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The Universal Nature, Unequal Distribution and Antioxidant Functions of Melatonin and Its Derivatives

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Abstract: Melatonin is an uncommonly widely distributed molecule. It is found throughout the plant and animal kingdoms, i.e., perhaps in every living organism. Within vertebrate organisms, melatonin also has an extremely wide distribution, seemingly being capable of entering every cell and all subcellular compartments. So-called morphophysiological barriers, e.g., the blood-brain barrier, are no impediment to the passage of melatonin and it has a multitude of confirmed functions. We have hypothesized that melatonin originally evolved as a free radical scavenger and during evolution it acquired other important and essential actions. Due to the multi-faceted actions of melatonin and its metabolites as direct free radical scavengers and indirect antioxidants, these agents have been used to abate oxidative damage in a diverse variety of experimental models where free radical destruction is a component. When compared with classic, better-known antioxidants, melatonin is better in terms of limiting destruction of intracellular macromolecules when the damage is a consequence of excessive oxygen or nitrogen-based toxic reactants. Considering the vast array of experimental data that has accumulated which documents melatonin's high efficacy and lack of, or minimal, toxicity over a very wide dose range, it is essential that the usefulness of this agent be more thoroughly tested at the clinical level. The findings from experimental models of numerous diseases overwhelming confirm that this indoleamine would likely have great benefit in aiding humans suffering with conditions that have as their basis tissue and molecular damage resulting from oxygen and nitrogen-based reactants.

Keywords: Animals, plants, intracellular concentrations, melatonin, free radicals, oxidative stress.

INTRODUCTION

Only a little over fifty years ago the pineal gland was considered a vestigial relic in the central nervous system of vertebrates. Since the discovery of melatonin (N-acetyl-5-methoxytryptamine) in extracts of the bovine pineal gland in 1958 [1], however, identifying the functions of the gland and its secretory product has proceeded at a feverish pace. Some of the original discoveries linked change in pineal activity to the regulation of reproduction, particularly in seasonal breeding mammals [2-4]. Despite the obvious role of the pineal gland in these circannual fluctuations [5], proving melatonin was the responsible agent was somewhat more difficult and was delayed for a decade or more [6-8]. While the actions of melatonin on the reproductive axis continue to be a major interest of scientists [9, 10], more recent discoveries have shown that the functional repertoire of melatonin is much wider than its actions on the neuroendocrine-reproductive axis. Thus, melatonin is now known to be linked to the inhibition of cancer [11-13], retinal physiology [14, 15], immune system function [16, 17], oral cavity pathophysiology [18, 19], circadian biology [20, 21], viral infections [22, 23], free radical scavenging [24-26], etc. Indeed, melatonin may be one of the most ubiquitously acting of all endogenously-produced molecules [27]. These diverse and multiple functions involve membrane receptors for melatonin [28-30] and possibly binding sites in the nucleus [16, 31, 32] and cytoplasm as well [33-35]. Additionally, due to its direct free radical scavenging activity, some of the melatonin's basic physiological actions do not involve a receptor, i.e., they are receptor independent [36-38].

MELATONIN: ITS UNIVERSAL NATURE

As already noted, melatonin was initially extracted and characterized in bovine pineal tissue by Lerner and colleagues [1, 39]. Since it was isolated from the pineal gland, at the time it was assumed that this indoleamine was only produced in this organ and, by extension, it would only be found in species that had a pineal gland. Thus, the activities of the enzymes that produce melatonin from serotonin, i.e., arylalkylamine N-acetyltransferase (AANAT) and hydroxyindole-O-methyltransferase (HIOMT) (now named N-acetyl-serotonin methyltransferase of ASMT), and in most cases the associated indoleamines were measured in the pineal gland (organ) of mammals, birds, reptiles, amphibians and fish [40-43]. Certainly, in every vertebrate where a search has been performed, melatonin has been found; this is even true for those species that do not have a well circumscribed pineal gland, e.g., the alligator and the crocodile [44, 45]. It seems likely that although these reptiles may not have an organized structure that can be identified as a pineal gland, it is presumed they possess pinealocytes diffusely distributed in the epithalamic region. In the crocodile a day:night serum melatonin rhythm was reported

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during one season (early dry season); however, under other conditions (end of dry season) a dark-associated nighttime melatonin rise was not apparent [45]. These findings suggest that under some environmental circumstances or seasons, melatonin does not function in the synchronization of circadian and/or circannual rhythms in these vertebrates as in most other species [46].

