Editorial

Melatonin in Brain: From Circadian Signal to Neuroprotection

INTRODUCTION

Due to the new findings on melatonin actions in the body, and the availability of melatonin analogs, the interest in the clinical applications of the neurohormone is increasing. The recent authorization of melatonin as a drug for insomnia treatment by the European Medicines Agency does it necessary to improve our knowledge regarding the melatonin synthesis, regulation, and actions in the brain. In this issue, the authors of the six contributions analyze the recent findings regarding melatonin-brain interactions, from chronobiological aspects to neurogenesis regulation. Here, a brief summary of the state of the art regarding the functions of melatonin, its brain receptors, and the neuroprotective properties of the indoleamine are shown.

CHRONOBIOLOGY OF MELATONIN

Melatonin is the paradigm of a chronobiotic. Produced by the mammal pineal gland in a circadian manner, the acrophase of melatonin occurs at 2-4 am in humans, whereas its levels remain low at day time [1]. Pineal synthesis of melatonin is regulated by multiple pathways, although the most important of them is the signal delivery from the suprachiasmatic nuclei (SCN), the seat of the biological clock [2]. In turn, the SCN activity depends of a signal provided by the retina in response to the photoperiod and, thus, daily changes in the pineal melatonin production reflect the environmental light:dark changes. The nocturnal surge of melatonin (also called the hormone of the darkness) is used by the organism to synchronize a series of endocrine and non-endocrine rhythms, such as the sleep/wake cycle [3, 4]. Thus, sleep alterations from chronobiotic origin are being successfully treated with melatonin administration [5]. Similarly, melatonin is being used for years as treatment for jet-lag manifestations [6]. The efficacy of melatonin in insomnia led to the discovery of synthetic analogs of melatonin with chronobiotic properties, some of them currently available as prescription drugs in some countries [7]. Melatonin itself is now considered a prescription drug in the European Union. Melatonin and its analogs constitute a new series of pharmacological tools for sleep disorders treatment, mainly those from chronobiological origin. Additionally, a series of reports have shown that melatonin and some of its analogs, ameliorate mild cognitive impairment and exerts antidepressant activity, two effects of remarkable clinical significance [8, 9].

MELATONIN MECHANISMS OF ACTION

One of the most fascinating features of melatonin is its multiple mechanisms of action. Melatonin membrane receptors were reported in several animal species, and they were cloned and pharmacologically characterized. Two of them, MT1 and MT2, display high affinity for melatonin, and they are related to multiple actions of the neurohormone, including seasonal reproduction in lower mammals, regulation of hypothalamic hormones, dopamine release in the striatum and the retina, and central pain and blood pressure regulation [10-13]. The action of melatonin through MT1/MT2 receptors is related to multiple intracellular signaling pathways [14]. Melatonin receptors, and melatonin production itself, are reduced with age, with may have implications in memory, mood, and movement [15, 16]. Thus, age-related diseases, both with non-diseased and diseased aged, are promising targets for melatonin therapy.

Nuclear melatonin receptors were also identified. Firstly, biochemical and pharmacological characterization in liver nuclei revealed the existence of nuclear binding sites that fullfit the criteria for a receptor [17]. Soon after, the receptor was identified as belonging to the orphan family of nuclear receptors, specifically to the retinoic orphan receptor (ROR/RZR) subfamily [18]. Three subtypes, i.e., RORα, RORβ, and RORγ, bind melatonin with high affinity. The activation of nuclear receptors of melatonin yields genomic effects, and the expression of a series of genes is under melatonin regulation; these include the inhibition of the pro-inflammatory enzymes inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX 2), and induction of anti-oxidant enzymes, such as glutathione peroxidase and reductase, superoxide dismutase, and catalase [19-21]. A connection between membrane and nuclear melatonin receptors have been proposed, explaining some of the paradoxical actions of the neurohormone.

