Review

Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions

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This review summarizes some of the recent findings concerning the long-held tenet that the enzyme, *N*-acetyltransferase, which is involved in the production of *N*-acetylserotonin, the immediate precursor of melatonin, may in fact not always control the quantity of melatonin generated. New evidence from several different laboratories indicates that hydroxyindole-*O*-methyltransferase, which O-methylates *N*-acetylserotonin to melatonin may be rate-limiting in some cases. Also, the review makes the point that melatonin's actions are uncommonly widespread in organs due to the fact that it works *via* membrane receptors, nuclear receptors/binding sites and receptor-independent mechanisms, i.e., the direct scavenging of free radicals. Finally, the review briefly summarizes the actions of melatonin and its metabolites in the detoxification of oxygen and nitrogen-based free radicals and related non-radical products. *Via* these multiple processes, melatonin is capable of influencing the metabolism of every cell in the organism.

INTRODUCTION

Melatonin, a secretory product of the vertebrate pineal, is now know to be produced in multiple cells and organs. However, blood melatonin levels are still derived almost exclusively from the pineal gland of mammals. Melatonin generated in other organs seems to be used locally as a paracoid, autocoid, tissue factor and antioxidant (Tan *et al.*, 2003).

The mechanisms controlling the production of melatonin were thought, until recently, to be well defined. As summarized below, however, new evidence indicates that the initial suggestions may have been premature. The other fact that has come to light is that melatonin's actions are much broader than defined several decades ago. Herein, the evidence that melatonin functions *via* multiple receptors, both membrane and nuclear, and it also scavenges free radicals by processes that require no receptor/binding site.

NEW FINDINGS CONCERNING MELATONIN SYNTHESIS AND ITS MECHANISMS OF ACTION

Melatonin (*N*-acetyl-5-methoxytryptamine) was initially isolated from and chemical identified in bovine pineal tissue (Lerner *et al.*, 1959). Subsequently, it was documented that in mammals the nocturnal increase in blood levels of melatonin is almost exclusively a result of its night-time synthesis in and secretion from the pineal gland (Reiter, 1991a). The discovery of the unique nocturnal production of pin-

eal melatonin launched an extensive series of studies designed to identify the mechanisms mediating the nighttime surge and also to clarify the enzymes that convert the amino acid, tryptophan, to melatonin.

The results of the earliest investigations in this area suggested that the O-methylation of N-acetyl serotonin (the immediate precursor of melatonin) by the enzyme hydroxyindole-O-methyltransferase (HIOMT) determined the quantity of melatonin produced on a nightly basis (Wurtman & Axelrod, 1965). The results of subsequent research implied that this may be invalid and a case was made for the enzyme that N-acetylates serotonin, i.e., *N*-acetyltransferase (NAT), being rate limiting in melatonin production (Klein & Weller, 1970). This idea persisted for several decades but recent findings have again shifted the emphasis to HIOMT as possibly being responsible for controlling the amount of melatonin produced in the pineal gland at night, at least under some conditions.

In support of HIOMT being the rate limiting enzyme in pineal melatonin production, Ribelayga and colleagues (2000) noted that melatonin levels in the Siberian hamster pineal gland do not correlate with the activity of the serotonin acetylating enzyme but rather levels of the indoleamine fluctuate in synchrony with the activity of HIOMT. Furthermore, it was observed that, also in the same species, stimulation of the pineal gland with α and β-receptor agonists caused a clear dichotomy between the responses of NAT and melatonin whereas HIOMT activity correlated directly with melatonin (Ceinos *et al.*, 2004). This disconnect between the activity of pineal NAT and the quantity of melatonin in the gland has also been noted in the sheep (Johnston *et al.*, 2004).