Invertebrates definitely lack a pineal gland and pinealocytes and yet they contain and likely synthesize melatonin [47-54]. What this means is that the synthesis of melatonin in the vertebrate pineal gland is more-or-less coincidental given that invertebrates lack an equivalent of this tissue. Moreover, even the unicell dinoflagellate, *Lingulodinium polyedra* (formerly *Gonyaulax polyedra*), contains melatonin and it also fluctuations over the light:dark cycle [55]. This organism obviously has no organs since it is a single cell. Following this discovery, melatonin was also found in other unicellular organisms [56] as well as in many other taxa [57, 58] including a bacterium [59] and yeast [60, 61].

In 1995, melatonin was also discovered in plant tissues [62, 63]. Since then, melatonin has been identified in a very large number of plant species [64-68] and, moreover, plants are already being genetically engineered to produce more or less melatonin [69, 70]. The function of melatonin in plants seems highly diverse ranging from antioxidant [71, 72] to function as a growth promoting anxin [73, 74]. When plant products are eaten, melatonin is absorbed through the gut and influences the levels of the indoleamine in the blood as well as improving the antioxidant status of this fluid [75].

Based on the discoveries to date, it seems likely that melatonin is found and perhaps synthesized in every species of the plant and animal kingdoms. This suggests melatonin is a phylogenetically ancient molecule and, indeed, we have predicted that it probably evolved two to three billion years ago, possibly when oxygen became the basis of metabolism. Molecular oxygen, especially in the mitochondria, is a major source of free radicals. Since this is so, it seems likely that the original function of melatonin was as a free radical scavenger with its other functions, e.g., as a circadian regulator, having appeared much later in evolution [27, 46].

MELATONIN: ITS UNEQUAL DISTRIBUTION

Within multicellular organisms, melatonin is not in equilibrium. In mammals, for example, melatonin concentrations vary widely between different fluids. This has led to a discussion as to what constitutes a physiological level of melatonin in a given species [76]. Considering the widely different melatonin concentrations in the body fluids of mammals, the definition of a physiological melatonin level must vary with the compartment in which it is measured. This also means that at least membrane melatonin receptors are exposed to greatly different melatonin concentrations depending on their location. How or whether this influences the function of these receptors remains unknown. The different concentrations of melatonin also have implications for the antioxidant capability of individual fluids. Higher melatonin levels would mean a greater capability of reducing oxidative damage. Even the very low levels of melatonin in the peripheral blood positively correlate with the total antioxidant status of that fluid [77]. It is also documented that surgical removal of the pineal gland, which prevents especially the nighttime melatonin rise, diminishes the ability of the blood to detoxify free radicals [78, 79]. Thus, even the relatively low levels of circulating melatonin provide some protection against oxidative stress.

Daytime levels of melatonin in the blood of mammals are typically very low (below 10 pg/mL) while at night these values are much higher (often in excess of 100 pg/mL). In the cerebrospinal fluid (CSF) of the third ventricle of the sheep brain, melatonin concentrations are orders of magnitude higher than in the blood, especially at night [80, 81]. Given the antioxidative potential of melatonin, the clear implication of these highly elevated levels is that the melatonin in the CSF may be especially important in protecting the brain from oxidative damage. The high concentrations of melatonin in the CSF are likely a consequence of its direct release into this fluid via the pineal recess rather than due to its extraction from the blood [81]. Even some mammals, e.g., the Syrian hamster, in which the pineal gland is apparently not in close proximity to the third ventricle, there are structural modifications in the epithalamus that would allow the discharge of melatonin into the ventricular system without the necessity of first entering the systemic circulation [82, 83].

