mice did not show hepatic recovery. Nevertheless, this was restored by recombinant IL-6. Inhibition of the IL-6 co-receptor GP130 also prevented the protective effect of melatonin. Melatonin was concluded to promote liver regeneration through monocyte-released IL-6 and downstream IL-6/GP130-STAT3 signaling.[116] These data show that a general association of beneficial effects by melatonin with downregulation of proinflammatory cytokines would be an oversimplification. Moreover, the spectrum of actions of a specific cytokine such as IL-6 is more complex than just the induction of a proinflammatory response. The multiple interrelationships within the immune system and the contextuality of an intervention have to be considered. Additionally, the precise experimental conditions have to be taken into account. When melatonin was added prior to an LPS challenge to isolated alveolar, splenic or peritoneal macrophages and to a microglial cell line, it did not prevent increases in IL-1β and TNFα.[117] These findings were interpreted as an inability of melatonin to prime macrophages or microglia before an inflammatory challenge. However, this would not be contradictory to a protective, anti-inflammatory action after the onset of inflammation, as observed many times and summarized above.

Any discussion of details concerning especially changes in cytokine regulation has to consider species differences and also disease-specific variations. For instance, murine autoimmune encephalomyelitis used as a model for multiple sclerosis (MS) demonstrated upregulation of IL-10 by melatonin and reduced mononuclear infiltration.[118] Additionally, a reduction of the Th1 response was observed,[118] whereas conclusions concerning a Th17 response did not find their expression in humans.[119] In PHA-stimulated PBMCs from patients with a remitting type of MS, melatonin decreased the Th1 (release of IL-2, IL-12, IFNγ, TNFα) and Th22 responses (IL-22 release), whereas the Th17 response remained uneffected.[119] In an animal model of autoimmune encephalomyelitis, melatonin elevated IFNγ levels, increased lymphocyte infiltration and astrocyte activation.[120] In a study on systemic lupus erythematosus using PHA-stimulated PMBCs, healthy controls responded to melatonin by downregulating IFNγ, IL-1β, TNFα, IL-5, and IL-9, whereas patients upregulated these cytokines either demonstrably or, at least, tendentially.[54] Additional effects concerning increased numbers of Treg cells were somewhat obscured by a high variability.[54] These differences between patients and controls shed light on the problem of disease manifestation and also on the particular role of melatonin in autoimmune diseases, in which melatonin may, at least, in a number of cases, exhibit proinflammatory rather than anti-inflammatory properties.[12,121-123]

5 | MELATONIN AND LOW-GRADE INFLAMMATION IN AGING

Low-grade inflammation is a common feature of senescence.[16,124] Although this can be observed in apparently healthy subjects, it gains particular relevance in

neurodegenerative diseases, in which vicious cycles lead to a progressive severity of inflammatory processes that end up in massive losses of neuronal connectivity and of cells, as will be discussed in section 6. Inflammaging in widely healthy subjects is associated with numerous contributing changes. These comprise aspects of immunosenescence, SASP, garb-aging, circadian malfunction and, notably, also decreased levels of melatonin.[14-19,21-25,124-126] With regard to the immune modulatory and numerous protective actions of melatonin, the usefulness of this agent in attenuating age-related inflammation is an important objective. Concerning the ambiguities of melatonin in acting in an either pro- or anti-inflammatory way, it is of particular relevance to clarify the prevalent effects in aging. Although it may be too early for a definite decision, the actually available data indicate that a distinction has to be made between patients that have acquired an autoimmune disease, such as rheumatoid arthritis, which is not uncommon in otherwise healthy elderly subjects, or multiple sclerosis, and individuals that are devoid of such immunological maldevelopments. Concerning arthritis, a caveat remains because of proinflammatory actions of melatonin that aggravate the disease.[12,121-123] With regard to the beneficial effects of melatonin in aging, the actual judgment has to mainly rely on preclinical data. Nevertheless, the increased secretion of proinflammatory cytokines in human aging has been emphasized in the context of reduced melatonin levels.[127]

The mainly anti-inflammatory actions of melatonin in the gerontological context were documented in several organs. In the liver of aged, ovariectomized female rats, levels of the proinflammatory cytokines TNFα, IL-1β and, particularly strongly, IL-6 were reduced, whereas the anti-inflammatory IL-10 was substantially upregulated.[128] In the same animal model, TNFα, IL-1β, and IL-6 were downregulated by melatonin in dentate gyrus of the hippocampus.[129] Anti-inflammatory actions of melatonin have been also studied in microglial cell lines. In BV-2 cells, melatonin inhibited a fluoride-induced upregulation of NADPH oxidase, iNOS expression, the phosphorylation of c-Jun N-terminal kinase (JNK) and reduced the release of TNFα and IL-1β. [130] In HAPI cells, melatonin attenuated the amphetamine-induced rise in iNOS, an effect that should dampen inflammatory responses,[131] as well as the methamphetamine-induced overexpression of TNFα, IL-1β, and IL-6.[132] In SH-SY5Y neuroblastoma cells, melatonin likewise suppressed the upregulation of iNOS and TNFα by methamphetamine.[133] In aging rats, TNFα, IFNγ, IL-6, and IL-8 were shown to be increased, effects that were attenuated by melatonin.[134] Further rises in proinflammatory cytokines were observed in aged rats subjected to constant light, a melatonin-suppressing treatment, which was, again, antagonized by melatonin.[135] In menadione-treated HUVECs (human umbilical vein endothelial cells), increases in TNFα were reduced by melatonin.[136] In the same study, old mice subjected to femoral artery ligation exhibited rises of TNFα and IL-6 in the ischemic quadriceps, effects that were also reversed by melatonin.[136]

Several investigations have followed effects of melatonin on age-related inflammation in the senescence-accelerated mouse strain SAMP8, usually in comparison with the largely isogenic, but normally aging control strain SAMR1. In the SAMP8 liver, TNFα and IL-1β were downregulated and anti-inflammatory IL-10 upregulated by melatonin.[137] Similar anti-inflammatory effects were observed in pancreas[138] and heart.[139,140] TNFα and IL-1β were also shown to be upregulated in the aging SAMP8 lung, changes that were again corrected by melatonin.[141] Similar decreases of inflammatory markers were observed upon long-term treatment of SAMP8 mice with melatonin, in a study on the proinflammatory role of perivascular adipose tissue.[142]

