Melatonin and Its Relation to the Immune System and Inflammation

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ABSTRACT: Melatonin (*N*-acetyl-5-methoxytryptamine) was initially thought to be produced exclusively in the pineal gland. Subsequently its synthesis was demonstrated in other organs, for example, the retinas, and very high concentrations of melatonin are found at other sites, for example, bone marrow cells and bile. The origin of the high level of melatonin in these locations has not been definitively established, but it is likely not exclusively of pineal origin. Melatonin has been shown to possess anti-inflammatory effects, among a number of actions. Melatonin reduces tissue destruction during inflammatory reactions by a number of means. Thus melatonin, by virtue of its ability to directly scavenge toxic free radicals, reduces macromolecular damage in all organs. The free radicals and reactive oxygen and nitrogen species known to be scavenged by melatonin include the highly toxic hydroxyl radical (·OH), peroxynitrite anion ($ONOO^-$), and hypochlorous acid (HOCl), among others. These agents all contribute to the inflammatory response and associated tissue destruction. Additionally, melatonin has other means to lower the damage resulting from inflammation. Thus, it prevents the translocation of nuclear factor-kappa B (NF-κB) to the nucleus and its binding to DNA, thereby reducing the upregulation of a variety of proinflammatory cytokines, for example, interleukins and tumor neurosis factor-alpha. Finally, there is indirect evidence that melatonin inhibits the production of adhesion molecules that promote the sticking of leukocytes to endothelial cells. By this means melatonin attenuates transendothelial cell migration and edema, which contribute to tissue damage.

INTRODUCTION

A few milligrams of a novel indoleamine were initially extracted from 250,000 ovine pineal glands, and the extracted molecule was structurally identified as *N*-acetyl-*5*-methoxytryptamine in 1958.[1] It was given the common name melatonin because it is a methoxy derivative of sero*tonin* and, in amphibians, it has a regulatory influence on *mela*nin dispersion in epidermal melanocytes. In mammals, it was initially widely investigated for its effects in determining annual fluctuations in reproductive competence in seasonally breeding mammals.[2] In the last two decades,

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however, the documented functional implications of this secretory product have expanded markedly. It is possible, considering its known receptor and nonreceptor mediated actions, that melatonin may well influence the physiology of every cell in the organism.

MELATONIN SYNTHESIS, SECRETION, AND METABOLISM

Virtually everything that is known of melatonin synthesis has been determined by examining its production in the mammalian pineal gland.[3] As will be seen below, however, it is by no means the only organ in which melatonin is produced.

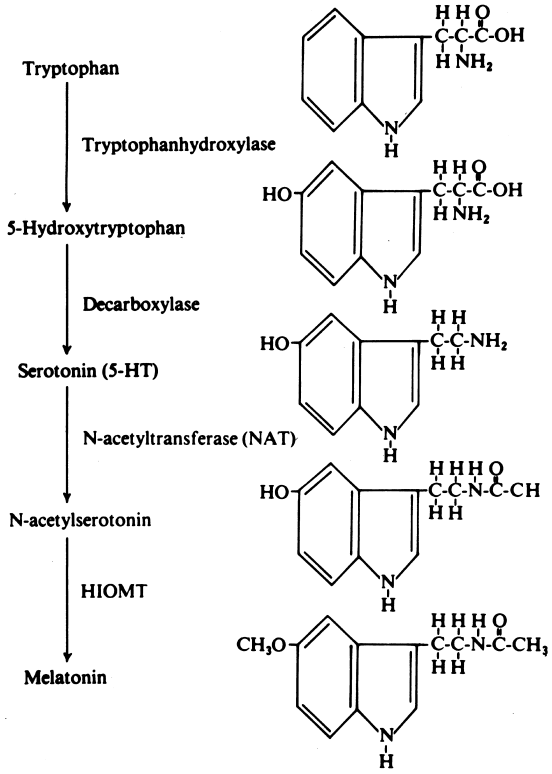

FIGURE 1. Metabolic pathway by which the amino acid tryptophan is converted to melatonin. The first two enzymes in this pathway, that is, the hydroxyase and decarboxylase, have a wide distribution, so serotonin is produced in a number of tissues, most noteworthy in neural tissues. The two enzymes that convert serotonin to melatonin have a more limited distribution. The pineal gland is a primary site of melatonin production, but other organs that produce or possibly produce melatonin include the retina, lens, ovary, gut, and several blood elements. Additionally, some sites, for example, bone marrow cells and bile, contain very high levels of melatonin of unknown origin.