The release of melatonin directly into the ventricular fluid obviously would be highly important in terms of the ability of the indoleamine to protect neural tissue from the persistent production of free radicals due to its high metabolic activity. The advantages of this route of secretion include; a) less rapid metabolism (melatonin is normally quickly broken down as it passes through the liver); b) in the CSF melatonin would have ready access to the neural tissue, especially that in close proximity to the ventricles; and c) because of the small amount of CSF relative to the quantity of blood, the dilution effect is much less so greater concentrations of melatonin are achieved [80, 81] at sites where it is needed. In summary, the secretion of melatonin directly into the ventricular fluid would put it in a position to readily protect the highly vulnerable brain from toxic free radicals [84].

While the best evidence for the release of pineal melatonin directly into the third ventricle comes from publications that used sheep in which an indwelling cannula had been placed directly into the third ventricle [80, 81], there is also similar evidence from the human as well. A clinical study in which melatonin levels were compared in the ventricular fluid from the third and lateral ventricles with that in the blood revealed the following relationships regarding the melatonin concentrations: third ventricular fluid > lateral ventricular fluid > blood. The authors of this report theorized that melatonin in the CSF from the human cerebral ventricles is a consequence of the direct release of melatonin into the fluid although this was obviously not proven [85]. For obvious reasons, to collect human CSF, especially from the ventricular system, for the purpose of determining the concentrations of melatonin in this fluid is technically difficult and usually ethically questionable and even in large mammals it is difficult. So the amount of information in this area remains sparse.

Like the CSF, bile has an unexpectedly high concentration of melatonin. When Tan and co-workers [86] measured melatonin in the bile, collected from the gall bladder of six different mammalian species (human, rat, monkey, guinea pig, rabbit, pig) during the day, the levels varied, according to the species, from 2,000 to 11,000 pg/mL. Bile acids and oxidized cholesterol derivatives are highly corrosive and could easily inflict damage on the biliary tree and on the intestinal epithelium via free radical mechanisms after the discharge of bile into the gut. We hypothesized that the high levels of melatonin in the bile are in fact for the purpose of protecting vulnerable tissues from the toxicity of bile. There are data suggesting that biliary melatonin may be important in reducing the incidence of gallstones [87, 88]. Melatonin has also been proposed to reduce impaired calcium homeostasis in the gallbladder and the associated cholecystitis [89].

In the follicular fluid of the human Graafian follicle, the melatonin concentration also exceeds that in simultaneously collected serum samples [90-92]. While it has been assumed that the melatonin in the follicular fluid is extracted from the serum, the possibility remains that in fact it derives from the follicular cells which contain the two enzymes that convert serotonin to melatonin, namely, AANAT and ASMT, as well as the necessary precursors for melatonin production [93]. Melatonin in the ovary may assist in stimulating ovarian growth, protect the ovum from oxidative damage, and promote luteinization of the corpus luteum after ovulation [94]. Whereas each of these potential functions of melatonin at the ovarian level are obviously of importance, protecting the ovum from toxic free radicals is of particular interest given this cell is the next generation and it is important that it be healthy after being shed during ovulation. It has also been shown that incubating recovered human ova for *in vitro* fertilization/embryo transfer in a melatonin-enriched solution enhances the success rate of this procedure [95]. The benefit derived from the melatonin was proposed to be a consequence of its antioxidative activity given that these ova had less measureable oxidative damage. While little is known about the melatonin levels in human seminal fluid [96], incubating sperm in a melatonin-enriched solution enhanced sperm quality and motility [97]. Again, the protection afforded by melatonin was speculated to relate to its ability to scavenge damaging free radicals.

Melatonin easily crosses all morphophysiological barriers. As a result, melatonin concentrations in the fetal umbilical vessels are equivalent to those in the maternal blood [98]. Melatonin that enters the fetus is capable of protecting it from free radical damage [99]. Additionally, melatonin is measurable in the amniotic fluid, but its function at this site remains uninvestigated [100]. Some of the melatonin that enters the fetus and is present in the amniotic fluid could also be of placental origin, given that this tissue has the molecular machinery to synthesize the indoleamine [101].

