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Molecules in focus

Melatonin

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Abstract

Melatonin, originally discovered as a hormone of the pineal gland, is produced by bacteria, protozoa, plants, fungi, invertebrates, and various extrapineal sites of vertebrates, including gut, skin, Harderian gland, and leukocytes. Biosynthetic pathways seem to be identical. Actions are pleiotropic, mediated by membrane and nuclear receptors, other binding sites or chemical interactions. Melatonin regulates the sleep/wake cycle, other circadian and seasonal rhythms, and acts as an immunostimulator and cytoprotective agent. Circulating melatonin is mostly 6-hydroxylated by hepatic P_{450} monooxygenases and excreted as 6-sulfatoxymelatonin. Pyrrole-ring cleavage is of higher importance in other tissues, especially the brain. The product, N^1-acetyl-N^2-formyl-5-methoxykynuramine, is formed by enzymatic, pseudoenzymatic, photocatalytic, and numerous free-radical reactions. Additional metabolites result from hydroxylation and nitrosation. The secondary metabolite, N^1-acetyl-5-methoxykynuramine, supports mitochondrial function and downregulates cyclooxygenase 2. Antioxidative protection, safeguarding of mitochondrial electron flux, and in particular, neuroprotection, have been demonstrated in many experimental systems. Findings are encouraging to use melatonin as a sleep promoter and in preventing progression of neurodegenerative diseases.

Keywords: Kynuramines; Melatonin; Mitochondria; Radicals

1. Introduction

Melatonin, a name designating a melanophore-contracting hormone, was originally discovered as a skin-lightening molecule acting on frog and fish melanocytes (Lerner, Case, & Takahashi, 1958). Subsequently, melatonin was found to be present in all vertebrates, rhythmically secreted by the pineal gland, and involved in regulation of circadian and, sometimes, seasonal rhythms (Reiter, 1993). In this role, it peaks at night, transmits the information "darkness" and is suppressed by light. Meanwhile, melatonin is known to be synthesized in many vertebrate tissues and almost ubiquitously present from bacteria, protozoa to plants, fungi and invertebrates (Hardeland & Fuhrberg, 1996; Hardeland & Poeggeler, 2003). The spectrum of known effects exceeds by far the original discoveries.

2. Structure

Melatonin (*N*-acetyl-5-methoxytryptamine) is an indoleamine (Fig. 1), whose two functional groups are not only decisive for specificity of receptor binding, but also for its amphiphilicity allowing the molecule to enter any cell, compartment or body fluid and, sur-

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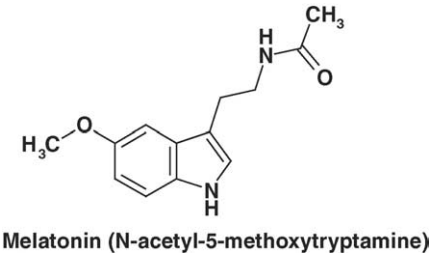

Fig. 1. Structure of the molecule.

prisingly, for its oxidation chemistry (Poeggeler et al., 2002).

3. Synthesis and degradation

The major pathway of melatonin biosynthesis consists, in any organism or cell type tested, of tryptophan 5-hydroxylation, decarboxylation, *N*-acetylation and *O*-methylation, in this order. Alternately, but at lower flux rates, melatonin can be formed via *O*-methylation of serotonin and subsequent *N*-acetylation of 5-methoxytryptamine, or by *O*-methylation of tryptophan followed by decarboxylation and *N*-acetylation (Sprenger, Hardeland, Fuhrberg, & Han, 1999). Usually, *N*-acetylation of serotonin is rate-limiting for melatonin formation. In amphibian eyes, tryptophan hydroxylation is another controlled step. In some insects tested (e.g. *Drosophila*), in which *N*-acetylserotonin exceeds melatonin by three orders of magnitude, *O*-methylation is rate-limiting, owing to the loss of a functional hydroxyindole-*O*-methyl transferase gene and low activities of other *O*-methyl transferases (Hardeland & Poeggeler, 2003).

In the mammalian pineal, the rate-limiting enzyme, arylalkylamine *N*-acetyltransferase (AA-NAT) is under control of the suprachiasmatic nucleus (SCN), the circadian pacemaker, which causes a nocturnal sympathetic release of norepinephrine acting via both β- and α_1-adrenergic receptors. Regulation in the cAMP/PKA pathway comprises gene expression through pCREB binding and a negative feedback loop involving the inducible cAMP early repressor (ICER; Maronde et al., 1999). Additionally, cAMP mediates AA-NAT phosphorylation, which enhances enzyme activity and promotes a stabilizing complex with 14-3-3 proteins (Ganguly et al., 2001). Noradrenergic stimulation also upregulates MAP kinase phosphatase 1, which prevents suppression by the MAP kinase pathway (Link et al., 2004). Another Ca^{2+}-dependent feedback involves binding of downstream regulatory element antagonist modulator (DREAM) to the downstream regulatory element (DRE). In non-mammalian vertebrates, AA-NAT seems to be directly controlled by circadian clock genes in the pineal.