Melatonin is a product of the amino acid tryptophan (see FIGURE 1). After its uptake into cells, tryptophan is first hydroxylated and then decarboxylated, resulting in the formation of 5-hydroxytryptamine (serotonin). The enzymes required for this conversion are widely distributed in neural and other tissues. The metabolism of serotonin to melatonin is likely more restricted due to the limited number of organs that contain the necessary enzymatic machinery to do so, although the list of cells known to contain/produce melatonin is increasing substantially. In organs that produce melatonin, serotonin is *N*-acetylated, with the resulting formation of *N*-acetylserotonin (FIG. 1). The final enzyme in the production of melatonin, hydroxyindole-*O*-methyltransferase (HIOMT), seems to be the most restrictive in terms of organ distribution. It *O*-methylates *N*-acetylserotonin to form *N*-acetyl-5-methoxytryptamine, melatonin.

In the pineal gland in particular, but also in the retina, melatonin generation is strictly photoperiod phase dependent. Thus, during the day pineal melatonin production is curtailed by mechanisms that involve a circuitous route for the transmission of neural messages from the eyes, via the suprachiasmatic nuclei (the biological clock) and the peripheral sympathetic nervous system, to the gland.[4] Conversely, at night pineal melatonin production proceeds unabated.

The nocturnal synthesis of melatonin, however, is interrupted by exposure of animals (and humans) to light of appropriate intensity and wavelength.[5] Considering this, only humans have the capability of determining the quantity of melatonin they produce with the use (or misuse) of artificial light.

Once melatonin is synthesized in the pineal, it is quickly released, generating a blood melatonin rhythm reminiscent of that seen in the gland. Being an amphiphilic molecule, melatonin is capable of entering every cell in the organism; additionally, it readily crosses all morphophysiological barriers, including, as examples, the blood–brain barrier[6] and the placenta.[7] Melatonin is enzymatically degraded in the liver to 6-hydroxymelatonin;[3] however, the indoleamine, in the process of scavenging free radials and reactive species, generates other metabolites, as well, including cyclic 3-hydroxymelatonin (when it scavenges two highly toxic hydroxyl radicals).[8] In the process of scavenging the activated form of peroxynitrous acid, melatonin is reportedly converted to 6-hydroxymelatonin, the same degradation product enzymatically produced in the liver.[9] Besides its direct free radical scavenging activities, which are accomplished without interaction with a receptor, melatonin modifies cell physiology via the membrane and possibly nuclear receptors as well.[10]

MELATONIN IN BODILY FLUIDS AND TISSUES

In all mammalian species, the pineal gland is a major source of circulating melatonin, inasmuch as, after surgical removal of this organ, blood melatonin concentrations are uniformly low during both the day and night.[3] Tissues that take up melatonin from the blood may differentially distribute it intracellularly. Although the number of studies related to the subcellular distribution of melatonin is limited, evidence suggests that highest concentrations are in the nuclei of cells.[6,11] Sufficient

quantities of melatonin get into other organelles, however, as indicated by their actions at these sites, for example, cellular membranes[12] and mitochondria.[13]

The synthesis of melatonin in nonpineal organs certainly occurs; it is only a question of how many different organs actually produce the indoleamine. Extra-pineal sites of melatonin production in mammals should not be unexpected considering that it is also produced in plants, unicellular organisms, bacteria, and invertebrates, all of which lack a pineal gland.[14] The retinas of mammals are accepted as being a site of melatonin formation, but it may also be synthesized by the gut, lymphocytes, monocytes, other bone marrow cells, ovary, and the lens of the eye. Recently, melatonin has been identified in very high concentrations in the bile of a variety of mammals, including humans.[15] The levels in this fluid are two to three orders of magnitude higher than in the blood at night, and its origin remains unknown. Other bodily fluids that are known to contain melatonin include saliva, cerebrospinal fluid, ovarian follicular fluid, and the fluid from the anterior chamber of the eye.[3]

Recently, high melatonin levels were also found in the bone marrow.[16,17] This is particularly noteworthy because this tissue gives rise to many cells that are functionally related to the immune system. Melatonin was identified in bone marrow tissue using a variety of techniques, including radioimmunoassay, immunocytochemistry, high-performance liquid chromatography, and mass spectrometry. The immunocytochemical studies revealed melatonin in roughly 50% of the cells in bone marrow smears;[17] the cells that contained immunoreactive melatonin could not be specifically identified as to their type, but it was usually present in the smallest cells in rat bone marrow. Also, considering the high nuclear to cytosol ratio of the immunoreactive cells, they may be lymphocytes, cells that have already been shown to possess membrane and possibly nuclear receptors for melatonin.[10,16] In general, the immunoreactive product in these cells appeared to be located primarily in the cytosol.

