Medical implications of melatonin: receptor-mediated and receptor-independent actions

Reiter RJ, Tan D-X, Manchester LC, Pilar Terron M, Flores LJ, Koppisepi S*

Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, TX, USA

Abstract

The functional versatility and diversity of melatonin has exceeded everyone's expectations. The evidence is substantial that melatonin has multiple receptor-mediated and receptor--independent actions. Considering the unexpectedly widespread distribution of cellular membrane receptors as well as the existence of nuclear binding sites/receptors and the fact that some of melatonin's actions are receptor-independent means that melatonin likely functions in every cell with which it comes in contact. This is highlighted by the fact that there are no morphophysiological barriers to melatonin, e.g., the blood-brain barrier. In addition to its widespread actions, melatonin synthesis occurs in widely diverse tissues with its production not being relegated to the pineal gland. This should not be unexpected given that it is present throughout the animal kingdom including species that lack a pineal gland, e.g., insects, and in single cell organisms. In this review, only a few of melatonin's effects that involve the interaction of the indoleamine with receptors are described. These functions include the control of seasonal reproduction, modulation of sleep processes and influences on bone growth and osteoporosis. Among the actions of melatonin that are likely receptor independent and that are reviewed herein include its ability to neutralize free radicals which leads to a reduction in cataract formation, reducing oxidative stress due to exposure to hyperbaric hyperoxia, ameliorating hyperthyroidism and abating the toxicity of sepsis and septic shock. These actions alone speak to the diversity of beneficial effects of melatonin; however, the review is no way near exhaustive in terms of what melatonin is capable of doing. Because of its ubiquitous benefits, the pharmaceutical industry is developing melatonin analogues which interact with melatonin receptors. Clearly, the intent of the drugs is to take advantage of some of melatonin's numerous beneficial effects.

Key words: antioxidant, cataracts, free radicals, hyperbaric hyperoxia, hyperthyroidism, osteoporosis, sepsis, sleep.

Introduction

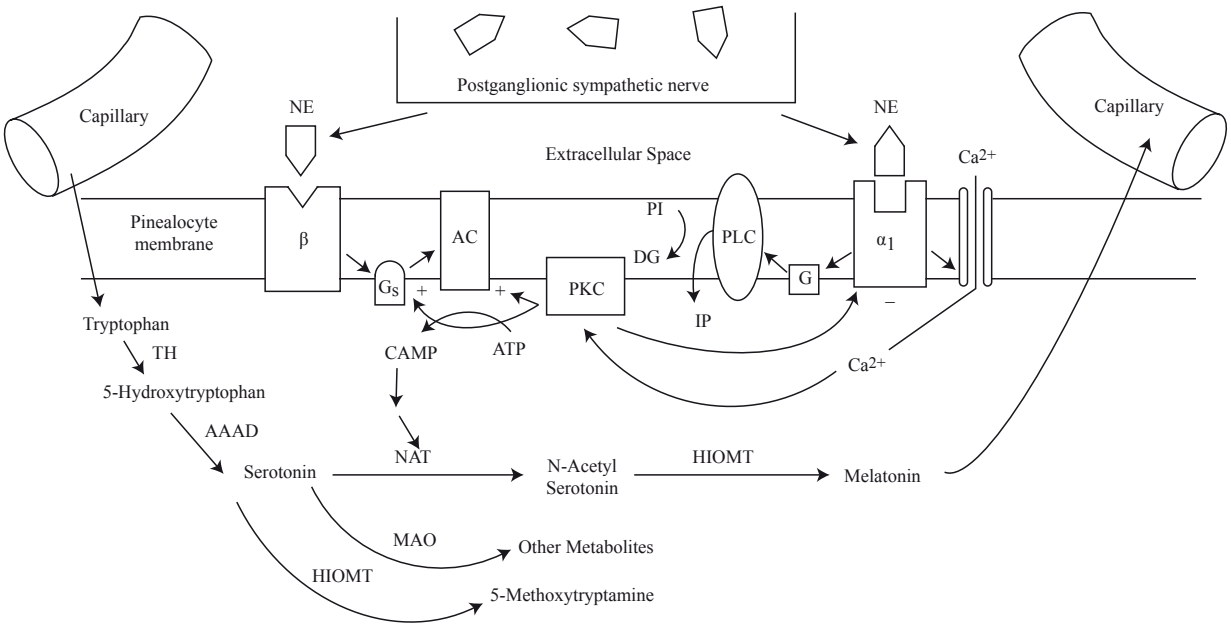
Melatonin is often referred to as a hormone. By conventional definition, a hormone is defined as a molecule that is synthesized in an organ, released into a bodily fluid from where it travels to another cell or group of cells were it acts via specific receptors to mediate its effects. Melatonin does not always act in this manner. Sometimes it carries out its actions without the intervention of a receptor, e.g., when it directly scavengers free radicals [1]. In other circumstances, it is released from a cell and acts on another cell in the immediate vicinity, i.e., it functions as a paracoid. Because of this diversity of actions, melatonin is not, in the strictest sense, a hormone. Rather it is a tissue factor, a paracoid, an autocoid, an antioxidant and sometimes a hormone depending on the physiological situation [2].

Also, typically hormones are regulated, either positively or negatively, by other hormones. Thus, hypothalamic releasing/ /inhibiting hormones act at the level of the anterior pituitary to release or inhibit, respectively, other hormonal products present in the adenohypophysis. In turn, these hypophyseal hormones act on peripheral endocrine organs to induce the synthesis and release of yet another set of hormones which then exert either positive or negative feedback effects on the anterior pituitary and/or brain. By comparison the control of pineal melatonin synthesis and release is primarily under control of the sympathetic innervation to the gland (*Fig. 1*) [3,4] and conventional hormones are without a marked influence on its production [5].

* CORRESPONDING AUTHOR:
Department of Cellular and Structural Biology
The University of Texas Health Science Center
7703 Floyd Curl Drive, San Antonio, TX 78229-3900
e-mail: reiter@uthscsa.edu (Russel J. Reiter)

Received 17.04.2007 Accepted 20.06.2007

Figure 1. **Interactions of the postganglionic sympathetic innervation with the pinealocyte (the major cell in the pineal gland). During darkness these nerve endings release norepinephrine (NE) which acts primarily on β-adrenergic receptors (β) and to a lesser degree on alpha1-adrenergic receptors (α1) to promote the nocturnal synthesis of melatonin. Melatonin is formed from the amino acid tryptophan which is taken up from the blood into the pinealocyte. Four enzymes, i.e., tryptophan hydroxylase (TH), aromatic acid decarboxylase (AAAD), N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT), are responsible for converting tryptophan to melatonin (N-acetyl-5-methoxy-tryptamine). Once produced, melatonin is quickly released into the adjacent capillaries and possibly into the third ventricle of the brain. Hormones from endocrine glands throughout the body do little to perturb the circadian production of melatonin and have only a minor influence on the total amount of melatonin synthesized**



Thus, the pineal gland itself is also not a conventional endocrine organ; rather it is probably best described as a neuroendocrine transducer.