Further evidence for NAT not limiting melatonin production has been provided by Liu and Borjigin (2005) who used a genetic mutant rat model, the Long Evens cinnamon (LEC) rat. In this rat strain, a point mutation is associated with low NAT expression and an unstable NAT protein with the actual enzyme activity being reduced by 90%. Despite the apparent large deficiency in NAT activity, the melatonin rhythm in the pineal gland remains essentially unaltered. Moreover, Liu and Borjigin (2005) also observed the concentrations of *N*-acetylserotonin, the product of the N-acetylation of serotonin, had essentially no relationship with melatonin values. Thus, in the presence of unusually high levels of *N*-acetylserotonin, melatonin values remained depressed. This argues in favor of a mechanism downstream from *N*-acetylserotonin, i.e., HIOMT activity, being a major determinant of melatonin production. It is also pointed out that in some evolutionarily-low ranked organisms the concentration of *N*-acetylserotonin may exceed melatonin levels by three orders of magnitude indicating

that, in fact, HIOMT is rate limiting in melatonin synthesis in these organisms. Finally, whereas light is certainly the major impeller of the circadian melatonin rhythm, there is also controversial evidence that suggests non-visible electromagnetic radiation may influence the 24-hour rhythm of melatonin (Lerchl *et al.*, 1990). Clearly, what enzyme is most important in the control of melatonin synthesis may have to be re-evaluated. Indeed, the activity of what enzyme actually determines melatonin synthesis may depend on the physiological situation.

MELATONIN RECEPTORS

The idea that all the actions of melatonin are mediated *via* specific receptors in cellular membranes has also been modified markedly in recent years. Early studies indicated that the receptors for melatonin were primarily associated with cells in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Reppert *et al.*, 1994). While the cells of the SCN do contain large numbers of membrane receptors, they have also been found to be more widely distributed not only in the brain but in many other organs as well (Witt-Enderby *et al.*, 2006). This immediately implies that the actions of melatonin are extremely widespread. In mammals the membrane receptors, classified as MT1 and MT2, are high-affinity and G-proteins coupled (Nosjean *et al.*, 2001; Dubocovich & Markowska, 2005).

Given that melatonin readily passes through cell membranes, one might expect to find binding sites in the cytosol or nucleus as well. In 1993, Menendez-Pelaez and co-workers noted that, based on immunohistochemical studies, intracellular melatonin concentrations appeared to be usually high in the nuclei of cells. This observation precipitated a search for nuclear binding sites/ receptors which has led to their identification in several tissues (Carrillo-Vico *et al.*, 2003; Naji *et al.*, 2004). A recent review by Tomas-Zapico and Coto-Montes (2005) essentially suggests that all cells that possess membrane melatonin receptors may also contain binding sites for the indoleamine in the nucleus of these cells. In fact, they present a theory as to explain how the membrane and nuclear receptors cooperate in the control of the activity of antioxidative enzymes. This theory still awaits definitive testing.

Besides working *via* receptors to execute its multiple actions, melatonin is also a direct free radical scavenger as described below. Thus, wherever melatonin is located, either intracellularly and/or extracellularly, it can act merely by detoxifying a radical species *via* electron donation. What this means is

that melatonin's action extends to every cell in an organism, or throughout the cell in the case of unicellular organisms.

MELATONIN AS AN ANTIOXIDANT

Although melatonin was discovered to be a free radical scavenger just over a decade ago (Tan *et al.*, 1993), the data documenting its ability to overcome oxidative stress has accumulated at a rapid pace and it is now abundant (Reiter 2000; Reiter *et al.*, 2001a; Allegra *et al.*, 2003; Hardeland *et al.*, 2003; Hardeland, 2005). The efficacy of melatonin in functioning in this capacity relates to its direct free radical scavenging actions (Allegra *et al.*, 2003; Reiter *et al.*, 2003), its ability to enhance the activities of a variety of antioxidative enzymes (Antolin *et al.*, 1996; Rodriguez *et al.*, 2004; Tomas-Zapico & Coto-Montes, 2005), its stimulatory actions on the synthesis of another important intracellular antioxidant, glutathione (Winiarska *et al.*, 2006), its efficacy in reducing electron leakage from the mitochondrial electron transport chain (Leon *et al.*, 2005), and its synergistic interactions with other antioxidants (Lopez-Burillo *et al.*, 2003).

Moreover, in recent years it has become apparent that when melatonin scavenges radicals and related reactants, the products that are generated are also free radical scavengers thereby greatly exaggerating the antioxidant potential of melatonin (Hardeland, 2005).