The remarkable features of melatonin continue. In fact, non-mediated receptor actions of the indoleamine were reported, including its interaction with calcium binding proteins, calmodulin and calreticulin. The binding of melatonin to calcium-calmodulin (CaCaM) complex was studied in detail, showing that melatonin blocks the CaCaM-dependent pathways in a concentration-dependent manner [22]. Two of these pathways have been profusely evaluated: the cytoskeleton regulation and the nNOS inhibition. The interaction of melatonin with CaCaM occurs at low concentrations of melatonin, and it may have important consequences in terms of neuronal survival and neurogenesis. In this regard, the anti-excitotoxic activity of melatonin may depend, at least in part, on its inhibition of nNOS activity and, thus, nitric oxide (NO) production [23].

Among the non-receptor-mediated actions of melatonin, the antioxidant and anti-inflammatory effects are of special interest. Melatonin and other tryptophan metabolites, give up electrons easily, becoming them in reductor agents. This is

particularly important in free radical pathophysiology. Melatonin scavenges hydroxyl radical, the most harmful oxygen radical, at a rate constant of 2.7 x 10^{10} M^{-1} s^{-1}, closed to that of glutathione. Melatonin also scavenges superoxide anion and hydrogen peroxide, thus reducing the oxidative stress status in the cell [24]. Because melatonin is an amphipathic molecule, it reaches every subcellular organelle, thus scavenging a molecule of reactive oxygen just when it is produced. But melatonin also scavenges nitrogen-based reactive species, such as nitric oxide and peroxynitrites, thus reducing the potential injury of these radicals to the cell [24]. The antioxidant activity of melatonin also involves the regulation of the expression of some of the main antioxidant enzymes, including glutathione peroxidase and reductase, superoxide dismutase, and catalase. Together, these properties of melatonin become this molecule in the most important antioxidant in the body. Some of the melatonin's metabolites are also antioxidants, leading to propose the 'melatonin antioxidant cascade', which make melatonin the most important endogenous antioxidant in the body [25].

The anti-inflammatory properties of melatonin have been evaluated in several experimental models of inflammation, including sepsis and aging. Melatonin reduces the expression of pro-inflammatory enzymes including COX 2 and iNOS, reducing the production of inflammatory mediators [21]. The discovery of the iNOS isoform in the mitochondria of several tissues including the brain, explained the high NO levels in these organelles during inflammation. NO, which is a physiological regulator of the respiration under normal conditions, is toxic for the mitochondria at high concentration, because it reacts with superoxide anion yielding peroxynitrites, a highly toxic compound able to irreversibly damage to the respiratory chain and other mitochondrial enzymes. Mitochondrial failure is the common final pathway in numerous diseases and, from excitotoxicity to neurodegeneration, and in aging itself, neuronal cell death depends on the mitochondrial dysfunction and ATP loss due to oxygen and nitrogen reactive species. Mitochondria seem to be a specific target for melatonin action, which prevents the NO-dependent mitochondrial impairment [26].

NEUROPROTECTIVE EFFECTS OF MELATONIN

The brain has a series of features that distinguish it from other tissues. Firstly, it is considered a post-mitotic tissue, which means that its cells, mainly neurons, are unable to further divisions to yield more cells. This observation is not absolutely true, and the presence of neuronal stem cells in some brain areas suggests the possible production of new neurons (see below). Anyway, the neuronal pool should be maintained along the life to prevent as far as possible neuronal death and loss of cognitive functions. Secondly, the brain is the tissue in the body with the highest oxygen consumption, i.e., 20% of the total oxygen respired, which means that neurons are exposed to a high chronic oxygen stress. Thirdly, the brain has a relative low antioxidant defense compared with other organs in the body, which means that this tissue is highly susceptible to oxidative stress. On the other hand, it is known that the cerebrospinal fluid contains high melatonin concentrations [27], and one wonder whether these levels may compensate the deficit in the other brain antioxidants, maintaining an adequate redox status in this tissue. The high levels of melatonin in cerebrospinal fluid do not depend only on the pineal production of the neurohormone, but also from its extrapineal sources, including the choroid plexus. Extrapineal production of melatonin is fully common in the body, and most of the organs and tissues produce melatonin for *in situ* protective purposes [28].