Another feature that characterizes melatonin is its widespread distribution in animals and its uncommonly widely diverse functions. Melatonin exists possibly in every species of the animal kingdom [6,7] and even in some plants [8,9], perhaps all plants. It seems possible that melatonin exists in all living organisms. Also, while the pineal gland of vertebrates is the best known site of melatonin synthesis, it is by no means relegated to this organ given that invertebrates and unicells produce melatonin but are devoid of a pineal organ. Indeed, unicells have no organs whatsoever. Given these observations, it should also be no surprise that even in vertebrates, including mammals, the production of melatonin is not restricted to the pineal gland, e.g., it synthesis reportedly occurs in the retinas [10], the gastrointestinal tract [11], the bone marrow [12,13], and a number of other organs [14-17]. When the mRNAs for the two enzymes that generate melatonin from serotonin are localized, they are found in many tissues that are not yet known to produce the indoleamine [18].

Receptor-mediated actions

In mammals, melatonin signals intracellular processes via activation of two high-affinity G-protein-coupled receptors designated MT1 and MT2 [19-21]. These receptors are distinguishable on the basis of their molecular structures [22], their pharmacological characteristics [23] and their chromosomal localization [24]. The MT1 and MT2 receptors signal by coupling to heterotrimeric Gi proteins. Activation of these receptors causes dissociation of G-proteins into α and a βγ dimer which then interact with various effector molecules related to transferring the signal [25]. The effector systems involved in MT1 and MT2 receptor signaling through G-protein coupling include adenyl cyclase, phospholipase C, phospholipase A2, potassium channels and possibly guanylyl cylase and calcium channels [26-28].

The following paragraphs describe conditions where melatonin receptors are involved in mediating physiological changes. In these cases either the MT1 or MT2 receptors, or both, are likely involved. Although these actions of melatonin are described as being receptor-mediated, the specific signal transduction and effector mechanisms are not always clearly defined.

Seasonal reproductive physiology. The first action of melatonin to be well substantiated is its ability to mediate seasonal changes in reproductive competence in photoperiodically-dependent seasonal breeders [29,30]. These studies were initiated by observations made in the mid-1960s which showed that short day exposure (winter-type photoperiods) of Syrian hamsters markedly depressed reproductive function in both male [31-33] and female [34,35] Syrian hamsters and that these reproductive degenerative changes were prevented by either surgical removal of the pineal gland [31,32,35,36] or by superior

cervical ganglionectomy [3], which interrupts the major nerve supply to the pineal gland and renders it non-functional. While the ability of short days to depress reproductive physiology in long day-breeding Syrian hamsters was first documented under stable laboratory conditions, soon thereafter it was also shown that, in animals maintained under natural photoperiod and temperature conditions, pineal removal allowed the hamsters to maintain large functional gonads during the winter months [37]. Furthermore, the pinealectomized animals were capable of successful reproduction during the winter [38], a time at which they would have normally been incapable of doing so. In most cases, delivery of young during the winter months is not conducive to survival of the offspring due to the reduced environmental temperature and the shortage of food supplies for both mother and newborns.

The importance of these findings is that the results document that the circannual rhythm in reproductive competence in photoperiodic species is mediated by seasonally-changing day lengths. Moreover, it is the pineal gland, via the elevated nocturnal secretion of melatonin, the duration of which is proportional to the daily dark period [39,40], that is the critical determinant of the seasonal reproductive cycle. Indeed, in a cleverly designed series of studies, Carter and Goldman [41] confirmed, using melatonin infusion, that long duration daily melatonin elevations (typically of those that occur during the short days of the winter) in pinealectomized Djungarian hamsters caused reproductive collapse while shorter duration (typical of those that occur during the long days of the summer) daily infusions did not. Moreover, Stetson and Tay [42] documented that late afternoon melatonin administration, which then synergizes with nocturnally produced endogenous nighttime melatonin to prolong the duration of elevated melatonin, induced gonadal quiescence even in long day-exposed animals. Collectively, the data summarized above provide compelling evidence that melatonin, and specifically the changing duration of elevated nocturnal melatonin level over the seasons is the impeller of seasonal reproductive breeding. The observations, originally derived from studies on the Syrian hamster, have been shown to also exist in many other long day-breeding species, e.g., the Djungarian hamster [43], vole [44], white-footed mouse [45], ferret [46], etc. This information is now so commonplace that it is considered textbook material.

At the time of these observations, melatonin was generally referred to as an antigonadal [47] or antigonadotropic [48] agent since long duration elevated melatonin levels were associated with reproductive involution. However, the use of these terms was premature and an incorrect designation. Melatonin seems not per se to be directly inhibitory to the reproductive system of seasonally breeding animals inasmuch as many species breed at a time of the year (the winter) when maximal duration melatonin levels exist. These are what are referred to as short day-breeders and include some strains of sheep [49,50], white-tailed deer [51], etc. Clearly, in these species melatonin does not inhibit reproduction and the associated hormone levels. In humans, although melatonin was initially thought to be inhibitory to reproductive development and physiology and was tested (in combination with progestin) as a contraceptive agent [52], it is now generally accepted that melatonin has a minor or no influence on the reproductive physiology of the human. While many individuals take melatonin on a regular basis, there have been no reports of suppressed reproductive function.

The site at which melatonin acts to synchronize seasonal reproductive capability has not been unequivocally proven. The evidence, however, is very strong that the neuroendocrine-hypophyseal axis is the site of this interaction. Hence, the medial basal hypothalamus [53-55] and the anterior pituitary gland itself [56,57] have received a great deal of attention. These cites certainly contain melatonin receptors which are capable of modifying cellular events that would alter reproductive physiology [21,58].

Sleep. Circadian rhythms play an important role in determining optimal functioning of organs and physiological processes in all animals. The sleep/wake cycle in man is internally synchronized with the body temperature rhythm and with the 24 hour blood melatonin cycle [59]. These three rhythms normally have a stable phase relationship with maximal sleepiness coinciding with highest melatonin levels and minimal core body temperature. In normally-entrained individuals, major sleep occurs during the night with the late evening rise in melatonin preceding slightly the propensity to sleep [60]. When humans enter darkness at 22:00-23:00 h, circulating melatonin levels reach their peak 3 to 5 hours thereafter and then begin to drop as the time of awakening approaches [61]. Core body temperature usually reaches its peak in the early evening and decreases to a nadir between 03:00 and 06:00 hours [62] with sleep onset usually occurring 5 to 6 hours before the lowest core body temperature [63].