It should be noted that the studies mentioned in this section also contained a lot of information on possible regulation mechanisms. They frequently showed a relationship to oxidative and nitrosative stress, based on determinations of NADPH oxidase subforms as well as iNOS and, additionally, markers of oxidative stress. Moreover, relationships to mitochondrial malfunction and induction of apoptosis or mitophagy were addressed. Counteractions by melatonin against these unfavorable processes were typically demonstrated, as discussed elsewhere in this article or other publications.[14,16,80,82] Repeatedly, the suppression of NF-κB signaling by melatonin was discussed in this context and also its positive effects on the antioxidant regulators DJ-1 and Nrf2.[134,135,141] Additional information is related to neurodegenerative diseases or to the contribution of sirtuins, findings that will be discussed in sections 6 and 8, respectively. A particular aspect related to inflammation shall be briefly mentioned. It concerns the protein family of suppressors of cytokine signaling (SOCS). Especially in the intestine and the heart, melatonin was shown to increase the expression of the *Socs1* gene.[143] However, the interaction of melatonin with SOCS subforms is complicated by additional roles of these proteins concerning the circadian system and developmental processes.

6 | MELATONIN AND INFLAMMATION IN NEURODEGENERATIVE DISEASES

Low-grade brain inflammation, which is already developing in the course of normal aging, is further aggravated in neurodegenerative diseases.[16] This is particularly evident in the most severe forms of dementias such as Alzheimer's disease (AD). Nevertheless, neuroinflammation is also a characteristic of other diseases of entirely different etiologies, such as Huntington' disease, Parkinson's disease, amyotrophic lateral sclerosis, spinocerebellar ataxia, and multiple sclerosis.[144]

Inflammation leads necessarily to oxidative stress and, under conditions of strongly elevated NO levels, to nitrosative/nitrative stress, too. However, oxidative stress, especially when caused by mitochondrial malfunction, can also induce inflammatory responses. The pivotal role of oxidative stress in these pathologies has been recently reviewed.[145] Melatonin is a broad-spectrum antioxidant that acts by various mechanisms that comprise direct and indirect detoxification of free radicals, mitochondrial protection, reduction of free radical formation by inhibiting electron leakage and NADPH oxidases, prevention of neuronal overexcitation and several anti-inflammatory actions.[2,14,16,80,82,146,147] From this point of view, melatonin can be assumed to counteract inflammation by reducing both possible causes of oxidative stress and proinflammatory signaling. In fact, several encouraging findings have been obtained by using melatonin in various neurological disorders, however, not yet on a sufficiently broad basis that would justify a more general clinical application.[148] Moreover, it seems important to avoid inappropriate generalizations. The existence of antioxidant and anti-inflammatory actions does not exclude additional undesired effects of melatonin in certain neuropathologies. In the case of multiple sclerosis, the autoimmune etiology has to be taken into consideration. As melatonin can also display proinflammatory properties, for example, in arthritis,[121-123] caution is due in all autoimmune diseases. In the case of Parkinson's disease (PD), numerous preclinical findings have indicated a beneficial role of melatonin,[149-151] however, mainly in models based on mitochondrial dysfunction in the nigrostriatum and mostly without considering the early pre-motor symptoms that reflect the initial stages of the disease. Moreover, several findings have remained contradictory.[13,152] Melatonin had been even regarded as a detrimental factor in PD and treatment with melatonergic antagonists had been reported to be beneficial.[153,154]

In the case of AD, several peculiarities have to be considered that exceed the usual interplay of neuronal excitation, astrocytic and microglia activation, with consequences for expression and release of proinflammatory cytokines. These specific changes concern additional proinflammatory signals, among which the most important effects are caused by Aβ peptides and oligomers, which fuel neuroinflammation in multiple ways.[155] Toxicity of amyloid plaques seems to be less severe and to be largely related to two effects. Physical contact with cells, especially microglia but also astrocytes and neurons, leads to responses of activation and enhanced generation of free radicals. Additionally, amyloid plaques are capable of sequestering copper and zinc and, therefore, can deplete the stores of these metals which are required for a functional Cu,Zn-superoxide dismutase.[155] This may cause impairments of superoxide removal. Microglia has been shown to be directly activated by Aβ peptides and oligomers,[156] and also, by amyloid plaques.[157] $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ oligomers were reported to induce oxidative stress by upregulating NADPH oxidase in both astrocytes and neurons.[158] A vicious cycle seems to result from responses of astrocytes to Aβ peptides, which induce a further upregulation of $A\beta_{1\text{-}42}$ formation and secretion.[159] An additional enhancement of neuroinflammation can be deduced to occur under the influence of the AD risk factor, APOE4, a conclusion supported by experimental data.[157] A further peculiarity of AD has emerged by its relationship to brain insulin resistance, which was shown to represent an early event in the AD etiology.[160-164] Insulin resistance, which is a hallmark of type 2 diabetes, has a strong relationship to low-grade, sterile inflammation, as shown, for example, by substantial upregulation of IL-6,[165] and activation of the NLRP3 inflammasome.[166] Whether brain insulin resistance has consequences similar to those in type 2 diabetes,[167] or whether it may represent a separate type of pathology referred to as type 3 diabetes,[168,169] remains to be a matter of debate. Recently, effects of hyperinsulinemia were reported concerning the clearance of Aβ peptides over the blood-brain barrier, however, with divergent results in $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$,[170] details that will need further clarification with regard to their pathological consequences. Type 2 diabetes is known to represent a risk for cognitive impairment and dementia.[171-173] However, its involvement in AD pathology has remained less clear, since other forms of cognitive deficits and vascular dementia are rather common in diabetes and have to be distinguished. Concerning the human, the relationship between melatonin and type 2 diabetes has still remained uncertain, despite numerous demonstrations of antidiabetic actions in preclinical animal studies.[174] The uncertainties are connected to differences in energy metabolism between the nocturnal laboratory rodents and the diurnally active human in relation to the melatonin maximum and, additionally, to the reduction of insulin secretion by an overexpressed melatonin receptor variant (G allele of *MTNR1B*).[12,21,108,175-177] However, the situation concerning the diabetogenic action of melatonin in humans has remained ambiguous, insofar as the suppression of insulin secretion by enhanced melatonergic signaling has to be distinguished from insulin resistance.[21]