These data probably reflect the importance of melatonin in brain homeostasis. Aging, excitotoxicity, neurodegeneration, ischemia/reperfusion and trauma, induce neuronal brain damage through multiple mechanisms. However, all of them course with oxidative stress and inflammation, and mitochondrial impairment, which may lead to neuronal death. In multiple models of brain injury, melatonin administration was highly effective in counteracting neuronal damage and recovering brain function. Studies with melatonin administration to human patients showing epilepsy, Parkinson's disease, Alzheimer's disease, and other brain diseases, have been reported in the literature. Generally, melatonin was able to improve physical and cognitive performances in these patients. In experimental models of the diseases in which melatonin was assayed, it was possible to determine the biochemical and molecular basis of its protective role. Recently, a series of papers have reported a role for melatonin in neuronal stem cell differentiation and neurogenesis [29]. Further experiments will be necessary to evaluate the mechanism(s) involving melatonin in neurogenesis, and this opens a promising new field of research.

The data obtained from these experiments support the existence of multiple pathways of action of melatonin. Even more, the multiple mechanisms of action of melatonin seem to converge to prevent or counteract the cell damage. Only a last consideration should be taken in mind. Whereas melatonin exerts antiapoptotic effects in normal cells, it induces apoptosis in cancer cells. This duality in melatonin action supports a role of the neurohormone as a housekeeper in the organism responsible for the maintenance of the cell function integrity.

ARTICLES IN THIS ISSUE

The objective of this issue is to update the information regarding melatonin mechanisms of action in the brain. The multiple effects of melatonin in brain made it necessary to revise specifically these items. For this purpose, the authors in this issue were chosen by the guest editor since they are outstanding specialists in each one of these fields of melatonin research, and their respective points of view will clarify the meaning of melatonin in brain physiology and pathophysiology.

The article of Dr. Cardinali and his colleague constitutes an update of the basic concepts of melatonin as a chronobiotic hormone, and its utility in human therapy. This review analyzes the relationships between melatonin and the SCN in the control of the circadian clock. The alterations in the circadian clock yield a number of circadian rhythm sleep disorders (CRSD). Dr. Cardinali and colleague provide enough information implicating endogenous melatonin as an important mediator in the pathology of CRSD. Melatonin improves sleep efficiency and cognitive function. They also provide information regarding the

mechanism of action and effects of some synthetic melatonin analogs. Accordingly, the author's data support the use of melatonin and its analogs as therapeutic tools in the treatment of CRSD.

The paper of Dr. Zisapel analyzes the sleep alterations with age, and the role of melatonin in its recovery. There is a decrease in the SCN function with age, thus reducing the efficacy of the circadian control over the brain functions. In parallel, there is also a decline in melatonin production with age. Together, the age-dependent SCN and melatonin dysfunction may favor a sleep alteration in aged subjects. Dr. Zisapel shows that insomnia patients over 55 years treated with melatonin improved sleep quality 3 weeks later, an effect maintained up to 6 months later. This clinical trial was done with a prolonged-release formulation of melatonin, which mimics its physiological release. Interestingly, no side effects of melatonin therapy were found. The data support the efficacy and safety of chronic melatonin administration. Additionally, Dr. Zisapel also shows some clinical data regarding the efficacy of this formulation of melatonin in children and blind people.

The third paper corresponds to a review of Dr. Witt-Enderby and colleague. They evaluate the CNS membrane receptors and signaling of melatonin in aging and age-related diseases. Brain melatonin receptors are distributed along the different areas, although the SCN display both MT1 and MT2 membrane receptors. In addition to the control of sleep rhythm, these receptors for melatonin are also responsible for the regulation of some of the hypothalamic/pituitary hormones related to the reproduction and stress responses. Moreover, MT1 and MT2 receptors are also related to pain control and blood pressure regulation. Additionally, memory, mood and movements are also affected with age. The affectation of these functions with age is the main topic cover in this review, and Dr. Witt-Enderby analyzes in deep the mechanisms of melatonin receptor activation and cell signaling in aging, and their relation with age-dependent diseases.