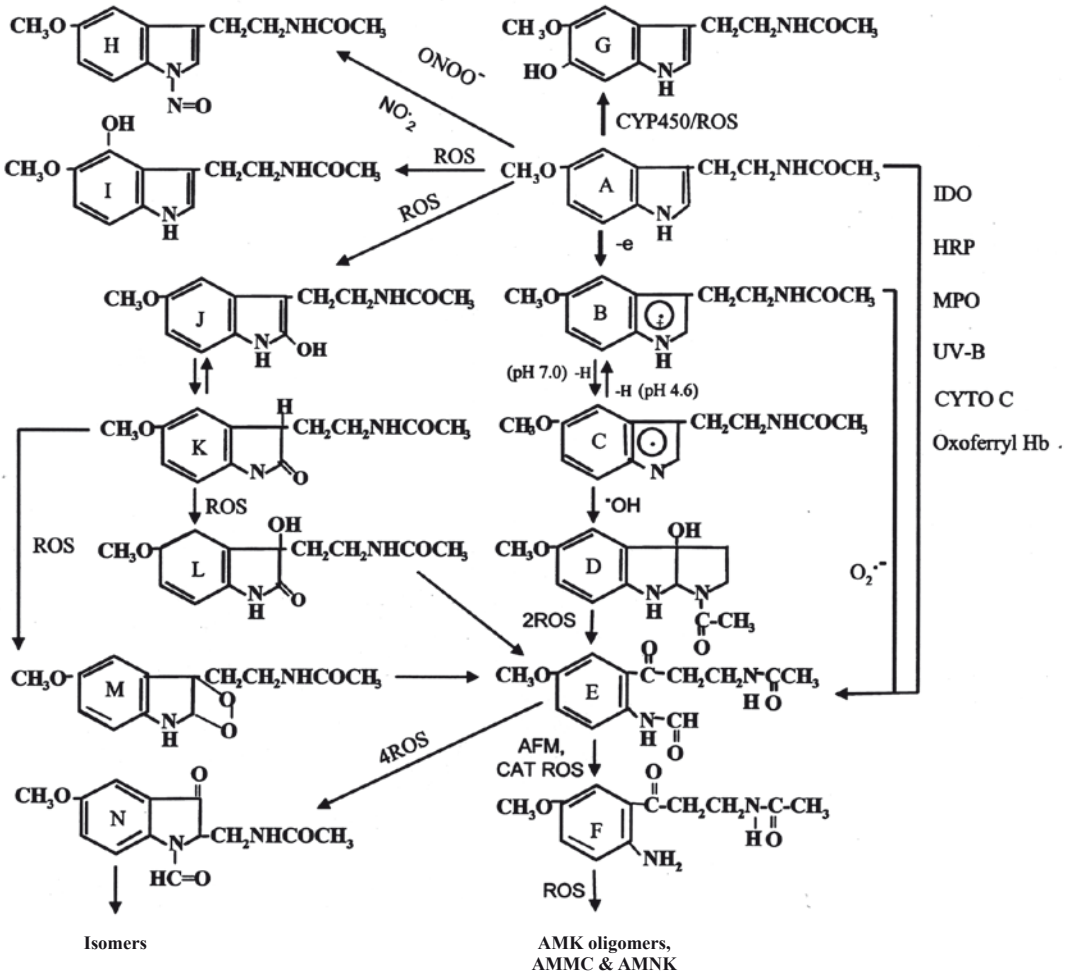
Given the obvious association between sleep propensity and rising melatonin levels, investigations into the effects of exogenously-administered melatonin on sleep induction would be expected. Many studies in the last two decades have suggested an association between sleep an elevated induced melatonin levels [64] although there are contrary reports [65].

Exogenously-administered melatonin functions as a non-photic Zeitgeber when its administration is appropriately timed. As a result it can phase advance or phase delay the circadian system including sleep onset when it is given during the proper interval. The human phase response curves for melatonin have been defined [66,67]; when exogenous melatonin is given in the late afternoon/early evening, the endogenous melatonin rise is phase advanced. When melatonin is given in the morning, a delay in the melatonin rise is seen in the evening of the same day, i.e., it is phase delayed [68].

Some chronic sleep disorders are a consequence of disturbances of the relationship between components of the circadian system. Besides sleep disorders, difficulties in alertness, fatigue, etc., in shift workers and during jet lag have a similar dyssynchronization of their circadian cycles. The ability of melatonin to correct or partially alleviate these conditions has been examined.

Individuals with delayed sleep phase syndrome (DSPS) experience difficulty in falling asleep at their desired bedtime and an inability to wake spontaneously in the morning [69]. Exogenous melatonin has been given to individuals with DSPS with the intent of correcting this problem. Because of different doses of melatonin given to patients at different times, it

***Figure 2*. Melatonin (A), during the process of scavenging toxic reactants, generates a number of metabolites that are equally effective or better scavengers than is the parent molecule. Some of the most important of these are cyclic 3-hydroxymelatonin (D), N1-acetyl-N2--formyl-5-methoxykynuramine (E), and N-acetyl-5-methoxykynuramine (F). 6-hydroxymelatonin (G), is the major hepatic enzymatic metabolite of melatonin**



is difficult to compare the results of the studies that have been published. In general, however, evening melatonin administration to DSPS patients often phase advances sleep onset [70-72], improves sleep and results in less daytime fatigue. Furthermore, evening melatonin treatment combined with early morning bright light therapy induces even a greater phase advance suggesting that these two treatments have additive effects and that it may be the best treatment to improve sleep in individuals with severe DSPS [73].

Sleep disorders in children have also been successfully treated with melatonin. Melatonin therapy in children with neurodevelopmental disorders (NDD) has yielded a 70-90% response rate in terms of sleep improvement [74,75]. DSPS in children, as in adults, as well as other circadian rhythm sleep disorders can usually be improved or corrected with melatonin. On the other hand, early morning awakening, which is common in children with NDD, is more difficult to treat with evening melatonin. This may relate to the rapid metabolism of melatonin after it is given. Thus, it can reduce sleep onset latency but early morning awakening still occurs [76,77].

The dose of melatonin required to induce sleep seems to be highly variable among individuals and some patients show no sleep improvement whatsoever in response to the indoleamine. Once the therapeutic threshold is established for sleep promotion in an individual, higher doses generally do not result in additional sleep promoting benefits [78]. Also, there are some individuals who exhibit a rapid response to melatonin treatment while others are slow responders [77]. Thus, some patients exhibit sleep improvement essentially the first night while in others melatonin must be given for weeks or months before sleep improvement becomes apparent.