Melatonin readily enters cells and, at least in prostate cancer cells, this may involve an active transport process [102]. In normal cells, melatonin does not distribute to all compartments equally [103, 104]. When melatonin is administered to rats, the levels of melatonin in subcellular compartments in brain cells and hepatocytes vary with concentrations having the following relationships: cell membranes > mitochondria > nucleus > cytosol. Giving extra melatonin to intact or pinealectomized rats caused dose-dependent increases in melatonin in these subcellular compartments [104]. Mitochondria are a site of major free radical generation so the relatively high levels of melatonin in these organelles may be of special importance in reducing oxidative cellular damage.

In plants, the concentrations of melatonin measured with various methods and using different procedures of extraction differ markedly [64]. Indeed, it has been proposed that the inefficiency of a specific procedure used for melatonin extraction from plants and plant organs may account for at least some of the reported variation in melatonin concentrations [105]. Of special interest are the very high melatonin levels that were measured in some plants that have been used as Chinese herbal medicines for centuries [106]. If these high levels are valid measures, it is conceivable that some of the alleged benefits of the regular use of these plant products may be due to their high melatonin concentrations. Melatonin, when consumed in plants, is absorbed and elevates circulating melatonin concentrations in vertebrates [63, 75].

Also as in animals, the various organs of plants seem to contain very different concentrations of melatonin [64], perhaps related to the specific function of the indoleamine in the cells in question. For example, a variety of seeds and nuts, which represent the next generation and therefore need special protection against molecular damage from free radicals, contain widely different concentrations of melatonin [107] (Table **1**). While in animal cells knowledge related to the subcellular distribution of melatonin is limited, in plant cells it is non-existent.

MELATONIN AND ITS DERIVATIVES AS ANTIOXIDANTS

When melatonin was discovered as an antioxidant in 1993 [36], it presumably came as a surprise to all scientists working on this indoleamine since no one had seriously suspected this possibility during in the early phase of melatonin research. The observation has, however, been reconfirmed many times within the last two decades using a wide variety of methodologies [108-115]. There is a general consensus that melatonin is a highly effective hydroxyl radical scavenger while it neutralizes the peroxyl radical somewhat less efficiently [116, 117]. Detoxifying the hydroxyl radical is of extreme importance since it is very highly destructive to all molecules and is believed to account for more than half the free radical damage that normally accumulates in organisms. While melatonin less effectively detoxifies the peroxyl radical, which is generated during the peroxidation of lipids, nevertheless the indoleamine is a potent protector against the breakdown of cellular lipids [118-120]. From this we surmise that melatonin scavenges the radicals and non-radicals that initiate this devastating degenerative process. For example, melatonin neutralizes the peroxynitrite anion which indiscriminately damages numerous molecules and is capable of initiating the process of lipid peroxidation [121, 122]. Other less reactive oxygen metabolites are also scavenged by melatonin [123, 124].

Table 1. Melatonin Levels Measured in Seeds of Representative Edible Plants.

Scientific Name	Common Name	Melatonin Concentration (ng/g dry seed)
Brassica hirta	White mustard	189
Brassica nigra	Black mustard	129
Prunus amygdalus	Almond	39
Linum usitatissimum	Flax	12
Apium graveolus	Celery	7
Papaver somniferum	Poppy	6
Silybum marianum	Milk thistle	2

The data in this table show the wide variation in the melatonin concentration in this plant organ. The publication of Manchester *et al.*, [107], from which these data were taken, includes melatonin levels in many other seeds as well. Plant organs in general, even within the same plant, often have widely different melatonin contents.