A study of the activities of the enzymes, that is, *N*-acetyltransferase (NAT) and HIOMT, which convert serotonin to melatonin, led Conti *et al*.[17] to suggest that melatonin is formed in bone marrow elements. They also reported that some bone marrow cells also express mRNA encoding HIOMT. These findings are consistent with our own observations, which show that long-term pinealectomy diminished but certainly did not eliminate melatonin from the bone marrow.[18] The levels of melatonin in bone marrow, even in animals lacking their pineal gland, are much higher than those normally measured at night in the blood of pineal intact animals. This strongly suggests that much of the melatonin in bone marrow is from nonpineal sources. We also showed that supplementing rats with melatonin by injecting it peripherally significantly increased its concentration in bone marrow tissue, proving that these cells are able to concentrate melatonin against a gradient.[18] This indicates the presence of intracellular binding molecules that assist in retaining the indoleamine intracellularly. The ability of selected cells to concentrate melatonin is not restricted to bone marrow, because it has been shown that neural tissue and human breast cancer cells (especially estrogen receptor–positive breast cancer cells) also can have higher levels of melatonin than those present in the blood.[6,11,19]

MECHANISMS BY WHICH MELATONIN INFLUENCES THE IMMUNE SYSTEM

It has already been mentioned that at least some immunocompetent cells possess either membrane and/or nuclear receptors for the indoleamine.[10,16] Membrane receptors for melatonin have been well characterized, and they likely mediate some of the many actions of melatonin.[20,21] Additionally, however, nuclear binding sites/receptors for melatonin have been identified (see FIGURE 2), and an interaction of melatonin with these receptors presumably relates to its ability to modify immune function.[22] Although less well characterized than the membrane receptors,[20,21] physiological and pharmacological evidence supports their existence[23,24] and melatonin-nuclear receptor interactions in the regulation of immune function.[10,25]

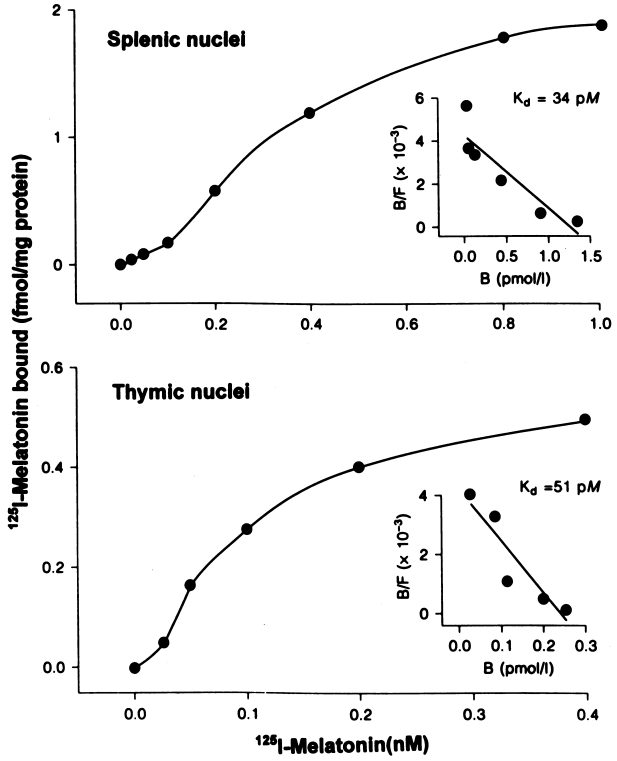

FIGURE 2. A summary of high-affinity binding of melatonin to purified cell nuclei from rat spleen (*top*) and thymus (*bottom*). Diagrammatically represented in the figure are the saturation isotherms and, as shown in the inserts, the Scatchard plots of 2[^{131}I]melatonin binding to these nuclei. In this study, nuclei were incubated with increasing concentrations of 2[^{131}I]melatonin; the incubation was carried out at 15°C for 45 minutes. (Reprinted by permission from Guerrero *et al.*[10])

Besides its obvious interactions with membrane and nuclear receptors in immune cells, melatonin has non-receptor-mediated actions in all systems, including the immune system. These effects are a consequence of melatonin's ability to directly scavenge free radicals.[26,27] In 1993 it was shown that melatonin directly neutralizes the highly toxic hydroxyl radical (·OH).[28] The ·OH is one of several by-products formed during the metabolism of molecular oxygen (O_2) (see FIGURE 3). Any metabolite that possesses an unpaired electron in its outer orbital is identified as a free radical, whereas the metabolic intermediates whose electrons are paired are referred to as reactive intermediates. Radicals and their intermediates are all differentially reactive and often toxic.