Insomnia is a prevalent problem in elderly individuals with up to 50% of that population exhibiting inefficient and/or non-restorative sleep despite ample opportunities to sleep [79]. Given that endogenous melatonin wanes with increasing age and because of the proposed association of melatonin with sleep improvement, it has often been surmised that insomnia in the elderly is related to the diminished melatonin levels. The soporific effect of melatonin has been tested in elderly subjects with varying degrees of success [80,81]. The use of melatonin

to treat insomnia in older people is supported by the findings of Zisapel [82] who reports that it is effective in promoting earlier onset and more restful sleep in the elderly and, although no specific melatonin formulation has been approved for sleep, its efficacy and high safety profile provide a rational for its use.

One novel approach to improve nighttime sleep and daytime activity in elderly institutionalized subjects was to give them melatonin-rich milk [83]. In this case, milk containing 10-40 mg melatonin per liter was given as a drink with meals. The amount of milk consumed by each subject was, on average, 0.5 liters daily. In this study, there was some subjective improvement of sleep quality as judged by the caregivers and a more noticeable improvement in daytime activity. The authors suggested that even ultra-low doses of melatonin, which do not measurably change circulating melatonin values, may nevertheless have some beneficial effects in elderly humans, particularly in relation to increased daytime activity.

Due to the very wide variety of factors that lead to sleep disturbances, it is not surprising that the use of melatonin to improve these problems has not been uniformly successful. While many investigators conclude that melatonin is efficious for improving sleep, the most effective doses and the ideal time of administration may vary among individuals, making generalizations about treatment difficult. Despite a significant amount of data to the contrary, as mentioned above, there are some clinicians/scientists who contend that melatonin is not beneficial in terms of sleep promotion [65]. This brief resume is certainly not exhaustive in discussing all the reports on melatonin in relationship to sleep and the interested reader can consult other reviews on this subject. Also, when melatonin does influence sleep processes the authors of the reports usually assumed that this result is a consequence of melatonin's interaction with neural membrane receptors, probably in the suprachiasmatic nuclei.

Osteoporosis. In 1992, despite the absence of any compelling data indicating an association between melatonin and bone metabolism, Sandyk et al. [84] made the suggestion that perhaps the indoleamine would be beneficial in reducing the severity of postmenopausal osteoporosis. The idea is interesting inasmuch as osteoporosis becomes most obviously manifested after menopause/andropause when melatonin levels, along with a variety of other hormonal agents, wane. While individuals have subsequently pointed out the temporal relationship between the age-related reduction in melatonin and the progression of bone loss in the elderly, neither directly suggested melatonin be used to treat this condition [85,86]. Considering the data from recent publications, melatonin administration may yet prove to be a feasible treatment to improve bone health in the elderly. In 2003, Cardinali and co-workers [87] reviewed the world's published literature on this subject and defined the rational for the potential use of melatonin therapy to augmented bone mass in diseases characterized by low bone density and increased fragility of osseous tissue.

Circulating levels of melatonin can be reduced in young animals by surgical removal of the pineal gland. When this procedure is performed in chickens, the development of scoliosis is a common finding, a change consistent with the loss of bone mass and deterioration of skeletal microarchitecture [88-92]. While pineal ablation does not by itself result in scoliosis in the rat, this may relate to the fact that rats do not walk upright like chickens and, therefore, the amount of pressure on the spine of the rat is greatly relieved.

Theorizing that being bipedal was potentially a requirement for the development of scoliosis after pinealectomy, Machida et al. [93] published a series of studies indicating this is the case. To create bipedal rats, this group surgically removed the forelegs and the tail from pups shortly after birth. These animals then learned to walk upright, i.e., they became bipedal, by using their hind legs only. When these rats were subsequently pinealectomized, they too developed spinal malformations similar to those in chickens lacking their pineal gland. Importantly, treating pinealectomized bipedal rats with a subcutaneous melatonin pellet prevented the deterioration of the vertebrae and the development of idiopathic scoliosis. Thus, the authors concluded that postural processes along with a melatonin deficient state are critical factors in the development of a weakened vertebral column in pinealectomized rats [93].

Machida and co-workers [94] have now extended these studies to another rodent, the C57BL/6J mouse. This strain of mouse is an ideal model in which to examine the role of melatonin in preventing bone loss given that it is genetically deficient in melatonin [95,96]. As in their studies using rats, Machida et al. [94] surgically-induced bipedalness and some of the animals were supplemented with an intraperitoneal injection of melatonin daily (8 mg/kg). After 5 months the mice were killed and the vertebral column was examined by spine X-ray and 3 dimensional computerized tomography. Scoliosis and rib humps developed in 29 or 30 genetically melatonin-deficient bipedal mice; interestingly, even 5 of 20 quadrupedal mice that had genetically-depressed melatonin levels exhibited spinal curvature. When melatonin was given as a daily supplement, no mice developed scoliosis.

A recent study from the same laboratory extended these findings using this unique strain of mouse [97]. Again, the animal selected for their studies was the C57BL/6J mouse because of its melatonin deficiency [95,96]. These mice were again rendered bipedal by surgical removal of the forelimbs. These animals, even though they were not pinealectomized (but they were melatonin deficient) developed scoliosis in a large percentage of the cases, i.e., 7 of 9 mice. In another strain of mice, the C3H/HeJ, which is not deficient in melatonin, bipedal ambulation by itself caused 25% of the animals to develop spinal curvature, this was increased to 70% when bipedal C3H/HeJ mice were additionally pinealectomized. The conclusion of these studies is that spinal deformations occur as a result of a melatonin deficiency combined with bipedal ambulation.

These findings attracted the interest of clinical researchers who surmised that bipedal primates, e.g., humans, may develop scoliosis and/or osteoporosis in later years since at this time endogenous melatonin levels are depressed. To test this they selected the rhesus monkey [98], 18 of which were pinealectomized when they were 8-11 months of age; the completeness of pineal removal was assessed by the minimal levels of a major melatonin metabolite, 6-hydroxymelatonin sulfate, in the urine. Following the surgical procedure, the follow-up interval varied from 10-41 months during which bone structure was analyzed

by means of monthly radiographs. Of the 10 monkeys that had complete pineal excision, none developed scoliosis (average follow-up was 29 months).