Regardless of these complications concerning the contribution of insulin resistance to inflammation in AD, direct evidence has been obtained in a couple of studies for anti-inflammatory actions of melatonin in this pathology. Additionally, various findings on reduction of Aβ formation by melatonin should be taken as indirect evidence for a counteraction against inflammation by decreasing the proinflammatory factor Aβ. In AD models, changes in proinflammatory cytokines by melatonin have been only occasionally observed. In organotypic mouse brain slice cultures, $A\beta_{1\text{-}40}$ induced IL-1β and IL-6, effects that were partially inhibited by melatonin.[178] In hippocampal slices, melatonin reduced

glial activation and the release of TNFα and IL-6 that had been induced by $A\beta_{25-35}$.[179] In APP + PS1 (amyloid precursor protein and presenilin 1) double transgenic mice, melatonin was shown to reduce the hippocampal levels of TNFα.[180]

Numerous studies have addressed the actions of melatonin in AD. Apart from the few studies mentioned concerning direct anti-inflammatory effects, many investigations have dealt with melatonin's antioxidant properties, with inhibition of tau hyperphosphorylation, tangle formation, and plaque formation.[181-185] Although one may conclude that attenuation of oxidative stress and decreased free radical formation by mitochondrial protection will also reduce one stimulus of enhanced inflammation, the extent and clinical relevance of this potential anti-inflammatory action has not been sufficiently documented. However, with regard to the proinflammatory effects of Aβ peptides and their derivatives, reductions in Aβ formation may presumably represent a substantial contribution to a decrease in neuroinflammation. In fact, melatonin was shown to lower Aβ peptide levels,[186-188] and also to inhibit amyloid plaque deposition in transgenic murine models of AD.[189,190] Several effects seem to contribute to this reduction of Aβ. Melatonin was reported to decrease the mRNA expression of the βAPP (β-amyloid precursor protein) in the pheochromocytoma cell line P12.[191] Whether or not this action is decisive and applicable to other cells in the CNS remains to be shown. Interestingly, melatonin also modulates substantially the processing of βAPP. In HEK293 and N2a cells overexpressing βAPP, melatonin increased the activity of α-secretase.[192] Importantly, this enzyme competes with the amyloidogenic β- and γ-secretases for βAPP, but generates the nonamyloidogenic and neuroprotective fragment sAPPα. Moreover, inhibition of β- and γ-secretases was observed under the influence of melatonin in the human neuroblastoma SH-SY5Y cell line.[193] The shift from β- and γ-secretase to α-secretase expression was recently also observed in the aged murine hippocampus.[194,195] Based on further experiments in SH-SY5Y cells, it was shown that $A\beta_{1-42}$ stimulates the pathogenic change of βAPP processing as well as PS1 upregulation and it was concluded that the reversal by melatonin involves a signaling pathway via peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1), GSK3β, and NF-κB.[196]

The reductions of Aβ formation, which include an attenuation of neuroinflammation, have, in fact, also been demonstrated in transgenic AD mouse models. However, the efficacy of a melatonin treatment depended on its early onset. It only slowed the progression of the disease, but did not prevent it. In mice treated from the 2nd or 4th month of life, Aβ was strongly reduced for several months and lifespan was extended.[180,189] However, Aβ levels started to rise around 11 months and a progressive, unhalted increase was observed until death of the animals, starting at about 15.5 months.[189] Consequently, an onset of treatment at 14 months, when Aβ was already strongly elevated, could no longer be successful.[197]

7 | NEURODEGENERATIVE ASPECTS OF MOOD DISORDERS: A ROLE FOR MELATONIN?

Neurodegeneration is becoming an emerging topic in mood disorders.[19] To date, the role of melatonin in counteracting neurodegenerative processes in depression has been insufficiently addressed. However, with regard to the high future potential of this topic, the processes by which mood disorders or their preceding states can lead to neuroinflammation and, thereby, to cell death will be briefly discussed in the context of possible protective actions by melatonin.

The association of mood disorders with sleep disturbances is known since long and sleep loss may be even prodromal to depressive episodes.[198-202] The deviations in sleep, which are also typically observed in aging, can be mostly interpreted in terms of circadian malfunction. Based on genetic risk factors, these changes can already occur in subjects of middle age or younger. In fact, several forms of depression have been found to be associated with either polymorphisms or underexpression of circadian genes, as recently summarized.[202] Such deviations were reported in bipolar disorder (BP) for the core or accessory oscillator genes *Per3*, *Cry2*, *Bmal1*, *Bmal2*, *Clock*, *Dbp*, *Tim*, *CsnK1ε*, and *NR1D1*; in seasonal affective disorders (SAD) for *Per2*, *Cry2*, *Bmal1*, and *Npas2*; in subtypes of major depressive disorder (MDD) for *Per3*, *Cry1*, *Clock*, and *Npas2*. Because of the complexity of MDD, which comprises a number of subforms with different etiologies, a malfunctioning circadian system may not be of causal relevance to any of them, or only develop in the course of aging. In fact, a brain-specific aspect was revealed by determining the time-of-death expression of circadian genes in MDD brains, in which expression levels of the following oscillator genes were altered: *Per1*, *Per2*, *Per3*, *Bmal1*, *NR1D1*, *Dbp*, *Dec1,* and *Dec2*.[203]

If circadian deviations cause sleep disturbances, the known proinflammatory effects of sleep deprivation may be relevant to the development of neurodegenerative processes, especially when these changes are lasting for years. As recently summarized,[126] sleep disturbances caused elevations of TNFα, IL-6, and CRP in the human plasma, of TNFα and IL-6 in monocytes as well as enhanced mRNA expression of TNFα and its soluble receptor sTNFR1 in whole blood preparations. Moreover, sleep deprivation was shown to induce DDR and SASP in elderly subjects.[204] Sleep deprivation increased TNFα and the NO metabolite nitrite, induced oxidative stress in mouse brains, upregulated IL-1β, IL-6, and IL-8 in the hippocampus and cortex of rats and mice, and of IL-1β in basal forebrain and hypothalamus.[126] In the rat hippocampus, the levels of the anti-inflammatory cytokines IL-4 and IL-10 were found

to be reduced. Notably, inflammatory responses were also observed in peripheral tissues of rodents.[126] Other findings of possible importance concern increases in the CSF levels of $A\beta_{1\text{-}42}$ or $A\beta_{1\text{-}40}$ peptides by sleep deprivation[205] or slow wave sleep disruption.[206] These elevations that were observed in healthy humans may not necessarily result from a higher expression, but rather result from reduced Aβ clearance, an interpretation that remains to be confirmed. It should be clarified whether a correction of circadian deviations and sleep disturbances by melatonin will enhance Aβ clearance.