The subsequent paragraphs include a discussion of the metabolites of melatonin that are capable of neutralizing free radicals and non-radical oxygen-based reactants.

ACTIONS OF MELATONIN AND METABOLITES FORMED

While receptors for melatonin exist on the membranes and possibly in the nucleus of many cells, the direct free radical scavenging actions of melatonin are accomplished without the intervention of a receptor for the indoleamine. Since melatonin readily crosses all morphophysiological barriers and easily penetrates all cells, its actions as a free radical scavenger occur throughout the organism. Also, since all vertebrates and invertebrates produce melatonin (Reiter, 1991b; Hardeland & Poeggeler, 2003), its actions as a scavenger extend throughout the animal kingdom. Finally, its recent discovery in plants indicates melatonin may reduce oxidative damage in them as well (Hardeland & Fuhrberg, 1996; Reiter & Tan, 2002; Hardeland *et al.*, 2005).

Melatonin is also uncommonly effective in neutralizing a number of oxygen-based and nitrogen-based toxic agents, some of which are radicals and some of which are related metabolites (Reiter *et al.*, 2001b; 2003; Allegra *et al.*, 2003). Melatonin was originally shown to detoxify the highly toxic hydroxyl radical ($^{\bullet}$OH) (Tan *et al.*, 1993). Since this discovery, its scavenging repertoire has been expanded to include hydrogen peroxide (H_2O_2) (Tan *et al.*, 2000a), hypochlorous acid (HOCl) (Zavodnik *et al.*, 2004), singlet oxygen ($^1O_2$) (Matuszak *et al.*, 2003), superoxide anion radical ($O_2^{\bullet-}$), nitric oxide (NO$^{\bullet}$) (Ximenes *et al.*, 2005; Aydogan *et al.*, 2006), peroxynitrite anion (ONOO$^-$) (Reiter *et al.*, 2001a) and others (Hardeland, 2005).

Melatonin possesses an electron-rich aromatic indole ring and functions as an electron donor, thereby reducing and repairing electrophilic radicals (Martinez *et al.*, 2005). Melatonin seems not to undergo redox cycling and is considered a suicidal or terminal antioxidant (Tan *et al.*, 2000b). Cyclic voltametry indicates that melatonin donates an electron at a potential of 715 mV. In doing so, melatonin becomes irreversibly oxidized and is not recycled.

While melatonin scavenges a number of reactants, as enumerated above, the most obvious finding in the oxidation chemistry of melatonin is the scavenging of the $^{\bullet}$OH (Tan *et al.*, 1993; Hardeland & Fuhrberg, 1996; Poeggeler *et al.*, 1996). As such, melatonin is more effective than most of its naturally occurring structural analogues (Tan *et al.*, 1993; Poeggeler *et al.*, 1995; 2002).

This suggests that the substituents of melatonin's indole moiety strongly influence is reactivity and selectivity. The rate constant has been calculated for the interaction of melatonin with the $^{\bullet}$OH and, depending on the method used for measurement, it ranges from 1.2 to 7.5×10^{10} M^{-1} s^{-1} (Matuszek *et al.*, 1997; Stascia *et al.*, 1998a; 1998b; Chyan *et al.*, 1999; Mahal *et al.*, 1999).

An interaction of melatonin with the $^{\bullet}$OH was first documented by Tan *et al.* (1993) slightly over a decade ago. In this report the authors used electron spin resonance (ESR) spectroscopy to confirm melatonin's scavenging action. Also of importance in this report was that the scavenging of two $^{\bullet}$OH by melatonin generated the metabolite cyclic 3-hydroxymelatonin (C3-OHM). As it turns out, C3-OHM is an intermediate metabolite since it undergoes oxidation resulting in the formation of N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) (Fig. 1). Melatonin's ability to neutralize the precursor of the $^{\bullet}$OH, i.e., H_2O_2, has also been documented (Tan *et al.*, 2000b). The reaction of melatonin with H_2O_2 leads to the oxidative cleavage of the indole ring of melatonin to produce AFMK; this product has been identified by electrospray ionization (ESI) mass spectrometry
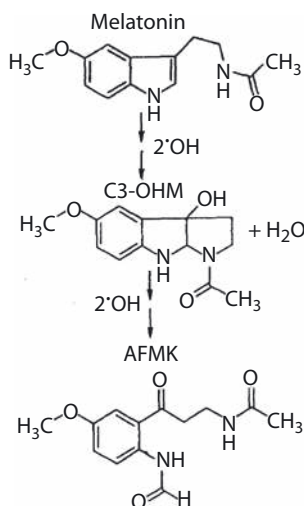
Figure 1. Hypothesized mechanism for the formation of cyclic 3-hydroxymelatonin (C3-OHM) during the oxidation of melatonin by hydroxyl radicals ($^\bullet$OH).
Once C3-OHM is formed it is further oxidized to N^1-acetyl-N^2-formyl-S-methoxykynuramine (AFMK).