The next paper corresponds to the review of Dr. Benítez-King and colleagues. They analyze the interaction of melatonin with the neurocytoskeleton and its polarization by the neurohormone. The authors show the mechanisms of these effects of melatonin, which includes a protein kinase C and Rho-dependent kinase activation at early stages of neurodevelopment. At later stages, melatonin antagonizes calmodulin inducing neurite and microtubule enlargement. The antioxidant properties of melatonin seem to be also involved in these effects. Dr. Benítez-King shows current evidences supporting melatonin as a cytoskeletal modulator affecting neuronal morphofunctional differentiation. In the light of these data, the therapeutic potential of the neurohormone in neuropsychiatric diseases involving loss of neuronal polarization is discussed.

The article of Dr. Reiter and colleagues emphasizes an important application of melatonin in brain diseases: its activity as antioxidant in ischemia/reperfusion brain injury. Brain damage resulting from ischemia is an example of brain impairment with no effective treatment to date. The authors emphasize the most that 15 years reporting experimental evidence in the ability of melatonin to reduce morphological damage, biochemical and molecular alterations, and behavioral disturbances. The mechanisms of these actions of melatonin are analyzed in deep. Among others, the free radical scavenger ability of the neurohormone; its stimulation of antioxidant enzymes, and its ability to inhibit the inflammatory responses, are here revised. These actions of melatonin prevent apoptosis, which is of great importance for brain integrity after ischemia/reperfusion. The clinical utility of melatonin in stroke is seriously considered.

The final article was co-authorized by the guest editor and colleagues. As an ending aspect of melatonin action in brain, its role in brain mitochondrial homeostasis and neurogenesis is revised. Mitochondrial impairment is a common alteration in aging and several age-associated pathologies, including neurodegeneration. All of them share mitochondrial dysfunction, oxidative/nitrosative stress and apoptosis. It is shown that neuronal loss may be associated to mitochondrial impairment in neurodegeneration, and preserving mitochondrial function may certainly prevent neuronal death. Additionally, brain preservation function may be reached if neurogenesis is activated, although neurogenesis itself and the role of melatonin on neuronal stem cell differentiation remain to be clarified. In this review current information on the unique mitochondrial protective roles of melatonin, in terms of preservation and recovery of mitochondrial function in different models of aging and neurodegeneration, are reported. Additionally, recent data regarding the role of melatonin on stem cell proliferation and neuronal differentiation, are also discussed.

CONCLUSIONS

We pretended here to update the information regarding the role of melatonin in brain along the age. The brain is unique in terms of some physiological functions: It consumes high amounts of oxygen and display low antioxidant capacity. But the brain was phylogenetically companied by an outstanding molecule, melatonin, present at the very early stage of evolution. Melatonin controls the circadian activity of the body; it is found at high concentrations in the brain, and many brain areas display melatonin receptors. Moreover, melatonin exerts a number of non-receptor-mediated actions in the brain, including cytoskeleton regulation and antioxidant and anti-inflammatory roles. The reviews in this supplement clarify how melatonin exerts its neuroprotective functions, proposing different pathways of action, and supporting its clinical relevance in age and some brain pathologies.

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REFERENCES

[1] Reiter RJ. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr Rev 1991; 12: 151-80.
[2] Ko CH, Takahashi JS. Molecular components of the mammalian circadian clock. Hum Mol Genet 2006; 15: R271-77.