Although it remains to be studied, several properties of melatonin may be promising for counteracting neurodegeneration in depressive disorders. Apart from antioxidant and anti-inflammatory actions, melatonin may also be beneficial in terms of correcting the circadian system by virtue of its chronobiotic properties.[19,126,202] This may not only include correction of deviating period lengths but also increases of circadian amplitudes and improvements of internal alignment of rhythms within the circadian multioscillator system. An additional circadian aspect has recently emerged from the relationship between melatonin and SIRT1, as will be discussed in section 8.

8 | DOWNSTREAM FACTORS OF MELATONIN'S ACTIONS WITH RELEVANCE TO INFLAMMATION

Melatonergic signaling is primarily based on G proteins that cause either cAMP reduction or ERK1/2 activation. ERK1/2 activation via both MT_1 and MT_2 receptors is a well-established pathway documented in many publications that would exceed the scope of this article.[207-211] This signaling

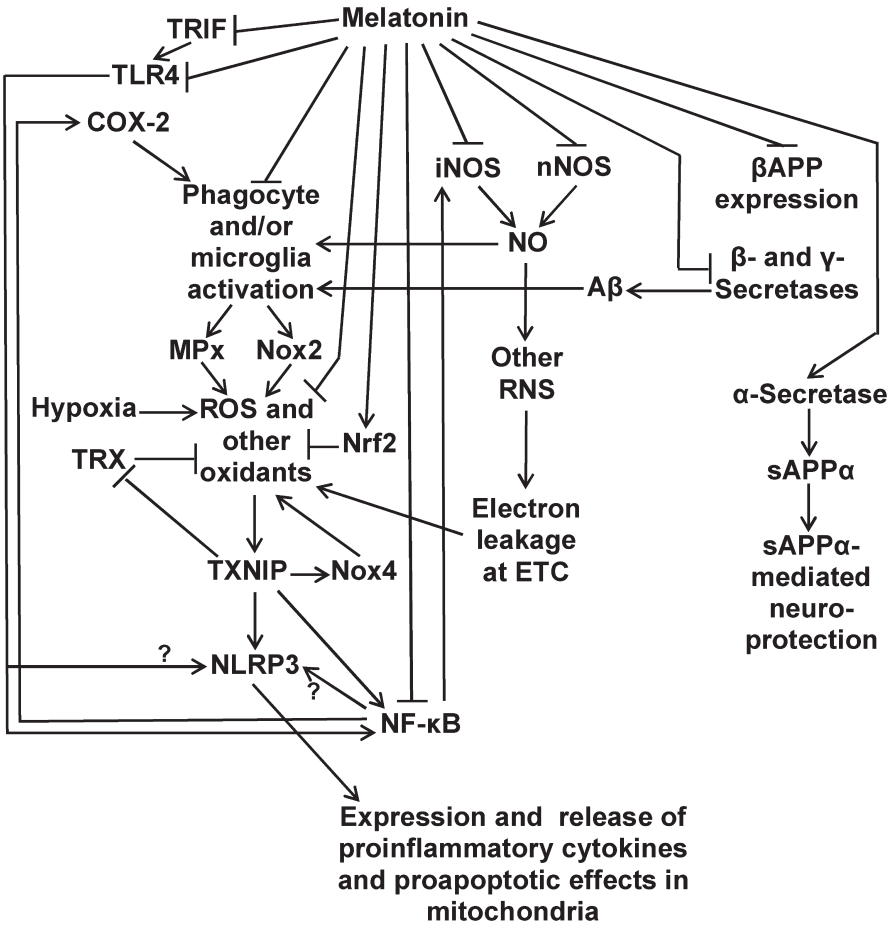

FIGURE 1 Overview of several anti-inflammatory actions of melatonin in a network that involves the generation of free oxygen and nitrogen radicals and various pro- and anti-inflammatory signaling molecules. Additional mechanisms are likely, especially in the context of inhibition of NF-κB activation. Question marks refer to the possibility that alternate activations may take place in parallel. For additional actions via sirtuin 1 see Figure 2. For changes in cytokines, see current text. Abbreviations: Aβ, amyloid-β peptide; βAPP, β-amyloid precursor protein; COX-2, cyclooxygenase 2; ETC, electron transport chain; iNOS, inducible NO synthase; MPx, myeloperoxidase; NF-κB, nuclear factor κB; NLRP3, NLR family pyrin domain-containing 3; nNOS, neuronal NO synthase; Nrf2, nuclear factor erythroid 2-related factor 2; Nox, NADPH oxidase; RNS, reactive nitrogen species; ROS, reactive oxygen species; sAPPα, soluble APP fragment α; TLR4, toll-like receptor 4; TRIF, toll-receptor-associated activator of interferon; TRX, thioredoxin; TXNIP, thioredoxin-interacting protein