In vertebrates, melatonin is not only formed in the pineal gland or comparable brain extrusions, such as retina or – in reptiles – parietal organ, but also in other organs and cells, including the Harderian gland, the membranous cochlea, mononuclear leukocytes, skin, and the gastrointestinal tract, which contains several hundred times more of melatonin than the pineal (Hardeland, 2005). Extrapineal sites contribute poorly, or only upon specific stimuli, to circulating melatonin.

For many years, melatonin was thought to be almost exclusively catabolized by hepatic P_{450} monooxygenases, followed by conjugation of the resulting 6-hydroxymelatonin to give the main urinary metabolite 6-sulfatoxymelatonin. This may be largely true for the circulating hormone, but not necessarily for tissue melatonin. Especially in the central nervous system, oxidative pyrrole-ring cleavage prevails and no 6-hydroxymelatonin was detected after melatonin injection into the cisterna magna (Hirata, Hayaishi, Tokuyama, & Senoh, 1974). This may be particularly important because much more melatonin is released via the pineal recess into the cerebrospinal fluid than into the circulation (Tricoire, Locatelli, Chemineau, & Malpaux, 2002). The primary cleavage product is N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), which is deformylated, either by arylamine formamidase or hemoperoxidases to N^1-acetyl-5-methoxykynuramine (AMK). Surprisingly, numerous – enzymatic (indoleamine 2,3-dioxygenase, myeloperoxidase), pseudoenzymatic (oxoferryl hemoglobin, hemin), photocatalytic or free-radical – reactions lead to the same product, AFMK (Hardeland, 2005). Recent estimations have revealed that pyrrole-ring cleavage contributes to about one-third of the total catabolism (Ferry et al., 2005), but the percentage may be even higher in certain tissues. Other oxidative catabolites are cyclic 3-hydroxymelatonin (c3OHM), which can also be metabolized to AFMK, and a 2-hydroxylated analog, which does not cyclize, but turns into an indolinone (Hardeland, 2005). Additional hydroxylated or nitrosated metabolites have been detected, which appear to represent minor quantities only. AFMK and AMK also form metabolites by interactions with reactive oxygen and nitrogen species.

4. Biological function

Melatonin is highly pleiotropic (Fig. 2). Classical effects are attributed to G_i protein-coupled membrane receptors, MT1 and MT2, differing in ligand affinity

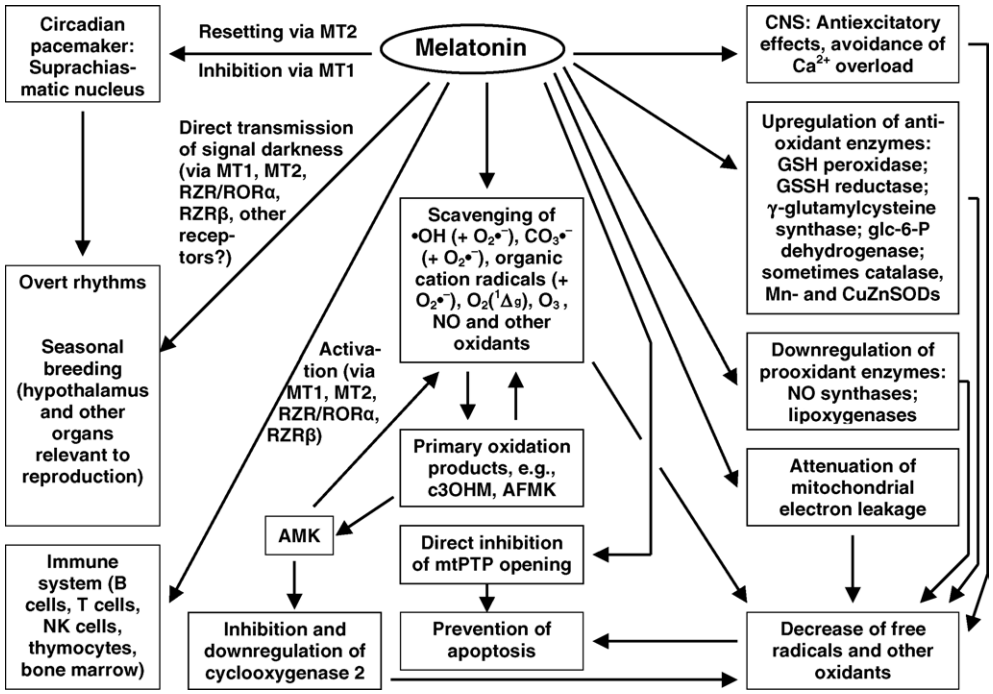

Fig. 2. Overview of some major effects of melatonin on circadian and seasonal rhythms, immunomodulation, as an antiinflammatory, antioxidant, and antiapoptotic agent, including actions of its metabolites c3OHM, AFMK, and AMK. For abbreviations, see text.