[3] Reiter RJ. Melatonin: The chemical expression of darkness. Mol Cell Endocrinol 1991; 79: C153-58.
[4] Moore RY. Suprachiasmatic nucleus in sleep-wake regulation. Sleep Med 2007; 8: 27-33.
[5] Pandi-Perumal SR, Trakht I, Spence DW, *et al*. The roles of melatonin and light in the pathophysiology and treatment of circadian rhythm sleep disorders. Nat Clin Pract Neurolo 2008; 4: 436-47.
[6] Arendt J. Managing jet lag: Some of the problems and possible new solutions. Sleep Med Rev 2009; 13: 249-56.
[7] Sateia MJ, Kirby-Long P, Taylor JL. Efficacy and clinical safety of ramelteon: an evidence-based review. Sleep Med Rev 2008; 12: 319-32.
[8] Rahman SA, Kayumov L, Shapiro CM. Antidepressant action of melatonin in the treatment of Delayed Sleep Phase Syndrome. Sleep Med 2010; 11: 131-36.
[9] Furio AM, Brusco LI, Cardinali DP. Possible therapeutic value of melatonin in mild cognitive impairment: a retrospective study. J Pineal Res 2007; 43: 404-09.
[10] Reppert SM, Weaver DR, Ebisawa T. Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron 1994; 13: 1177-85.
[11] Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005; 27: 101-10.
[12] Witt-Enderby PA, Radio NM, Doctor JS, *et al*. Therapeutic treatments potentially mediated by melatonin receptors: potential clinical uses in the prevention of osteoporosis, cancer and as an adjuvant therapy. J Pineal Res 2006; 41: 297-05.
[13] Doolen S, Krause DN, Dubocovich ML, *et al*. Melatonin mediates two distinct responses in vascular smooth muscle. Eur J Pharmacol 1998; 345: 67-69.
[14] Bondi CD, McKeon RM, Bennett JM, *et al*. MT1 melatonin receptor internalization underlies melatonin-induced morphologic changes in Chinese hamster ovary cells and these processes are dependent on Gi proteins, MEK 1/2 and microtubule modulation. J Pineal Res 2008; 44: 288-98.
[15] Feng Z, Cheng Y, Zhang JT. Long-term effects of melatonin or 17 beta-estradiol on improving spatial memory performance in cognitively impaired, ovariectomized adult rats. J Pineal Res 2004; 37: 198-206.
[16] Lewy AJ. Circadian misalignment in mood disturbances. Curr Psychiatry Rep 2009; 11(6):459-65.
[17] Acuña-Castroviejo D, Reiter RJ, Menéndez Peláez A, *et al*. Characterization of high-affinity melatonin binding sites in purified cell nuclei of rat liver. J Pineal Res 1994; 16: 100-12.
[18] Becker-André M, Wiesenberg I, Schaeren-Wiemers N, *et al*. Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. J Biol Chem 1994; 269: 28531-34.
[19] Carlberg C. Gene regulation by melatonin. Ann N Y Acad Sci 2000; 917: 387-96.
[20] Hardeland R. Melatonin: signaling mechanisms of a pleiotropic agent. Biofactors 2009; 35: 183-92.
[21] Escames G, Acuña-Castroviejo D, López LC, *et al*. Pharmacological utility of melatonin in the treatment of septic shock. J Pharm Pharmacol 2006; 58: 1153-1165.
[22] León J, Macías M, Escames G, *et al*. Structure-related inhibition of calmodulin-dependent nNOS activity by melatonin and synthetic kynurenines. Mol Pharmacol 2000; 58: 967-75.
[23] León J, Escames G, Rodríguez MI, *et al*. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynurenamine, a brain metabolite of melatonin. J Neurochem 2006; 98: 2023-33.
[24] Reiter RJ, Paredes SD, Manchester LC, *et al*. Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Crit Rev Biochem Mol Biol 2009; 44: 175-200.
[25] Tan DX, Manchester LC, Terron MP, *et al*. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007; 42: 28-42.
[26] Escames G, León J, Macías M, *et al*. Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. FASEB J 2003; 17: 932-34.
[27] Longatti P, Perin A, Rizzo V, *et al*. Ventricular cerebrospinal fluid melatonin concentrations investigated with an endoscopic technique. J Pineal Res 2007; 42: 113-18.
[28] Sánchez-Hidalgo M, de la Lastra CA, Carrascosa-Salmoral MP, *et al*. Age-related changes in melatonin synthesis in rat extrapineal tissues. Exp Gerontol 2009; 44: 328-34.
[29] Ramírez-Rodríguez G, Klempin F, Babu H, *et al*. Melatonin modulates cell survival of new neurons in the hippocampus of adult mice. Neuropsychopharmacology 2009; 34: 2180-91.

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