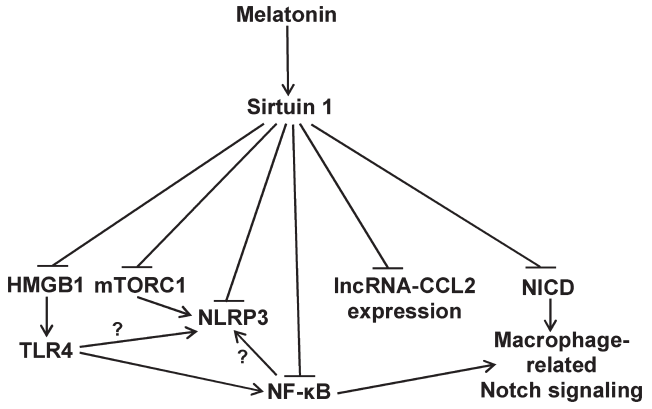

FIGURE 2 Anti-inflammatory actions that can be assumed to be mediated by sirtuin 1 (SIRT1), under conditions of SIRT1 upregulation by melatonin and suppression of melatonin effects by SIRT1 inhibition or knockdown. Again, additional mechanisms are likely. Question marks refer to the possibility that alternate activations may exist. Collectively, the SIRT1-dependent actions suppress proinflammatory cytokines and proapoptotic effects. Abbreviations: HMGB1, high-mobility group box-1; mTORC1, mechanistic target of rapamycin receptor complex 1; NF-κB, nuclear factor κB; NICD, intracellular domain of Notch; NLRP3, NLR family pyrin domain-containing 3; TLR4, toll-like receptor 4

route is also present in immune cells.[212] However, several other reports came to the conclusion of ERK1/2 inhibition by melatonin.[213-216] These latter effects are certainly not caused by primary signaling, but have to be interpreted on the basis of secondary actions induced by different pathways. It would be a matter of further clarification whether epigenetic mechanisms are involved that change the balance between histone acetylation and deacetylation.[215] In addition to the primary modes of signal transduction, various other regulators including cGMP, NO, metabolic sensors, and transcription factors can be secondarily up- or downregulated.[14,207] Apart from NO, which has been extensively discussed above, some of these regulators are relevant to inflammation and to melatonin's anti-inflammatory actions as well (Figure 1). Melatonin has been shown many times to inhibit NF-κB signaling and this had been convincingly related to the attenuation of inflammatory responses, under various experimental conditions.[94,212,217-224] In particular, this effect was found to be decisive in the downregulation of two relevant proinflammatory factors, iNOS and COX-2 (cyclooxygenase-2).[213,217-219] Another factor of relevance is the mediator of antioxidant functions, Nrf2, that has been shown to be upregulated by melatonin in the context of anti-inflammatory actions.[133,135,218,224-228] An additional proinflammatory route concerns TLR4 (toll-like receptor 4) activation, which can be induced by IFNγ via its adaptor proteins, toll-receptor-associated activator of interferon (TRIF). TRIF and TLR4 inhibition by melatonin was shown to suppress of the proinflammatory cytokines TNFα, IL-1β, IL-6, and IL-8.[229] As the proinflammatory actions of TLR4 are frequently also associated with NF-κB activation and prooxidant effects, inhibitory actions of melatonin on TLR4 are of particular mechanistic interest for the understanding of reducing inflammation. This mode of action by melatonin was repeatedly demonstrated and sometimes shown to be associated with inhibitions of NF-κB and of inflammasome activation as well as upregulation of Nrf2.[230-243] These findings were obtained in different organs and models, under various conditions, from endotoxemia, ischemia/reperfusion and other forms of brain injury to chemically induced inflammation. Such effects were even demonstrated in the pineal gland.[244] Collectively, these data show that inhibition of TLR4 represents an important and widely applicable anti-inflammatory action of melatonin. It may be possible that this mechanism extends to other TLRs, but the experimental basis for such a conclusion is still rather small. Inhibition of TLR3 was reported in two cases,[230,245] but no effect of melatonin was found in a study on TLR2.[235]

In studies on the suppression of NF-κB signaling, corresponding effects were observed concerning the inhibition of p300.[213,217,246] This effect is of further interest, as p300 displays histone acetylase activity and melatonin was shown to act on SIRT1, an important histone/protein deacetylase. These inverse effects of melatonin on the activities of these antagonistically acting players of chromatin remodeling and of acetylation-controlled regulators may be of more profound importance. In the case of SIRT1, information on changes by melatonin has accumulated during the last years. While earlier findings obtained in tumor cells indicated a strong downregulation by melatonin, this was not observed in the majority of studies in nontumor cells.[21] This divergence does not reflect a contradiction, but is explained by differences between dysregulated circadian oscillators in tumor cells, which have to epigenetically silence some oscillator genes with tumor suppressor properties, and normal oscillators in which SIRT1 plays an important role as an accessory clock component.[20,21,107,108,124,177] Literature concerning melatonin effects on sirtuins has been recently reviewed in detail.[21,247] With regard to the anti-inflammatory actions of melatonin, new insights are emerging concerning SIRT1 as a secondary mediator of melatonin effects. SIRT1 was not only shown to be upregulated by melatonin in a number of animal models and cell cultures, especially in the context of aging and/or inflammation,[21] but melatonin effects were also shown to be abrogated by sirtuin inhibitors, such as sirtinol and EX527 or knockdown by *Sirt1* siRNA. This was demonstrated in a murine model of ischemia/reperfusion, in which melatonin's mitochondria protecting and anti-apoptotic actions were blunted by EX527.[248] Similar results were obtained in brain injury by sepsis, induced in mice by cecal ligation and puncture,[249] in myocardial protection against ischemia/reperfusion in

normal rats,[250] against ER stress in diabetic rats and in cultured H9C2 cardiomyocytes,[251] and in a study on the suppression of glucocorticoid-induced chondrocyte impairment by melatonin.[252] In cultured neurons from senescence-accelerated SAMP8 mice, the protection by melatonin against oxidative stress by buthionine sulfoximine was strongly reduced by sirtinol.[253]

The involvement of SIRT1 signaling in various protective effects of melatonin is further supported by a number of recent data. Protective actions of melatonin in microglial cell lines stimulated by LPS were reduced by EX527.[254] Renal tissue of severely burned rats was protected by melatonin in multiple ways, including antioxidant and anti-inflammatory actions, including downregulation of TNFα and IL-1β, effects that were blocked by EX527.[255] However, in the same study, downregulation of the anti-inflammatory IL-10 was also described as well as a counteracting effect by the sirtuin blocker, findings that would require further elucidation. Moreover, melatonin was reported to increase in H_2O_2-exposed SH-SY5Y cells the levels of autophagic factors beclin-1 and LC3-II, effects that were moderately reduced by EX527.[221] Some mitochondrial actions of melatonin were shown to be absent in *Sirt1*$^{-/-}$ mice.[256] In a rat COPD model, beneficial effects of melatonin, which included reduced NLRP3 inflammasome formation and IL-1β levels, were inhibited by EX527.[257] Despite of the diversity of models in which a suppression of melatonin's actions by SIRT1 inhibition was observed, the outcome of these studies was remarkably consistent. Collectively, all data indicate that, at least, several effects of melatonin are mediated by SIRT1. In other words, SIRT1 has to be regarded as a downstream factor of melatonin. This is especially valid for the field of anti-inflammatory actions and should be also seen on the background of several similarities in the spectrum of effects known for melatonin and SIRT1. As summarized elsewhere, the overlapping field concerns mitochondrial protection, antioxidant signaling, control and enhancement of circadian amplitudes, anti-aging effects, and, in the context of this review, reduction of inflammatory responses.[107,108]