(MS), proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{12}C-NMR) (Tan *et al.,* 2000b; Carampin *et al.,* 2003). Tan and colleagues (2001b; 2007) have proposed that AFMK could be generated *via* a dioxetane intermediate or through the standard alkene epoxidation reaction followed by hydrolysis to the diol, which is then oxidized to AMFK. Like melatonin, AFMK is an effective free radical scavenger (Hardeland 2005; Onuki *et al.,* 2005; Tan *et al.,* 2007). These two schemes are summarized in Fig. 2.

The photosensitization of endogenous molecules and also lipid peroxidation within cells can generate $^1O_2$ (Miyamoto *et al.,* 2003). $^1O_2$ is highly
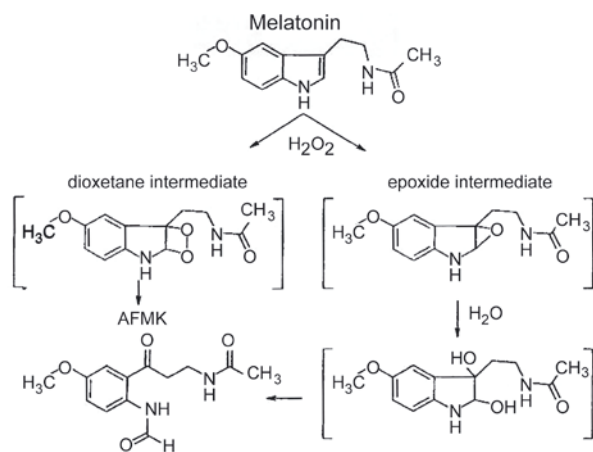

Figure 2. Hypothesized mechanism for the formation for N^1-acetyl-N^2-formyl-S-methoxykynuramine (AFMK) during the oxidation of melatonin by hydrogen peroxide (H_2O_2).
Two potential pathways are proposed; one invloves a dioxetane intermediate while the other involves standard alkalene epioxidation.

reactive toward electron-rich molecules and it is capable of damaging biomolecules (Cavalvante *et al.,* 2002). The product formed when melatonin is oxidized by $^1O_2$ is AFMK (Almeida *et al.,* 2003). Immune responsive cells are also capable of oxidizing melatonin; this reaction involves myloperoxidase and also produces AFMK (Silva *et al.,* 2000; Ximenes *et al.,* 2001).

Melatonin has a number of effects at the mitochondrial level that improve the well being of cells and prevents normal cells from undergoing apoptosis (Acuna-Castroviejo *et al.,* 2002; Leon *et al.,* 2005). Thus, melatonin influences both the electron transport chain (ETC) and oxidative phosphorylation by increasing electron transport and ATP production in normal cells. It also counteracts damage resulting from the exposure of mitochondria to *tert*-butyl hydroperoxide, and restores reduced glutathione levels and enhances the production of ATP. These actions of melatonin may not relate to its antioxidant activities since the indoleamine specifically interacts with complex I and complex IV of the ETC to achieve these changes (Acuna-Castroviejo *et al.,* 2003). Melatonin also rescues normal cells from apoptosis but this response, at least in some cases, is dependent upon the free radical scavenging activity of the indoleamine (Jou *et al.,* 2004). Interestingly, in tumors melatonin exaggerates the death of cells *via* apoptosis (Sainz *et al.,* 2003).