Given the apparent negative outcome of this study, Cheung et al. [98] reasoned that the observations made in bipedal rodents regarding melatonin deficiency, bipedalness and weakened bone structure cannot be extrapolated to bipedal primates or to the human. They surmised that the difference may be possible etiological factors that contribute to the development of idiopathic scoliosis in different animal groups. There are, however, several factors to consider. Firstly, whereas the rhesus monkey is classified as a bipedial primate, rarely does it walk upright. Also, in this case the animals were likely maintained in cages that greatly limited their mobility and stress on the vertebral column; if these animals would have been in their natural setting with free movement perhaps the outcome of the studies may have been different. Thus, it would seem premature to conclude that melatonin is without effects of bone growth, remodeling or deterioration in primates including man.

Ladizesky et al. [99,100] used two different animal models to test whether melatonin is influential in terms of bone formation and resorption. Ovariectomy in rats is accompanied by changes in bone metabolism and demineralization. To monitor these changes, they assessed urinary deoxypyridinoline (a marker of bone resorption) and calcium excretion as well as blood levels of calcium, phosphorus and bone alkaline phosphatase (a marker of bone formation); additionally, they evaluated bone mineral density (BMD), bone mineral content (BMC) and bone area (BA) of the entire skeleton at 60 days after ovary removal. Half of the rats had melatonin added to their drinking water (250 μg/ml). By 30 days after ovariectomy, urinary deoxypyridinoline increased by 50%, a change that did not occur in the ovariectomized rats that were ingesting melatonin in their drinking fluid. At 15 days after surgery, a significant rise in serum phosphorous and bone alkaline phosphatase was apparent in rats lacking their ovaries but receiving melatonin. The BMD, BMC and BA, although reduced after ovariectomy was not modified by melatonin in the drinking fluid. While not all parameters of bone remodeling were preserved by melatonin treatment, the authors were confident in concluding that melatonin does modify bone remodeling following surgical removal of ovaries, but for maximal benefit some estrogen may also have to be available [100]. Castration in male rats causes similar changes in indices of bone loss which are reduced when melatonin is given [101,102].

In a second study, Ladizesky et al. [103] treated male rats for 10 weeks with either melatonin or methylprednisolone or both agents. While each molecule independently had positive effects on indices of bond health, when given in combination the benefit was the greatest. The indices that exhibited positive effects included BMC, BMD, and BA and, during a femoral biomechanical test the combination of the glucocorticoid and the indoleamine produced the highest values of work to failure. Clearly, both methylprednisolone and melatonin independently reduced bone resorption and had bone protective effects.

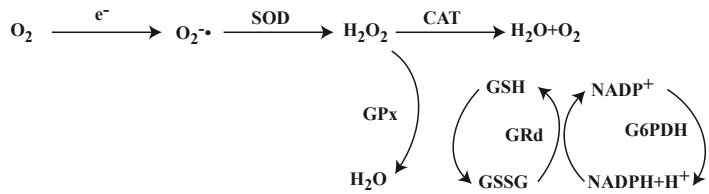
The consequences of melatonin on the proliferation of osteoblasts differ slightly among the findings that are reported in the literature. Roth et al. [104], using two rodent osteoblastic cell lines claimed that melatonin was either without effect or slightly depressed osteoblastic cell proliferation. This group also reported, however, that melatonin promotes osteoblast differentiation and enhances bone formation. In contrast, Nakade et al. [105] found that, in the presence of melatonin, human osteoblast cell proliferation was improved.

The most complete examination of the association of melatonin and bone growth is that recently published by Satomura et al. [106]. The intent of their studies was to test whether melatonin could be effectively used as a pharmacological agent to shorten the treatment period of bone fracture, osteotomies and bone distraction. For their *in vitro* studies they used human osteoblasts and for the *in vivo* experiments the mouse was the animal of choice. In terms of its effects on human osteoblasts, melatonin dose-dependently stimulated cell proliferation and alkaline phosphatase activity. Additionally, the indoleamine intensified gene expression of type 1 collagen osteopontin, bone sialoprotein and osteocalcin. As with cell proliferation, the degree of heightened gene expressions were related to the concentrations of melatonin used. Also, importantly the intraperitoneal administration of melatonin (100 mg/kg for 21 days) increased the volume of newly formed cortical bone in the femurs of mice. Finally, reverse transcription-polymerase chain reaction and Western blot analysis showed that human osteoblasts express the melatonin 1a receptor.

That melatonin influences the differentiation of progenitor cells toward osteoblasts was recently suggested by the studies of Sanchez-Hidalgo et al. [107]. In mammalian bone marrow, two of the major cell types that occur develop from a common precursor cell, the multipotent bone marrow-derived cell, or the mesenchymal stem cell [108]. Melatonin may be able to direct these undifferentiated cells toward the osteoblast line rather than to the adipocyte [104]. Using the ROS17/2.8 cell line which was also used by Roth and co-workers [104], Sanchez-Hidalgo et al. [107] found that melatonin inhibited oleic acid uptake by these cells reducing the formation of adipocytes and directing cell differentiation toward the osteoblast. They also found, with the aid of the melatonin receptor antagonists, luzindole and S20928, that melatonin inhibited triglyceride uptake by these cells via a receptor-mediated mechanism, although the signaling events were not identified. These findings were interpreted in light of the reduced melatonin production that occurs in the elderly. Normally, bone marrow cell differentiation with increased aging shifts toward the adipocyte line of cell development at the expense of osteoblast formation. This may contribute to osteoporosis which is common in aged individuals. These changes occur coincident with reduced endogenous melatonin production during aging [109-111]. The findings of Sanchez-Hidalgo et al. ([107] indicate that supplemental melatonin administration in the elderly may preserve bone strength and decrease fat cell accumulation in the bone marrow, a feature common to the marrow of aged individuals. The outcome of these *in vitro* findings are consistent with the observations summarized above related to melatonin's ability to prevent bone deterioration in melatonin-deficient bipedal rodents.

The structural integrity of the skeletal system relies on the persistent remodeling processes carried out by bone-resorbing osteoclasts and bone-forming osteoblasts. Melatonin may

***Figure 3*. Under elevated oxidative stress conditions melatonin either upregulates and/or prevents the loss of the activities of important antioxidative enzymes. The enzymes that melatonin protects include the superoxide dismutases (SOD, both the cytosol and the mitochondrial forms, i.e., CuZnSOD and MnSOD, respectively) and glutathione peroxidase (GPx) and glutathione reductase (GRd). Although less evidence is available, glucose-6-phosphate dehydrogenase (G6PDH) and catalase (CAT) may also be stimulated by melatonin. CAT and GPx act to metabolize H_2O_2 to harmless molecules thereby reducing the formation of the hydroxyl radical, which is highly destructive**



enhance bone formation by suppressing osteoclasts as suggested by Suzuki and Hattori [112] and Koyama et al. [113] via its free radical scavenging properties and actions on RANKL and/or by promoting osteoblastic activity [105,114] by mechanisms that involve membrane melatonin receptors on these cells [115,116]. The down-stream signaling mechanisms whereby melatonin enhances the activity of osteoblasts theoretically involve a number of mechanisms none of which have much support [117].