Importantly, many effects of SIRT1 that are relevant to the suppression or avoidance of inflammation have been also observed upon treatment with melatonin (Figure 2). In addition to the inhibition of NF-κB activation,[258-261] to Nrf2 upregulation,[262-265] and to the avoidance of NLRP3 inflammasome activation,[257,266-268] several further anti-inflammatory actions of SIRT1 have become known. This concerns, for example, an inhibition of TLR4, which was demonstrated in renal collecting duct cells by either SIRT1 overexpression or activation by SRT1720, effects that were blocked by *Sirt1* siRNA or the sirtuin inhibitors sirtinol and EX527.[269] In a murine model of allergic rhinitis, SIRT1 was shown to reduce TLR4 signaling.[270] Overexpression of SIRT1 in LPS-exposed periodontal ligament fibroblasts downregulated TLR4, along with reduced levels of the proinflammatory cytokines TNFα, IL-1α, IL-6, and IL-8.[271] Although the body of direct evidence for TLR4 inhibition by SIRT1 is still very limited, this relationship is highly suggestive, also because numerous other studies not discussed here in detail had shown negative correlations between SIRT1 and TLR4. With regard to the effects of melatonin on TLR4, as discussed above, and the frequently observed upregulation of SIRT1 by melatonin, this sirtuin might well be involved in melatonin's actions via TLRs. A specific aspect of TLR4 activation concerns the binding of HMGB1 (high-mobility group box-1), which acts as an inflammatory signal released by, for example, monocytes, macrophages, endothelial and several other cells. In a number of studies, SIRT1 has been shown to deacetylate HMGB1,[272] to decrease expression, nucleocytoplasmic transfer and release of this protein.[270,273-279] Similar findings on anti-inflammatory actions via HMGB1 inhibition have been obtained with melatonin.[75,114,230,249,280] Therefore, a mediation of melatonin effects by SIRT1 does not seem to be unlikely. Other cases in which anti-inflammatory actions of SIRT1 were reported concern mTOR (mechanistic target of rapamycin), in particular, via mTORC1 (mTOR complex 1). For instance, SIRT1 was shown to decrease adipose inflammation by inhibiting mTORC1 signaling.[281] In experiments on liver steatosis, hepatocyte-specific deletion of SIRT1 enhanced mTORC1 activity.[282] Corresponding effects by melatonin are not sufficiently documented. A single paper reported inhibition of mTORC1 signaling in hepatoma,[283] whereas other studies in squamous cell carcinoma and breast cancer rather indicated a promotion of mTORC1 signaling by melatonin, however, under concomitant oncostatic treatment.[284,285] To definitely judge the role of melatonin in this pathway, it would be important to investigate this under exclusively inflammatory conditions, in the absence of co-medication. This will be also necessary, because melatonin was shown to strongly reduce SIRT1 expression in several cancer cells,[21,286,287] whereas it is known to elevate SIRT1 in inflammation, as discussed above. Another possible anti-inflammatory action of SIRT1 concerns the inhibition of NICD (intracellular domain of Notch) and, therefore, of Notch signaling, which was observed under conditions of sepsis.[288] Notch is usually discussed in the context of developmental processes, but it has a specific proinflammatory effect in macrophages and related cells.[288] NICD was shown to be deacetylated by SIRT1.[289] In stellate cells, SIRT1 knockdown by *siRNA* caused an increased expression of Notch1 protein and mRNA.[290] It would be of interest to study the effects of melatonin on NICD and Notch proteins (Notch1, Notch 2, Hes1) under conditions of sepsis, in models that have been repeatedly and thoroughly investigated with regard to other protective actions. To date, two studies have described opposite effects of melatonin of Notch upregulation, one in myocardial ischemia-reperfusion,[291] the other in the rat hippocampus under treatment with

$A\beta_{1\text{-}42}$.[292] Cell specificity and contextuality of the different actions of SIRT1 and melatonin remain to be clarified and changes under same or similar conditions, such as sepsis or endotoxemia, should elucidate a possible role of SIRT1 as a mediator of melatonin in this context. Finally, another anti-inflammatory action of SIRT1 has been recently reported in macrophages upon treatment with LPS. A long noncoding RNA denominated *lncRNA-CCL2*, according to the gene locus of the chemokine CCL2, was shown to stimulate the release of proinflammatory cytokines. The expression of *lncRNA-CCL2* was downregulated by SIRT1, whereas SIRT1 expression was decreased by LPS treatment.[293] No effects of melatonin on *lncRNA-CCL2* have been investigated to date.

As outlined in this section, numerous anti-inflammatory actions are shared by melatonin and SIRT1. In conjunction with the upregulation of SIRT1 by melatonin especially under inflammatory conditions, it seems highly likely that SIRT1 mediates a number of melatonin effects or, at least, supports melatonin's actions. However, the relationship between melatonin and SIRT1 can be assumed to be a mutual one, since SIRT1 acts as an accessory component of circadian oscillators,[21] with amplitude-enhancing properties that have been also demonstrated in the SCN,[109] the master clock that controls rhythmicity in the mammalian pineal gland. As recently discussed, melatonin may also exert effects via other sirtuin subforms.[107,108] All sirtuin subforms are driven by the NAD^+ cycle, which is generated by NAMPT activity. However, their roles in gene expression are strongly differing, also according to their intracellular distribution. For example, SIRT6 is constitutively chromatin-associated and involved in global circadian chromatin remodeling, but cannot interact directly with oscillator components. SIRT3 also does not interact with the oscillator, because it is mitochondrially located. However, it is of particular interest with regard to free radical generation and resulting consequences for inflammation. While no consistent effects of melatonin have been observed to date in the cases of SIRT2 and SIRT6,[108] upregulation of SIRT3 by melatonin has been reported a couple of times, including conditions of enhanced oxidative stress and inflammation.[108,294-300]