Only a small percentage (1–4%) of the O_2 that aerobic organisms use is converted to reactive intermediates and free radicals. Despite this small percentage, over the course of a lifetime, because of their toxic nature, they gradually destroy tissue, which leads to cellular and eventually organ dysfunction. Even the longevity of

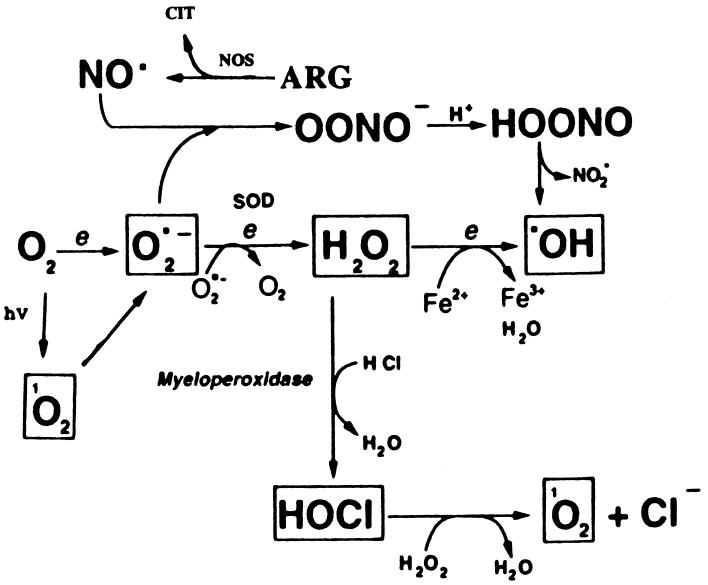

FIGURE 3. Molecular oxygen (O_2) is reduced metabolically to a variety of free radicals (molecules with an unpaired electron and therefore highly reactive) and reactive intermediates. A single electron reduction of O_2 produces the superoxide anion radical ($O_2^{\cdot-}$), which is either dismutated to hydrogen peroxide (H_2O_2) in the presence of superoxide dismutase (SOD) or is coupled with nitric oxide (NO·) to produce the peroxynitrite anion ($ONOO^-$). H_2O_2, via the Fenton reaction, generates the highly toxic hydroxyl radical (·OH). In activated neutrophils and monocytes, myeloperoxidase activity generates hypochlorous acid (HOCl). Many of these reactive agents are produced at sites of inflammation. Because melatonin has the ability to scavenge a number of these reactive products, that is, ·OH, H_2O_2, HOCl, singlet oxygen ($^1O_2$), and the $ONOO^-$, it reduces tissue damage during inflammatory reactions. As summarized in the text, melatonin has other anti-inflammatory actions as well.

aerobic organisms has been theoretically linked to the quantity of tissue destruction caused by free radicals. This is referred to as the free radical theory of aging, and free radical scavengers and antioxidants, that is, molecules that scavenge free radicals and their reactive intermediates, have likewise been implicated in preserving longevity and reducing pathophysiology due to their ability to prevent molecular damage.[29,30] Besides melatonin, there are a number of other molecules that effectively reduce the biomolecular destruction caused by oxygen metabolites, for example, ascorbic acid, α-tocopherol, and glutathione.[31]

MELATONIN AND INFLAMMATION

In the immune system, phagocytes play a critical role in warding off bacterial infections by engulfing these agents and destroying them via free radical mechanisms.[32] This process is part of what is known as the inflammatory reaction, and in the extreme it leads to extensive tissue injury mediated by the generated reactive species. The immune cells, which generate these oxidizing agents, include a variety of phagocytes and leukocytes, for example, neutrophils, monocytes, macrophages, and eosinophils. Phagocytes are normally activated by proinflammatory mediators of bacterial products that have receptors on the plasma membrane of the leukocytes. Following interaction with a receptor, the phagocytic cell assembles the multicomponent flavoprotein NADPH oxidase that catalyzes the formation of the superoxide anion radical ($O_2^{-\cdot}$):

$$NADPH + O_2 \; NADP^+ + O_2^{-\cdot}$$

$O_2^{-\cdot}$ has rather low reactivity toward most biological substrates. The major portion of this free radical is quickly dismutated to hydrogen peroxide (H_2O_2) (FIG. 3). H_2O_2, via the Fenton reaction, interacts with Fe^{2+} (or $C\mu^{1+}$) to yield the very highly reactive and destructive ·OH. H_2O_2 has another fate in certain immune cells. Thus, activated neutrophils and monocytes secrete the hemoprotein myeloperoxidase (MPO) (FIG. 3) into the extracellular fluid where it catalyzes the oxidation of Cl^- by H_2O_2 to produce hypochlorous acid (HOCl):

$$H_2O_2 + Cl^- + H^+ \; HOCl + H_2O$$

HOCl possesses the two oxidizing equivalents of H_2O_2, and as a result it is roughly 100 to 1,000 times more toxic than either $O_2^{-\cdot}$ or H_2O_2.