Increasing the efficacy of melatonin as a scavenger is the ability of several of its metabolites to also neutralize radical and related non-radical products [125, 126]. When melatonin detoxifies radicals, typically via electron donation, it is converted to metabolites that are likewise radical scavengers (Fig. **1**). Because melatonin sacrifices itself in this process, it is referred to as a suicidal antioxidant. The first metabolite that was identified to be a product of the scavenging actions of melatonin was cyclic 3-hydroxymelatonin (c3OHM) [127]. It is formed when melatonin interacts with two •OH [36]. c3OHM, like its parent molecule, is likewise a scavenger and yields N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) [125]. This latter derivative was actually discovered in the brain more than three decades ago and was thought to be exclusively a result of the action of indoleamine 2, 3-dioxygenase [128, 129]; only recently was it found to be a product of the metabolism of c3OHM. Besides being a product of c3OHM, AFMK can be formed directly from melatonin when the latter agent neutralizes the oxygen-based non-radical species, hydrogen peroxide (H_2O_2) [130]. AMFK likewise functions as a scavenger to generate N1-acetyl-5-methoxykynuramine (AMK) [131-134], a product that is further metabolized when it neutralizes free radicals [135-137]. This entire sequence of reactions is referred to as melatonin's antioxidant cascade and allows a single melatonin molecule, along with its metabolites, to scavenge multiple free radicals [132] thereby making it high efficacious in preventing oxidative damage to essential macromolecules.

The relative efficacies of melatonin and several classic antioxidants as scavengers of the ABTS cation radical have been compared by Tan *et al.* [138]. The results of this study revealed that the scavenging activities of some classic antioxidants [glutathione, vitamin C, trolox (water soluble vitamin E), NADH, and NADPH] were complete within 60 seconds, since they each scavenged a single ABTS radical. Conversely, melatonin continued to neutralize ABTS radicals for up to 10 minutes (Fig. **2**). This prolongation of the scavenging period was interpreted to be a consequence of the sequential quenching of radicals by melatonin's metabolites, i.e., a result of the antioxidant cascade [138]. In this scheme, the IC_{50} values for melatonin, glutathione, vitamin C, trolox, NADH and NADPH were 4, 11, 15.5, 15.5, 17 and 21 μM, respectively.

In a variety of *in vitro* and *in vivo* tests, melatonin has been compared to other better-known antioxidants in terms of their relative abilities to reduce oxidative damage. In these reports it was common to find that melatonin was equivalent to or better than the radical scavengers to which it was compared [139-144]. This higher efficacy of melatonin was often apparent since it required lower concentrations/doses of melatonin to achieve the equivalent degree of protection achieved by much greater levels of the other radical scavengers. Melatonin has also been shown to work synergistically with other antioxidants in scavenging toxic oxygen-based reactants [145-147].

The recent discovery of isomers of melatonin in plant products will likely broaden the number of indoleamines that may be proven to have efficacy as radical scavengers [148-151]. Only one of these molecules, which is structurally similar to melatonin with the identical molecular mass, has been tested for its antioxidant capability. Interestingly, it was found to be more effective than melatonin as a radical scavenger [152]. If other isomers of melatonin prove efficacious as radical scavengers, they could contribute significantly to the general protective actions of melatonin-type molecules in eliminating oxidative stress.

Interestingly, not all free radicals generated in organisms mete out molecular damage to normal cells. Some function as essential second messengers while others are important to resist invading bacteria, etc., as a result of the oxidative burst by neutrophils [153]. Melatonin, as well as other antioxidants, generally does not interfere with these essential anti-inflammatory actions of free radicals. Why the protective free radical reactions are not subject to suppression by antioxidants remains unknown. Additionally, melatonin and perhaps its metabolites, have strong intrinsic anti-inflammatory actions [16, 154, 155].