In addition to melatonin's activities as a direct free radical scavenger, it also reportedly enhances organisms to defend against oxidative stress by promoting enzymes that metabolize radicals and their products to innocuous agents (Fig. 3). These indirect antioxidative effects of melatonin may well be mediated *via* membrane and/or nuclear receptors. Examples include the often reported stimulation of glutathione peroxidase (GPx) (Barlow-Walden *et al.,* 1995; Kotler *et al.,* 1998; Reiter *et al.,* 2000; Okatani *et al.,* 2002; Rodriquez *et al.,* 2004; Tomas-Zapico & Coto-Montes, 2005), which converts hydroperoxides, including H_2O_2, to water and oxygen while oxidizing GSH. Once the oxidized form of glutathione, i.e., GSSG, is formed it is recycled to GSH by glutathione reductase (GRd), another enzyme whose activity is enhanced by melatonin (Reiter *et al.,* 1998; Liu & Ng, 2000). Likewise, the superoxide dismutases (Rodriquez *et al.,* 2004; Tomas-Zapico & Coto-Montes, 2005) (SODs, including Cu,Zn-SOD and Mn-SOD), which enzymatic dismutate $O_2^{\bullet-}$ to H_2O_2, are also under the stimulatory influence of melatonin. Finally, one report claimed that melatonin stimulated glucose-6-phosphate dehydrogenase activity (Hardeland, 2005); this would be important in providing reducing equivalents, i.e., NADPH, for the action of GRd. Less commonly, melatonin has been reported to promote catalase (CAT) activity (Reiter *et*
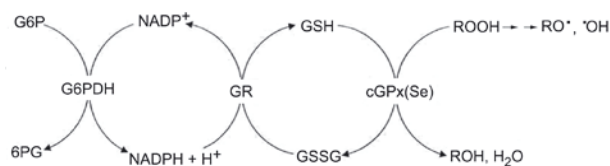
Figure 3. Metabolic recycling of glutathione by glutathione peroxidase (cGPx(Se)) and glutathione reductase (GR).
The activity of GR requires NADPH which is supplied by the activity of the enzyme, glucose-6-phosphate dehydrogenase (G6PDH). Melatonin reportedly stimulates the activity of GPx, GR and G6PDH. G6P, glucose-6-phosphate; 6PG, 6-phosphogluconate; RO$^\bullet$, alkoxyl radical; $^\bullet$OH, hydroxyl radical; ROOH, hydroperoxide.

al., 2001b; Rodriguez et al., 2004; Gomez et al., 2005; Tomas-Zapico & Coto-Montes, 2005).

The stimulatory actions of melatonin on these antioxidative enzymes have been observed in a variety of species and in many different tissues. Also, both physiological levels (Pablos et al., 1998; Mayo et al., 2002) and pharmacological doses of melatonin seem capable of promoting antioxidant enzyme activities. Nevertheless, whereas stimulatory effects of melatonin have repeatedly been described, not every publication found an augmentation of antioxidative enzyme activities after melatonin treatment (Balkan et al., 2004; Ohta et al., 2004). These apparent contrary findings could be a result of the unique features of the tissue investigated or a consequence of the timing of tissue collection after melatonin administration.

GSH is an important intracellular antioxidant which is typically in high concentrations within cells. Its production is controlled by the enzyme γ-glutamylcysteine synthase. In addition to the evidence showing that melatonin commonly preserves GSH levels when cells are under oxidative attack, two studies also report a stimulation of the activity of its rate limiting enzyme (Urata et al., 1999; Winiarska et al., 2006). How essential the promotion of GSH synthesis is in terms of melatonin's ability to limit oxidative stress has not been determined.

The down regulation of several prooxidant enzymes is also a reported function of melatonin. In particular, 5- and 12-lipoxygenases (Manev et al., 1998; Uz & Manev, 1998; Zhang et al., 1999) as well as NO synthase (Pozo et al., 1994; Bettahi et al., 1996; Hardeland et al., 2003; Escames et al., 2006) respond to melatonin in a negative manner. The almost universal observation of a down regulation of NOS by melatonin would markedly curtail the generation of the highly reactive and toxic ONOO$^-$; this down regulation could contribute significantly to melatonin's prevention of oxidative stress. Furthermore, the negative actions of melatonin on NOS would stymie the formation of radical prod-

ucts that derive from ONOO$^-$, i.e., $^\bullet$NO$_2$, CO$_3^{\bullet-}$ and $^\bullet$OH.