Secondary signaling may not only result from pathways originating at a certain receptor, but can also occur as a consequence of metabolism, if bioactive products are generated. With regard to melatonin, this is the case. Several of its metabolites contribute to the scavenging of ROS and RNS.[301] In the context of inflammation, especially two compounds formed from melatonin are of particular interest, namely, AFMK and its deformylated product, AMK. In neutrophils and PBMCs exposed to LPS, AFMK was reported to downregulate TNFα and IL-8, most efficiently in the neutrophils and more strongly than melatonin.[111] Both AFMK and AMK were shown to specifically downregulate COX-2,[302] a property shared with their parental compound, melatonin. The constitutively expressed COX-1 was not affected. Earlier literature from a time, at which COX-1 and COX-2 were not discriminated, reported that AMK was a more potent inhibitor of prostaglandin synthesis than acetylsalicylic acid (aspirin).[303] Three additional effects of relevance to inflammation were demonstrated for AMK, (a) inhibition of nNOS (neuronal NO synthase),[301,304-306] (b) downregulation of iNOS, including its mitochondrially targeted subform mt-iNOS,[301,305,307] and, in neutrophils, (c) inhibition of myeloperoxidase.[305,308] Again, all these properties were more or less shared with melatonin. The inhibition of nNOS by AMK was demonstrable down to concentrations of 10^{-11} M, although much higher levels were required for half-inhibition.[305] This kinetic peculiarity is reminiscent of recent data on antiproliferative effects in HaCaT keratinocytes and in a melanoma cell line.[309] As this is not explainable on the basis of usual affinity properties toward a saturable receptor, it has been assumed that it may be the consequence of protein AMKylation at tyrosyl residues,[305] a property detected under conditions of AMK oxidation.[310] If this interpretation is correct, this would mean that AMK acts differently on nNOS, compared to melatonin, which binds to calmodulin of the active enzyme. The situation may be entirely different in the case of iNOS and COX-2, in which downregulation by melatonin was attributed to the NF-κB pathway, as outlined above. To date, no receptors for AFMK or AMK have been identified. Nevertheless, it would be an attractive idea to investigate whether these substituted kynuramines may inhibit NF-κB signaling in a similar way as melatonin. In any case, AFMK and AMK remain to be of interest in the context of melatonin and inflammation. As melatonin can be transformed into these compounds via oxidative processes,[301] what also happens under inflammatory conditions by myeloperoxidase- and Nox-expressing cells,[301,308,311-313] this route of melatonin catabolism can gain relevance in quantitative terms. This was impressively shown under conditions of viral meningitis, in which AFMK attained levels sometimes above 50 nM in the CSF, that is, by orders of magnitude above serum melatonin levels.[314] Moreover, high AMFK correlated negatively with IL-1β and IL-8 levels. Based on such findings, the AFMK route of melatonin metabolism has been suggested to be particularly important under brain inflammatory conditions and these metabolites may contribute to the suppression of overshooting inflammation.[16,301,315]

9 | THE PRO- AND ANTI-INFLAMMATORY BALANCE: RISK FACTORS, LIMITS AND CONTRAINDICATIONS

The capability of melatonin of acting in either a pro- or an anti-inflammatory way is on the one hand remarkable, but

on the other hand a matter of uncertainties. Regarding this as a buffering function in the immune system[10] represents an attractive idea, but would require more mechanistic insights. A problem results from the fact that melatonin can also act in an anti-inflammatory way in low-grade inflammation, particularly in aging. Therefore, this cannot be easily understood as buffering, which would make sense in high-grade inflammation like sepsis. To date, we can mainly define functional areas in which pro- or anti-inflammatory responses are prevailing. As outlined in the course of this article, proinflammatory responses were mainly detected in preclinical studies on isolated cells or cell lines and, in the clinical field, in arthritis. Of course, the manifold immune-enhancing properties of melatonin[10-12] indicate a basically positive side of melatonin, namely, in enhancing the capacity of the organism to improve its defense. However, dysregulated responses of the autoimmune type, which typically appear during immune senescence, cannot profit from an overshooting immune enhancement induced by melatonin. So far, arthritis may be classified as a contraindication of melatonin or other melatonergic treatment. The consequences of melatonin in all other autoimmune diseases cannot yet be seriously judged because of the lack of clinical data. For the moment, autoimmune diseases of whatever kind should be regarded as a caveat.

As extensively discussed above, anti-inflammatory actions have been mainly reported for conditions of high-grade inflammation and aging. In the latter field, melatonin exerted anti-inflammatory properties in low- or medium-grade stages, in accordance with its numerous neuroprotective actions. Nevertheless, it would be precocious to recommend melatonin in any neurodegenerative disease. For instance, multiple sclerosis may be aggravated because of its autoimmune etiology.

Moreover, melatonin's protective properties may find their limits in a late onset of treatment, as soon as self-promoting vicious cycles have led to irreversible neurodegeneration. This was particularly evident in AD, but may be also of relevance in other neurodegenerative diseases. Under these aspects, it might be a matter of future research to identify risk factors early enough in a subject's life to allow a promising treatment. Perhaps, this should be done already in midlife, before levels of melatonin and SIRT1 are decreasing and age-related deteriorations of the circadian system occur.

Another field that requires clarification concerns the translation of preclinical findings to the human. Most of the details discussed in this article have been reported in studies in nocturnal laboratory rodents. As the maxima of melatonin are differently associated with other body functions in the diurnally active human, compared to rats and mice, identical actions of melatonin cannot be generally inferred. This seems also to be the case in the immune system, which is to a substantial extent controlled by the circadian system. In the human, several proinflammatory cytokines, such as IFNγ, TNFα, IL-1 (presumably IL-1β), IL-2, IL-6, IL-7, and IL-12, have been reported to peak at night.[316-318] A nocturnal peak was also described for the soluble IL-6 receptor.[318] Humans exposed to *Salmonella abortus-equi* endotoxin showed stronger increases in TNFα and IL-6 at night than during the day.[319] Symptoms of allergic diseases including forms of asthma are known to appear in higher intensities at night, despite lower exposure to the allergens.[320,321] These examples may illustrate that, in humans, these inflammatory responses are not suppressed by physiological levels of melatonin. However, this conclusion should not be misinterpreted, since higher levels by exogenous melatonin may well reduce inflammatory mediators, markers, and symptoms. This would, of course, require thorough investigations on dose dependency. Anyway, many of the preclinical studies that demonstrated anti-inflammatory actions of melatonin were based on high pharmacological doses. From this point of view and with regard to melatonin's ambivalence of behaving in an either pro- or anti-inflammatory way, many more investigations on the dose dependency of effects are desirable. This may also contribute to a better understanding of the dual, opposite actions of melatonin.