Non-receptor mediated actions

The ability of melatonin and its metabolites to expunge free radicals and related reactants possibly involves all of the following actions: a) direct detoxification of radicals and radical products, b) stimulation of the activities of several antioxidative enzymes, c) inhibition of the activities of prooxidative enzymes, d) promotion of the synthesis of glutathione, another essential antioxidant, e) synergistic actions with other antioxidants, and f) mitochondrial actions of melatonin that reduce free radical generation. Of these actions, some clearly require no specific receptor (are non-hormonal) while others may well be receptor-mediated (are hormonal). For the purposes of this presentation, however, they are listed under the non-hormonal category of actions.

While melatonin has the capability of donating one or more electrons to free radicals resulting in their detoxification [118--120], the metabolites that are formed during this process, i.e., cyclic 3-hydroxymelatonin (3-OHMel), N-acetyl-N-formyl--5-methoxykynuramine (AFMK) and N-acetyl-5-methoxykynuramine (AMK) [121-126] also have similar capabilities. This progressive annihilation of radicals and their products by melatonin and its metabolites is referred to the antioxidant cascade and is graphically depicted in figure 2 [1].

The activities of antioxidative enzymes may exhibit increases or decreases depending on the duration and severity of oxidative stress and when they are measured during the stress response. Initially, early in the oxidative stress response the activities of antioxidative enzymes may show a compensatory rise to overcome massive free radical generation. As the oxidative stress response is prolonged, free radicals either directly damage the enzyme molecule or its upstream processes leading to a reduction in enzyme activities. Thus, melatonin's apparent actions in terms of these enzyme responses may be at least two-fold, i.e., it prevents the upregulation of the enzymes presumably due to the fact that as melatonin and its metabolites scavenge radicals the oxidative stress environment is reduced and upregulation of antioxidative enzyme activity is less necessary. Secondly, melatonin due to its combined scavenging actions with that of its metabolites may reduce the likelihood that the enzyme itself or its upstream processes are damaged, thereby preventing a reduction in the activities of the antioxidative enzymes. These reported effects of melatonin have been summarized in several reviews [127-130].

The antioxidative enzymes that are influenced by melatonin include the superoxide dismutases (both the mitochondrial and cytosolic isoforms), glutathione peroxidase and glutathione reductase (*Fig. 3*) [129,130]. The antioxidative enzyme, catalase, has been less extensively investigated in terms of the effects of melatonin on its activity.

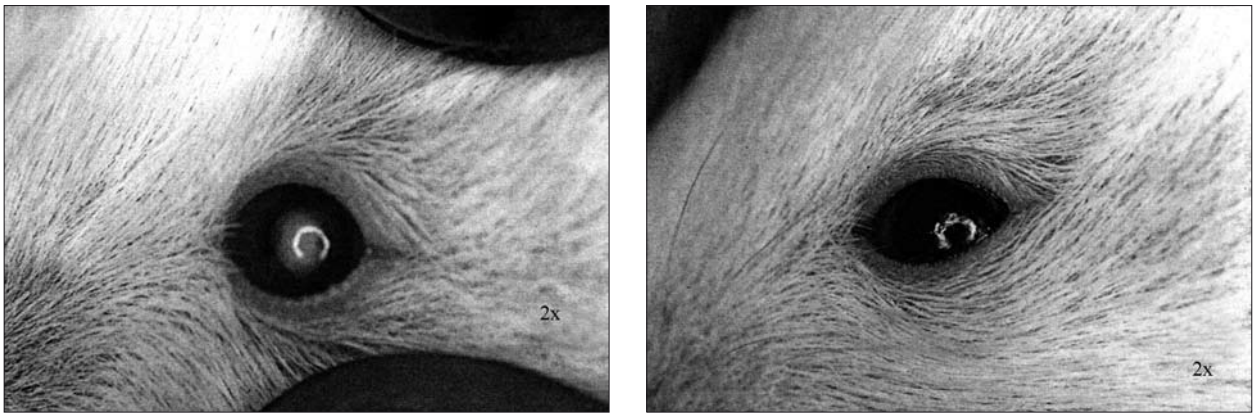
There are a number of prooxidative enzymes in multicellular organisms which generate free radicals. Examples include nitric oxide synthase which generates NO• and the lipoxygenases which result in the formation of the superoxide anion (O2•-). Whereas NO• is not a powerfully damaging free radical, when it couples with O2•- it forms the peroxynitrite anion (ONOO-) which is potently reactive and damaging. Melatonin inhibits lipoxygenase [120], while AMK reduces the activity of the enzyme that catalyze the formation of NO•, nitric oxide synthase (NOS) [131]. As of result, free radical and/or toxic reactant generation is alleviated.

The concentration of the intracellular antioxidant, glutathione, is very high in many cells. During high oxidative stress conditions total glutathione levels can be reduced. One action of melatonin seems to be to ensure that glutathione levels do not drop significantly. This may be achieved by melatonin's ability to stimulate the enzyme, gamma-glutamyl-cysteine synthase, the proposed rate limiting enzyme in glutathione production [132,133].

At least in *in vitro* experiments, melatonin has been shown to synergize with vitamins C and E and others to reduce free radical damage [134,135]. It is not uncommon for antioxidants to couple their actions such that the beneficial effect is greater than when the individual antioxidants act independently.

Prevention of free radical generation may be an important activity which allows melatonin to limit oxidative stress. A number of studies have documented that the fumbling of

***Figure 4*. Cataracts in the lens of 17-day-old rat (left) treated with L-buthionine-S,R-sulfoximine (BSO) at day 1 after birth to deplete intracellular levels of the antioxidant, glutathione. Prominent "cloudiness" of the lens was apparent at 17 days after birth. Melatonin, given as a daily intraperitoneal injection, prevented the formation of cataracts (right)**



electrons during their transfer though the electron transport chain may be reduced by melatonin [136,137]. When the escape of electrons from the chain is diminished, the number of radicals that are formed in the mitochondria is likewise reduced. Melatonin's ability to limit free radical generation at the mitochondrial level is readily apparent when cells subjected to high oxidative stress conditions are treated with melatonin [138].

In the following examples where melatonin has been used to reduce oxidative mutilation, one or more of the processes outlined above may have been operative. Indeed, it is impossible to determine what percentage of the protection is afforded by an individual action of a free radical scavenger/antioxidant. Also, the following list includes only a few of the experimental situations in which melatonin has been shown to attenuate free radical damage and improve tissue function.