Of additional interest regarding melatonin, is its reported binding to quinone reductase 2 (Nosjean et al., 2000; 2001; Witt-Enderby et al., 2003). This enzyme, which is considered a melatonin receptor, is important in the detoxification of prooxidant quinones. This function of melatonin should be more thoroughly examined relative to the ability of the indoleamine to reduce levels of oxidative stress that are a consequence of toxic quinones.

A final feature of melatonin that should be considered in its function as a ubiquitously acting protector against oxidative damage is melatonin's synergistic actions with the some classic antioxidants, e.g., vitamin C and E. When antioxidant treatment includes melatonin in combination with either vitamin C or E, the protective effect is better than the combined actions of both detoxifying agents, i.e., they synergize to produce a greater benefit (Gitto et al., 2001; Lopez-Burillo et al., 2003).

Melatonin has also frequently been compared with vitamins C and E *in vivo* in terms of their degrees of antioxidative protection. When equal doses of these antioxidants are given, melatonin has consistently performed better than either of these vitamins (Hsu et al., 2000; Montilla et al., 2001; Martinez-Cruz et al., 2002). Often vitamins C and E are given in larger doses than melatonin and yet their ability to reduce oxidative stress is less than that of a lower dose of melatonin.

METABOLITES OF MELATONIN IN ANTIOXIDATIVE PROTECTION

As seen in Fig. 1, when the melatonin metabolite C3-OHM is formed, it is also believed to scavenge two $^\bullet$OH to generate AFMK. In studies conducted *in vitro*, C3-OHM has been found to be a powerful protector against oxidative stress, at least in regard to reducing lipid peroxidation (D.X. Tan, R.J. Reiter, unpublished observation). In general, this metabolite has rarely been tested for its antioxidant activity; one factor that likely accounts for this paucity of studies relates to the fact that this molecule is not commercially available.

Tan and co-workers (2001) have shown that AFMK is also capable of donating electrons to detoxify radical species. With the aid of cyclic voltametry, this group showed that AFMK donates two electrons at potentials of 456 mV and 668 mV, respectively. Thus, like it parent molecule, its functions as a reducing agent. This was further documented when it was shown that AFMK preserves the integrity of DNA that is otherwise damaged when it is exposed to oxidizing agents. Likewise, the peroxida-

tion of lipids in hepatic homogenates is inhibited in the presence of AFMK. In reference to both oxidant-induced DNA and lipid damage, the protective actions of AFMK are concentration dependent. Finally, in cultured hippocampal neurons, AFMK eliminated the toxicity of hydrogen peroxide, glutamate and amyloid-β 25-35.

5-Aminolevulinic acid (ALA) accumulates in humans under conditions of lead poisoning, hereditary tyrosinemia and acute intermittent porphyria. ALA has significant oxidative potential and often damages DNA. The ability of AFMK to reduce DNA damage caused by ALA/Fe^{2+} *in vitro* was assessed by Onuki *et al.* (2005). Damage was estimated using DNA plasmid breaks and the detection of 8-oxo-7,8-dihydro-2-deoxyguanosine using HPLC. AFMK, in a dose-dependent manner, protected against free radical damage. In this study, AFMK was compared with melatonin, quercetin and resveratrol in terms of their relative protective actions. The efficacy of these antioxidants in resisting free radical damage depended on the model system used. AMK may even be a better free radical scavenger than is AFMK. This molecule if formed from AFMK *via* pyrrole ring cleavage. The scavenging action of AMK against the •OH was documented by competition with ABTS cation radicals in a Fenton reaction system at pH 5.0 and by competition with dimethylsulfoxide (DMSO) in a hemin-catalyzed H_2O_2 system at pH 8.0 (Ressmeyer *et al.*, 2003). The physiological protection against oxidative damage by AMK was demonstrated in an oxidative protein destruction assay where AMK proved to be highly protective. When AMK functions as an antioxidant *in vivo* the products formed may be 3-acetamidomethyl-6-methoxycinnolinone and 3-nitro-AMK (Guenther *et al.*, 2005).