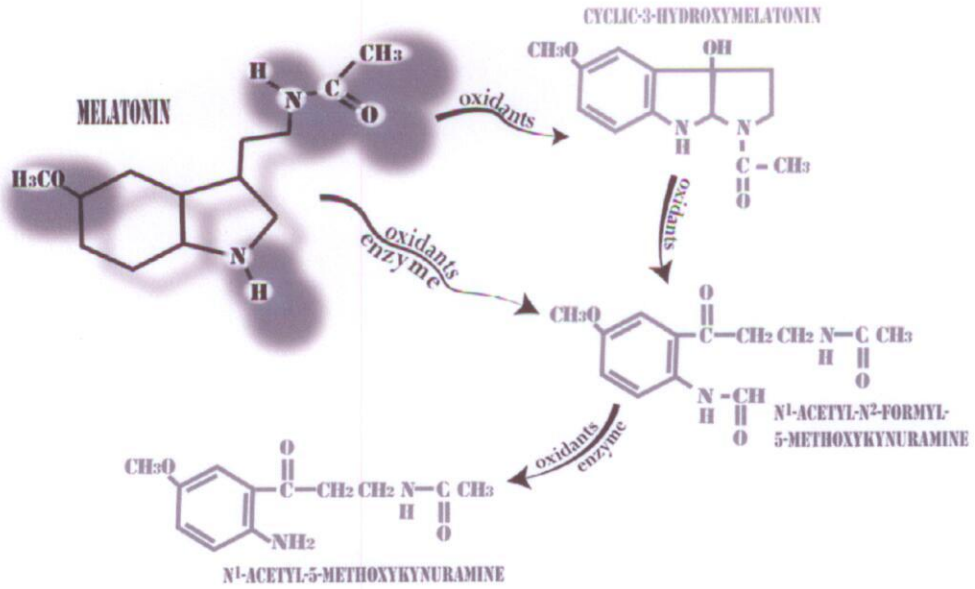

Fig. (1). Melatonin and the metabolites that are formed when it scavenges free radicals. Each of the metabolites, like melatonin itself, is likewise a free radical scavenger in what is referred to as the antioxidant cascade. N1-acetyl-5-methoxykynuramine (AMK) also functions in the detoxification of radical species. The resulting metabolites of this action are not shown since their definite structure remains unknown.

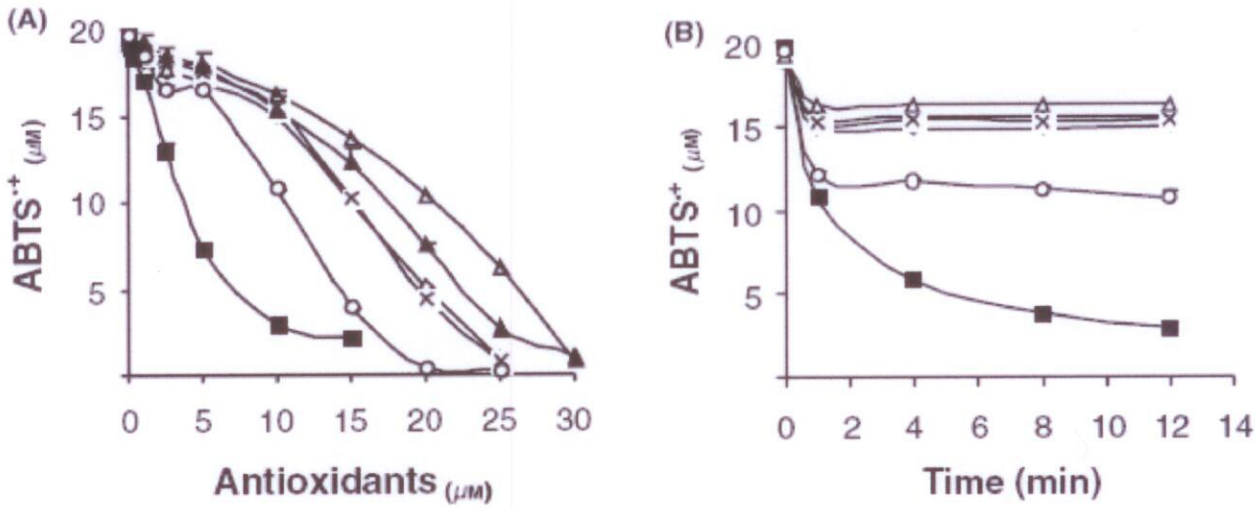

Fig. (2). (A) Concentration dependence of melatonin (solid square), glutathione (open circle), vitamin C (cross), trolox (diamond), NADH (solid triangle), NADPH (open triangle) in terms of scavenging the ABTS cation radical. The IC_{50} values for these molecules were 4, 11, 15.5, 15.5, 17 and 21 μM respectively. (**B**) Time dependence of ABTS cation radical scavenging by these same antioxidants (at a concentration of 10 μM). The scavenging activity of the classic antioxidants was complete within 60 seconds while that of melatonin (and its metabolites) continued for roughly 10 minutes. From Tan *et al.* [138] with permission.