(Jin et al., 2003). Both are involved in a circadian feedback to the SCN. MT2 is required for efficient phase-shifting. MT1, having a higher affinity, causes acute suppressions of neuronal firing. These actions involve decreases in pCREB levels stimulated by pituitary adenylyl cyclase activating peptide (PACAP). Other effects may be related to nuclear receptors of lower ligand sensitivity, RZR/RORα and RZRβ (Carlberg, 2000), but in these cases functional significance and target genes are less clear. Although a lot of good and solid work has been carried out on the control of circadian and seasonal rhythms (Reiter, 1993), including receptor-mediated actions on the mammalian SCN (Jin et al., 2003), we would like to focus here on selected non-classical effects, according to most recent developments.

Melatonin exhibits immunomodulatory properties, which are mediated via membrane and nuclear receptors (Guerrero & Reiter, 2002). Data were reported on activation of T, B, NK cells and monocytes, thymocyte proliferation, release of cytokines (IL-1, IL-2, IL-6, IL-12, and IFNγ), met-enkephalin, other immunoopioids, and anti-apoptotic effects, including glucocorticoid antagonism. Signaling mechanisms are only partially understood, and some findings are contradictory. In thymocytes and lymphocytes, cAMP is decreased via MT1 or MT2 receptors. However, melatonin also potentiated VIP-induced rises of cAMP in lymphocytes. Antiinflammatory actions of melatonin are related to the inhibition of PGE_2 effects, and in particular, COX-2 downregulation, which may be transmitted by its metabolite AMK (Mayo et al., 2005).

Immunomodulation seems to be part of antitumor effects described for melatonin. Other oncostatic actions involve MT1/MT2-dependent suppression of linoleic acid uptake or estrogen receptor downregulation (Blask, Sauer, & Dauchy, 2002).

A developing area is antioxidative protection. Even if unjustified claims based on suprapharmacological doses remain unconsidered, a remarkable body of evidence exists showing protection in numerous cell culture and in vivo systems (Hardeland, 2005; Hardeland & Fuhrberg, 1996; Poeggeler et al., 2002; Srinivasan et al., 2005). A special but important aspect is melatonin's role in neuroprotection. Antioxidant actions are observed at different levels, including attenuation of radical formation by antiexcitatory and antiinflammatory effects. This is not restricted to scavenging, although melatonin efficiently interacts with various reactive oxygen and nitrogen species as well as organic radicals, but includes upregulation of antioxidant enzymes (glutathione peroxidase, glutathione reductase, γ-glutamylcysteine synthase, glucose 6-phosphate dehydrogenase, sometimes Cu-, Zn- and Mn-superoxide dismutases and catalase) and downregulation of prooxidant enzymes (NO synthases, lipoxygenases; Hardeland, 2005). Mecha-

nisms of the enzyme inductions have not been identified, whereas suppression of Ca^{2+}-dependent NOS may involve melatonin binding to calmodulin, an effect also playing a role in cytoskeletal rearrangements. Other studies related NOS downregulation to membrane receptors. Additional antioxidant effects may be mediated by binding to quinone reductase 2, which had previously been assumed to represent another melatonin receptor. Antioxidative protection is particularly evident in senescence-accelerated mice. Recently, mitochondrial effects of melatonin have come into the focus of interest, which comprise safeguarding of respiratory electron flux, reduction of oxidant formation by lowering electron leakage, effects shared by AMK, and inhibition of opening of the mitochondrial permeability transition pore (mtPTP; Hardeland, 2005; Srinivasan et al., 2005).

5. Possible medical applications

Apart from the use in treating jet lag, melatonin has been tested in sleep disorders. It generally reduces sleep latency and improves sleep especially when circadian phasing is disturbed. In the latter case, this was particularly effective in patients with neurodegenerative diseases (Srinivasan et al., 2005). Numerous attempts have been made or are under current investigation to mitigate neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases and amyotrophic lateral sclerosis. Melatonin was shown to inhibit Aβ-fibrillogenesis. The suitability as an anticancer agent has been repeatedly investigated. Antiinflammatory actions, which may result from AMK formation, deserve enhanced attention, especially with regard to AMK as a natural COX-2 inhibitor and downregulator (Hardeland, 2005; Mayo et al., 2005).

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