Cataracts. Oxidative stress is identified as a major cause of cataracts [139,140]. In humans, cataracts usually develop late in life and are more common in individuals who have spent much of their time working outdoors where they were exposed to high levels of ultraviolet radiation. The lenticular damage that occurs is mainly in the epithelium and cortex [141].

A frequently-used experimental model to investigate processes associated with cataractogenesis and the means of inhibiting their development includes the newborn rat treated with L-buthionine-S, R-sulfoximine (BSO); this drug inhibits gamma-glutamyl-cysteine synthase, the rate limiting enzyme in glutathione production. Hence, the drug depletes tissues, including the lens, of the important antioxidant glutathione. This depletion leads to exaggerated oxidative stress, molecular damage and the formation of cataracts [142,143]. Typically, BSO is given on the day rat or mice pups are born and the cataracts are grossly apparent by the time the palpebral fissures open at 10-12 days after birth.

Abe and co-workers [144] used this model to induce cataracts and half of the newborn rats treated with BSO were given a daily intraperitoneal injection of melatonin (4 mg/kg BW) for the duration of the study period which ended 17 days after birth. They anticipated that melatonin would reduce cataract formation since it had been shown to be a free radical scavenger [145] and lenticular opacification is related to oxidative stress [146]. Moreover, melatonin had been identified in the fluid of the anterior chamber of the eye [147] and, therefore, it would likely have ready access to the lens. In this model, Abe et al. [144] indeed found that melatonin readily substituted for glutathione and almost totally prevented cataractogenesis (*Fig. 4*). In BSO-treated rats given only diluent, 18 of 18 pups had visually apparent cataracts while only 1 or 15 BSO-injected pups given melatonin on a daily basis had obvious bilateral cataracts. Since all rats treated with the glutathione-depleting drug had very significantly depressed (>90%) lenticular glutathione levels, the authors surmised that the protection against oxidative stress provided by melatonin was a consequence of its antioxidative actions.

Li and colleagues [148] used the same newborn rat model to study the efficacy of melatonin in reducing lenticular opacification and lipid peroxidation. In this case, the lenses were examined on both day 9 and day 17 after birth of the pups. When the data from the two days were combined, again virtually all pups (16 of 18) given BSO plus diluent developed cataracts while 3 or 18 of those that had the benefit of melatonin injections had cataracts. The levels of lipid peroxidation were elevated in many organs of pups given BSO with the amounts of oxidized lipid being reduced after daily melatonin treatment.

Bardak et al. [149] approached the problem of cataractogenesis and the protective effects of melatonin differently. This group specifically exposed rat lenses to ultraviolet light to induce cataracts and, again, melatonin reduced their incidence.

Of interest in relation to the ability of melatonin to limit the frequency of cataracts is that it was subsequently reported that the rat lens itself produces melatonin, at least after adulthood. This was initially reported by Abe et al. [150,151] and has been recently confirmed by Itoh and co-workers [17]. Abe and colleagues [150,151] detected melatonin and the serotonin acetylating enzyme, AANAT, in the rabbit and rat lens and in the latter species they described a circadian rhythm of AANAT activity. Most recently, Itoh et al. [17] documented that the mRNAs for both AANAT and HIOMT exist in the adult rat lens; immunocytochemical localization showed that AANAT is localized in the lenticular cortical fiber cells. The locally-produced melatonin may help to protect the lens from oxidative

stress and the formation of cataracts. Melatonin produced in the lens along with that synthesized in the ciliary body [152], which probably accounts for the concentrations of the indoleamine in the fluid of the anterior chamber of the eye [147], likely aids in protecting the lens from free radical-mediated oxidative damage.

Melatonin receptors are not required when the indole functions as a direct free radical scavenger. In the ocular tissues including in the lenticular cortical fiber cells of *Xenopus laevis*, however, receptors for melatonin are present [153]. This suggests that these lenticular cells are direct targets for melatonin and they could assist in reducing oxidative stress by mediating the effects of melatonin in stimulating antioxidative enzymes [130]. Whether any cells in the mammalian ocular lens possess melatonin receptors has not been determined.

Recently, Siu and co-workers [154] summarized the data related to the protective actions of melatonin against a variety of free radical-related ocular conditions/diseases. Besides cataracts, oxidative stress contributes to retinopathy of prematurity, retinoblastoma, age-related macular degeneration, retinitis pigmentosa, glaucoma, photokeratitis and ischemia/reperfusion injury in the orbital globe. Given the association of each of these conditions with excessive free radical damage, melatonin may help to attenuate the severity of these conditions as well.

Hyperbaric hyperoxia. Hyperbaric oxygenation (HBO) involves exposure to 100% oxygen at a pressure typically higher than atmospheric pressure. This treatment modality is a commonly-used procedure for a variety of disorders and has been successfully implemented in clinical situations involving ischemia and/or hypoxia [155]. The rational for HBO therapy is that it elevates pO_2 levels in the blood and tissues, especially those deficient in O_2. The higher concentration of O_2 administered and the higher pressure increases the level of dissolved oxygen entering the blood [156]. The down side to oxygen therapy, however, is the elevated levels of toxic oxygen-based free radicals and related products that are generated under elevated oxygen conditions [157,158]. Given the involvement of free radical-mediated molecular damage with this treatment paradigm, several studies examined whether melatonin would reduce oxidative damage to tissues of rats exposed either acutely or chronically to 100% oxygen.

In the first study in this series, Pablos and colleagues [159] exposed adult rats to 100% oxygen at 4 atmospheres for 90 minutes in a plexiglas chamber. Half of the rats were given a single intraperitoneal injection of 10 mg/kg melatonin before the onset of the exposure. As a result of the HBO, levels of lipid peroxidation products were elevated in the lungs, liver and brain; the rises in lipid degradation were prevented, in all organs, in the animals that had received melatonin. The antioxidative enzymes, GPx and GRd, were reduced as a result of hyperbaric oxygen exposure, changes also reversed by melatonin. Based on the indices measured, melatonin was highly effective in preventing oxidative damage resulting from hyperbaric oxygen exposure.

In the other two acute studies [160,161], adult rats were placed in a stainless steel oxygen chamber which was flushed with 100% oxygen with the chamber pressure being increased to 2.5 atmospheres pressure. Following the exposure session, the chamber was decompressed to normbaric air gradually over a 5 minute period. Immediately following the exposure, tissues were collected to evaluate the degree of oxidative stress.