The ability of melatonin and its derivatives to function as potent antioxidants, as summarized above, applies to their actions in normal cells. In cancer cells, melatonin has frequently been found to produce opposite effects, i.e., it promotes free radical generation [156-159]. Again, how melatonin functions as a radical scavenger, with few exceptions [157], in the context of a normal cell while having pro-oxidant actions in cancer cells has not been experimentally or theoretically resolved. It is, however, fortunate that melatonin enhances reactive oxygen species production in cancer cells since this has obvious advantages in terms of killing tumor cells. This is one of the proposed means used to explain melatonin's anti-cancer effects [156, 160].

Besides its direct scavenging actions, melatonin has other means by which it strengthens antioxidative defenses. Shortly

after its discovery as a direct free radical scavenger [36], the indoleamine was tested for its ability to promote indirect means of improving resistance against radical-mediated molecular destruction. Quickly, it was shown that exogenously administered melatonin [161] and the endogenous nocturnal melatonin rise [162, 163] stimulate enzymatic processes which remove free radicals from the intracellular environment. Although this beneficial antioxidative action of melatonin has not received the same degree of attention as has its free radical scavenging, it is likely equally, or possibly more, important in ameliorating oxidative stress. Thus, the enzymes that have been positively impacted by melatonin include both isoforms of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRd) [164, 165] (Fig. **3**). Also, in addition to possibly scavenging nitric oxide (NO) [166], melatonin also reduces its synthesis by inhibiting nitric oxide synthase [167]. Both these events would limit the generation of the strong oxidizing agent, $ONOO^-$, since NO would not be available to couple with $O_2^{\bullet-}$. Finally, several authors have shown that melatonin promotes the synthesis of glutathione, another important antioxidant that battles against free radical damage [168, 169].

The multiple actions by which melatonin forestall oxidative damage makes interpretation of its *in vivo* effects difficult in a sense. Thus, it is virtually impossible, in a given situation, to determine what action was most beneficial in warding off free radical destruction. The ability of melatonin is reduce the accumulation of oxidatively-damaged molecules is unexpectedly thorough, but what percentage of the reduction is accounted for by direct free radical scavenging as opposed to its indirect antioxidative effects remains to be determined.

CONCLUDING REMARKS

There is a massive amount of published data showing that total cellular oxidative stress is reduced when melatonin is in the vicinity. Indeed, melatonin has been tested under an uncommonly large number of situations relative to its ability to ameliorate oxidative stress. As examples, melatonin reduces molecular damage and physiological perturbations that result from ischemia/reperfusion injury [170-172] (including that which occurs during organ transplantation [173-175]), chemical toxins [176-178], bacterial endotoxins [179-181], heavy metals [182-184], pesticides [185, 186], drugs [118, 187, 188], free radical-related diseases [189-194], etc. Many