Besides its dismutation to H_2O_2, $O_2^{-\cdot}$ couples with nitric oxide (NO·) to generate the peroxynitrite anion ($ONOO^-$):[33]

$$O_2^{-\cdot} + NO\cdot \; ONOO^-$$

$ONOO^-$ and/or the products it subsequently generates, that is, peroxynitrous acid and the ·OH or a facsimile thereof,[34] participate in the tissue destruction induced by free radicals. Neutrophils are generally believed to produce both $O_2^{-\cdot}$ and NO· and thereby promote the formation of $ONOO^-$ and other destructive species.

In addition to directly scavenging the ·OH and a variety of other agents that cause macromolecular damage during inflammation, melatonin also scavenges both HOCl[35] and $ONOO^-$.[36] In so doing, melatonin has been shown to be a powerful anti-inflammatory agent in several experimental models.[37–39]

It is now accepted that, in addition to causing direct toxicity to biomolecules, the reactive species produced by phagocytic cells may also initiate, as well as exaggerate, the inflammatory response by virtue of their ability to stimulate a number of genes involved in inflammation. The stimulation of inflammation-related genes may occur, for example, by the activation of the transcription factor nuclear factor-kappa B (NF-κB). NF-κB is a wide-spread transcription factor that regulates a number of genes involved in immune and inflammatory responses.[40] Cytosolic NF-κB is activated by a variety of mediators, including oxidants and viral proteins, that enhance reactive oxygen species generation. This allows NF-κB to translocate to the nucleus and bind to DNA, thereby upregulating the production of a variety of enzymes and proinflammatory cytokines, including interleukin-2 (IL-2), IL-6, tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS) (see FIGURE 4).

Melatonin was recently shown to reduce NF-κB binding to DNA, probably by preventing its translocation to the nucleus.[41,42] This curtails the production of the proinflammatory cytokines referred to above. Additionally, because melatonin has been shown to reduce leukocyte-endothelial adhesion and leukocyte transendothelial cell migration,[43] it may also inhibit the production of adhesion molecules that are also upregulated by NF-κB. Finally, melatonin has been shown to reduce recruitment of polymorphonuclear leukocytes to inflammatory sites.[44]

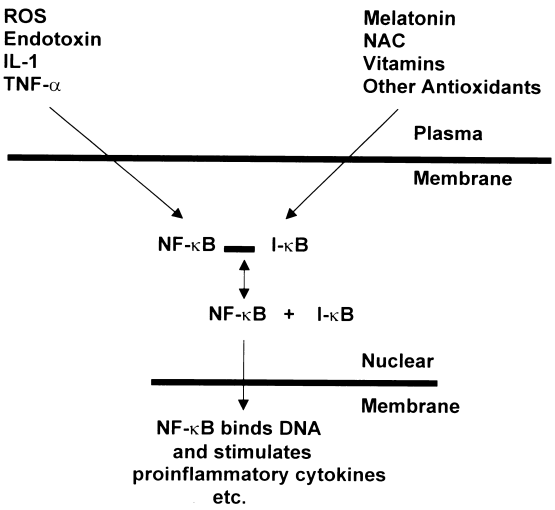

FIGURE 4. A summary of some aspects of the regulation of NF-κB translocation and inflammation. The proinflammatory cytokines produced, when NF-κB binds DNA, include several interleukins, tumor neurosis factor-α, and β-interferon. Melatonin and other free radical scavengers reduce the translocation of NF-κB to the nucleus, thereby reducing the induction of proinflammatory cytokines.

CONCLUDING REMARKS

From this brief discussion, it is obvious that melatonin may reduce tissue destruction during inflammatory responses via a number of means including direct free radical scavenging and indirectly by lowering the production of agents (cytokines and adhesion molecules) that contribute to cellular damage. It is anticipated that in the next decade these interactions will be clarified in greater detail and that melatonin will be experimentally and clinically exploited as an antioxidant and as an anti-inflammatory agent.

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