There are currently no studies related to the ability of any of the melatonin metabolites mentioned above to stimulate the activity of antioxidative enzymes. However, AMK has been shown to inhibit the prooxidative enzyme, NOS (Leon *et al.*, 2006); both *in vitro* and *in vivo* studies were performed. AMK, but not AFMK, inhibited neuronal NOS activity in a dose-response manner *in vitro*. A 20% inhibition of NOS was achieved at a concentration of 10^{-11} M AMK; in contrast, melatonin caused a 20% reduction in enzyme activity at 10^{-10} M. AMK was shown to reduce neuronal NOS *via* a non-competitive mechanism by binding with Ca^{2+}-calmodulin. *In vivo*, the potency of AMK in inhibiting striatal NOS activity was greater than that of melatonin. Thus, a 25% reduction in striatal NOS activity was seen in rats given 10 mg/kg AMK while it required a dose of 20 mg/kg melatonin to achieve the same degree of inhibition. One clear implication of these

findings is that AMK, rather than melatonin, may account for the inhibitory effect of the later molecule on NO synthesis.

As noted above, the formation of AMK during the catabolism of melatonin seems not to end with this molecule. Rather AMK, in the presence of NO donors, forms N^1-acetyl-5-methoxy-3-nitrokynuramine (AMNK) while in the presence of peroxynitrite AMK is converted to 3-acetoamidomethyl-6-methoxycinnolinone (AMMC) (Guenther *et al.*, 2005). These molecules were formed in a pure chemical system and whether they would be produced *in vivo* remains unknown. However, the authors of the report feel it is likely that they are formed in organisms, particularly AMMC. These findings suggest that the catabolism of melatonin does not end at the level of AMK and additional products may be generated in situations where both reactive oxygen (to produce AFMK and AMK) and reactive nitrogen species (to form AMNK and AMMC) are elevated. The observation of Guenther and co-workers (2005) extends the cascade of reactions whereby melatonin protects against oxidative/nitrosative stress.

CONCLUDING REMARKS

This brief review summarizes some of the vast literature related to the antioxidative actions of the indole, melatonin. Melatonin is an uncommonly diverse free radical scavenger that directly interacts with a variety of oxygen and nitrogen-based radicals and related reactants. Also, although not thoroughly discussed in this report, melatonin protects against molecular damage seemingly in all regions of the cell, i.e., in both the lipid and aqueous compartments. Furthermore, it protects against lipid peroxidation, protein mutilation and against both mitochondrial and nuclear DNA damage by radicals and their toxic products. These direct radical scavenging actions of melatonin are receptor-independent processes.

In reference to the ability of melatonin as well as its metabolites to directly neutralize free radicals is the issue of their respective intracellular concentrations. Relative to GSH, for example, the concentrations of melatonin, C3-OHM, AFMK and AMK seem to be low. If so, to function as effective scavengers in the presence of higher concentrations of GSH, they must have some positional advantage within the cell. How, or whether, this occurs remains to be established.

Many cells, however, contain specific receptors for the melatonin molecule. The stimulation of antioxidative enzymes, i.e., SODs, GPx, GRd and catalase, may well involve an interaction of melatonin with these conventional receptors, both mem-

brane and nuclear. The membrane receptors for melatonin are well characterized; however, nuclear binding sites/receptors also exist for this indole and may be involved in the signal transduction mechanisms whereby melatonin promotes the activities of antioxidative enzymes.

Finally, what is becoming increasing apparent is that metabolites that are formed when melatonin is oxidized, e.g., C3-OHM, AFMK and AMK, contribute to the reducing potential of melatonin. Indeed, at this point it is not possible to determine whether the protective actions of melatonin are due to melatonin *per se*, to its metabolites, to its ability to stimulate antioxidative enzymes, or due to its action in inhibiting prooxidative enzymes. It is likely that each of these processes will prove to be important.

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