10 | CONCLUSION

Melatonin is an immune modulator of considerable relevance. Contrary to many other agents, it can both enhance defense mechanisms, which include proinflammatory processes and can be assumed to contribute to the protection against pathogens, and reduce inflammatory responses. In an attempt to weigh the pro- and anti-inflammatory actions of melatonin, one may arrive at the impression that one of the two edges of the blade seems to be sharper than the other one. Although the proinflammatory effects do exist and can be problematic in a specific case, most publications have documented anti-inflammatory properties of melatonin. The suppression of inflammation is based on manifold mechanisms, which are only partially identified in their details. What is still missing in many cases is the bridge between primary signal transduction via G proteins interacting with the melatonin receptors and secondary signaling by downstream factors. Earlier assumptions concerning the involvement of nuclear melatonin receptors have to be dropped after the definite demonstration that RORα does not bind melatonin.[32,33] As far as indications for a role of RORα exist, these have not to be interpreted on the basis of melatonin binding, but rather with regard to its role in the circadian oscillator system. These statements do not exclude the existence of nuclear melatonin receptors, but, if they are really present, they remain to be identified. Concerning the bridge between primary and secondary signaling, several reports on the suppression of the MAP kinase

pathway, as studied, for example, on the basis of ERK1/2 phosphorylation, have not provided explanations that resolve the contradiction to MAP kinase activation as a primary signaling response via α_q, βγ, PLC and PKC activation or, alternately, via β-arrestin binding to multiply phosphorylated MT_1 or MT_2 receptors.[207,208]

Many other forms of secondary signaling are partially explainable, but not generally linked to the mechanisms of primary signal transduction. The inhibition of TLR4 and suppression of NF-κB activation are well in accordance with NLRP3 inactivation and downregulation of the various proinflammatory cytokines targeted by melatonin. Corresponding upregulations of Nrf2 are also well compatible and all these effects contribute to cell protection. Nevertheless, the connections to directly G protein-mediated processes would require further elucidation.

The diversity of actions, also with regard to primary and secondary signaling, may be seen in conjunction with the question of dose dependency. In cell cultures, physiologically relevant concentrations have been mostly used, such as 10^{-10}, 10^{-9} or 10^{-8} mol/L.[36,44-46] These concentrations are sufficient for almost complete or total receptor saturation. In several studies, doses have been varied up to 10^{-6} mol/L and stronger effects on cytokine release have been reported for the elevated levels. The actions above saturation of MT_1 and MT_2 were interpreted as being mediated by nuclear receptors. However, in the absence of melatonin binding to RORs,[32,33] the possibility of effects via other binding sites would now require their unambiguous identification. However, the respective studies in leukocytes or leukocyte-derived cell lines only concern proinflammatory actions. Anti-inflammatory actions have been investigated in microglial cell lines using 10^{-9} to 10^{-7} mol/L melatonin.[131,132] Other in vitro studies that demonstrate antioxidant effects via SIRT1 and are of interest for anti-inflammatory properties have used 10^{-6} mol/L[221] or even 10^{-3} mol/L[253] melatonin, however, to antagonize damage by 10^{-4} mol/L H_2O_2 or 2.5–10 m mol/L buthionine sulfoximine, respectively, that is, concentrations at which additional actions of melatonin come into play. At these elevated levels, the contribution of receptor-dependent effects via SIRT1 remains to be clarified. Numerous studies on anti-inflammatory effects in rodents under conditions of sepsis/endotoxemia have used pharmacological doses, which presumably exceed receptor saturation and comprise radical scavenging. For instance, 10 mg/kg,[58,60] 30 mg/kg,[75] 60 mg/kg,[64] or 5×30 mg/kg[68] have been employed in rats. In mice, 4×30 mg/kg has been used.[61,62,71] In human newborns with sepsis, melatonin was given in two doses of 10 mg.[57] In studies that were focused on cytokines in vivo, dosage was usually adapted to the severity of the inflammation-inducing insults. Under conditions of low-grade challenges, melatonin was frequently given in the drinking water, for example, in rats using 1 mg/kg daily.[128,129] In the context of aging, mice were typically treated with 1 or 10 mg/kg daily.[138,139,141] These data only represent examples, since a full record of doses used in all studies cited would exceed the frame of this article. Generally, it seems necessary to state that studies should often remain closer to physiological melatonin levels. However, this may only yield effects under conditions of low oxidative pressure and, thus, minor grades of inflammation, because elevated oxidants can easily destroy the redox-reactive molecule melatonin, even when present in high concentrations.[322]

A potentially important, newly emerged aspect of secondary signaling concerns the involvement of sirtuins in the effects of melatonin. As outlined in section 8, melatonin and SIRT1 display a remarkable overlap in their spectra of actions, and the suppression of melatonin effects by sirtuin inhibition or knockdown strongly indicates that SIRT1 may be a partial mediator of cell protection by melatonin. This seems to be especially the case in the reduction of inflammation, with regard to both aging-related neuroinflammation and acute high-grade inflammation because of sepsis, ischemia/reperfusion or other forms of brain injury. It will be an important task for the future to identify under which conditions SIRT1 or other sirtuins mediate effects by melatonin or act in parallel, thereby supporting or enhancing the actions of melatonin. This concerns, on the one hand, their common effects on TLR4, inflammasomes, NF-κB and Nrf2, but extends to several newly investigated or discovered pathways concerning HMGB1, mTORC1, NICD, and *lncRNA-CCL2*. Even in cases, in which melatonin does not directly interact with these factors, the role of SIRT1 as a possible mediator is highly likely under all conditions under which melatonin upregulates this sirtuin.

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