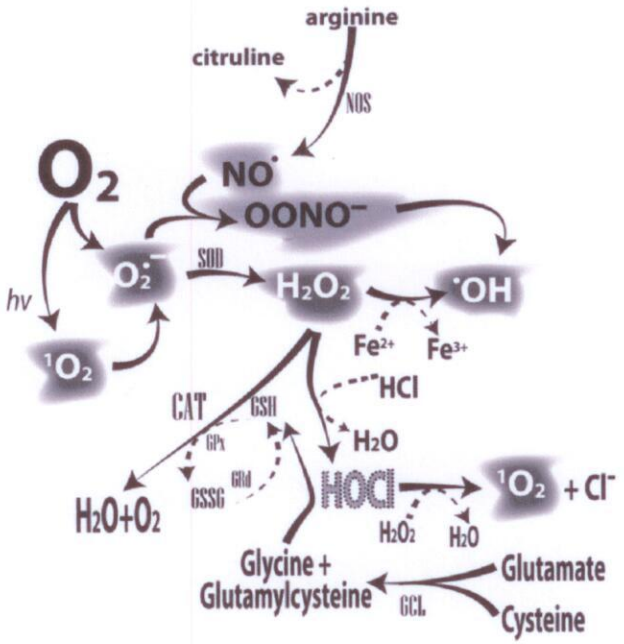

Fig. (3). Molecular oxygen (O_2) is a major source of free radicals and other toxic agents. The chemical reduction of O_2 generates the superoxide anion radical ($O_2^{\bullet-}$) which couples with nitric oxide (NO) to form the strong oxidizing agent, peroxynitrite ($ONOO^-$). $O_2^{\bullet-}$ is also dismutated (by SOD, superoxide dismutase) to form the non-radical product, hydrogen peroxide (H_2O_2). In the presence of transition metals, e.g., Fe^{2+}, H_2O_2 forms the highly destructive hydroxyl radical ($\bullet OH$). H_2O_2 can also be enzymatically removed from the intracellular environment by two antioxidative enzymes, catalase (CAT) and glutathione peroxidase (GPx). GPx, in the process of converting H_2O_2 to innocuous products (H_2O and O_2), uses reduced glutathione (GSH) as a substrate resulting in the formation of glutathione disulfide (GSSG); this latter molecule is recycled by glutathione reductase (GRd) back to GSH. Melatonin stimulates the antioxidative enzymes SOD, GPx, and GRd while inhibiting the pro-oxidative enzyme nitric oxide synthase (NOS). Melatonin also influences the levels of reduced glutathione (GSH) by stimulating the activity of its rate-limiting enzyme, glutamylcysteine ligase (GCL). By influencing the activities of the enzymes indicated, melatonin has substantial indirect antioxidant actions in addition to its ability to function in the direct detoxification free radicals ($\bullet OH$, $O_2^{\bullet-}$) and non-radical toxic products ($ONOO^-$, $^1O_2$, HOCl, H_2O_2). The combination of melatonin's ability to function as a direct scavenger and as an indirect antioxidant contributes to its ability to reduce oxidative stress both intracellularly and extracellularly.

of the benefits of melatonin in these situations are likely a result of its scavenging of toxic radicals at the mitochondrial level or its abatement of free radical generation due to its ability to improve the function of the complexes of the mitochondrial electron transport chain [195-199].

The literature summarized in this brief review documents that melatonin functions as a multi-faceted antioxidant by directly scavenging free radicals and other toxic agents [25, 26, 36, 108-117, 121, 122, 126], indirectly by stimulating antioxidant enzymes [161-165] and inhibiting pro-oxidant enzymes [167] and via its ability to reduce free radical generation, a process known as radical avoidance [126, 200]; this latter function occurs particularly at the level of the respiratory complexes in the inner mitochondrial membrane. As stated above, what percentage of the protection melatonin provides is actually due to each of these processes in any given situation is unknown.

Because of its high efficacy in reducing oxidative stress and especially because of its virtual absence of toxicity, it is imperative that melatonin be tested at the clinical level in situations where oxidative damage is normally a contributing factor to the pathophysiology. Examples of clinical situations in which controlled tests should be performed include ischemia/reperfusion injury (stroke, heart attack, organ transplantation, etc.), neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's diseases, etc.), as a protective agent against drugs which damage intracellular molecules because they induce free radicals (many prescription drugs, aspirin, ibuprofen, etc.), and as a protective agent against degenerative diseases of aging (atherosclerosis, diabetes, cataracts, skin deterioration, osteoporosis, etc.). In animal models of each of the conditions mentioned, cells and organs have been shown to benefit from melatonin administration but, to date, only occasional studies have been performed in humans [195].

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

ABBREVIATIONS

CSF = Cerebrospinal fluid

c3OHM = Cyclic 3-hydroxymelatonin

GCL = Glutamate cysteine ligase

GPx = Glutathione peroxidase

GRd = Glutathione reductase

GSH = Reduced glutathione

GSSG = Oxidized glutathione

HOCl = Hypochlorous acid

H_2O_2 = Hydrogen peroxide

NADH = Nicotinamide adenine dinucleotide

NADPH = Nicotinamide adenine dinucleotide phosphate

NO = Nitric oxide

NOS = Nitric oxide synthase

O_2 = Molecular oxygen

$O_2^{\bullet-}$ = Superoxide anion radical

$ONOO^-$ = Peroxynitrite anion

SOD = Superoxide dismutase

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