As in the study Pablos et al. [159], both Topal and colleagues [160] and Dunbar et al. [161] reported that exogenously administered melatonin (10 mg/kg) before HBO exposure reduced oxidative damage in both the lungs and brain. Perhaps of greater importance is that both groups also showed that performing the oxygen exposures a night produced less severe effects in terms of free radical damage than when the exposures were done during the day. These authors, therefore, concluded that the nocturnal rise in endogenous melatonin provides some antioxidative protection against HBO.

In the only chronic exposure study, the investigators also examined the effect of both exogenously-administered pharmacological melatonin levels and endogenously-produced physiological melatonin concentrations on oxidative damage in rats after repeated hyperbaric oxygen exposure [162]. In this case, rats were given 10 consecutive daily HBO sessions of 1 hour duration; the parameters of exposure were 100% oxygen at 2.5 atmospheres. Immediately prior to each session, half of the animals were given an intraperitoneal injection of 5 mg/kg melatonin. The HBO treatment caused rises in protein carbonyls in both the lungs and brain and a compensatory increase in SOD activity in the same tissues. The changes in both protein carbonyls and SOD activity were blocked by daily pharmacological melatonin treatment.

To test the potential protective effect of physiological melatonin levels, the hyperbaric oxygen exposures were performed for 10 consecutive days at night when circulating melatonin levels are elevated [162]. The study was carried out with the aid of a dim red light which does not depress endogenous melatonin levels [163]. While not as effective as exogenously-administered pharmacological melatonin, when the hyperbaric oxygen exposures were performed at night in the presence of elevated endogenous melatonin concentrations, the damage to proteins was in part prevented.

From these studies, it is apparent that melatonin would be a worthy adjunct therapy to be given in combination with hyperbaric oxygen treatment. Undoubtedly, this HBO exposure is limited by the toxicity of oxygen and the use of melatonin may allow more prolonged or frequent treatment periods which may benefit recovery and avoid the associated molecular damage.

Hyperthyroidism. The hypermetabolic state associated with hyperthyroidism generates an excess of free radicals in the heart and other tissues [164,165]. During hyperthyroidism, the heart undergoes hypertrophy [166]. Due to the increased metabolic rate imposed by elevated thyroid hormone, additional free radicals are produced which damage the heart (and other tissues); the dysfunctional heart then undergoes a compensatory growth response to counterbalance the altered cardiac function.

Ghosh et al. [167] tested whether melatonin would overcome cardiac hypertrophy and oxidative alterations associated with triiodothyronine (T3) administration for 15 days. Additionally, they examined whether depressed gene expression for GLUT4 and the reduced glucose uptake by cardiomyocytes would be

changed by melatonin treatment. T3 (8 μg/100 g) was given via an intraperitoneal injection as was melatonin (2 mg/100 g); melatonin was always given 1 hour in advance of T3.

Melatonin proved to be protective against the cardiac enlargement that resulted from T3 treatment and, furthermore, it reduced •OH generation in the myocardium. Melatonin also reversed the marked drop in CuZnSOD in the T3-treated rat heart as well as the reduction in glutathione levels. Gene expression for GLUT4 was lowered as a consequence of T3 administration, an effect also prevented by melatonin. Finally, the 50% reduction in insulin-stimulated glucose uptake by hyperthyroid-induced hypertrophic cardiomyocytes was restored by melatonin. GLUT4 normally mediates facilitative transport of glucose into cells. Thus, in this study it was theorized that the down regulation of the GLUT4 gene compromised glucose uptake by the cardiomyocytes. Given that glucose transport/utilization is obviously very important for the optimal functioning of the heart, restoration of these processes by melatonin is important as is the reduction in oxidative damage to the organ.

Sepsis and septic shock. Sepsis is a common cause of mortality in both children and adults in intensive care units [168,169] and is a consequence of the host response to a microbial invasion. Multiple organ failure is frequently associated with sepsis and is characterized by severe hypotension and hyperactivity of blood vessels to vasoconstrictor agents. There are a variety of highly negative consequences of sepsis including respiratory distress syndrome, acute renal failure, disseminated intravascular coagulation and central nervous system dysfunction. The predominant agent responsible for sepsis is lipopolysaccharide (LPS), a component of the cell walls of Gram-negative bacteria [170,171].

In addition to the deaths related to septic shock, this condition has considerable economic cost. An estimated 700,000 patients develop severe sepsis annually in the United States [172,173]. The cost of treatment for each of these individuals is about $24,000. Unfortunately, there is no specific treatment for this condition, possibly related to the lack of definition of the underlying mechanisms of sepsis [174]. Given that sepsis is an extremely high proinflammatory state and a condition in which multiple free radicals and related reactants are produced [175], attempts have been made to combat the toxic reactions associated with this deadly disease in both experimented animals and in humans. The initial animal experiments, Sewerynek and coworkers [176,177] reported that the injection of melatonin into rats that had been treated with LPS reduced the associated peroxidation of lipids in hepatic membranes and also limited the leucocytosis in this organ. Also using the rat model of sepsis, Crespo et al. [178] found that melatonin attenuated the degree of lipid peroxidation and the exaggerated nitric oxide production that accompanies LPS toxicity in both the liver and lungs. Melatonin, in a dose-response manner, lowered nitric oxide by inhibiting inducible nitric oxide synthase, a well known action of this indoleamine which is actually mediated by a melatonin metabolite, AMK [131].

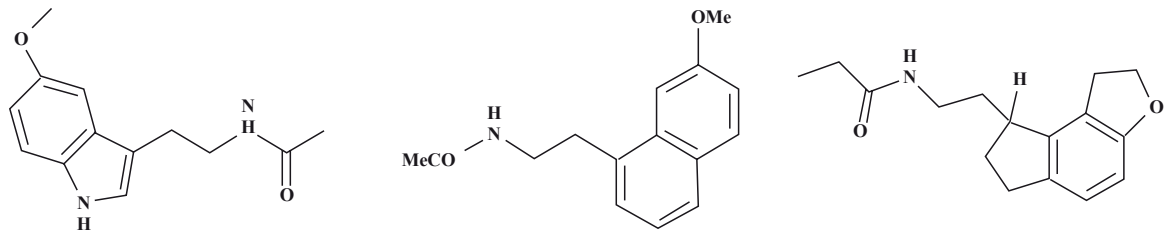
Besides reducing molecular damage resulting from LPS toxicity, melatonin also has been shown to lessen the death rate of endotoxemic rats [178,179]. Melatonin probably protects against death by improving the hemodynamics of these animals since the indoleamine was shown to counteract the release of TNF-α (and possibly other cytokines) into the plasma and reduce O2•- production by the aorta [180]. Moreover, melatonin also limits ONOO- formation and the activation of poly (ADP ribose) synthase [181], curtails the breakdown of lipids and replenishes GSH levels in a variety of organs [182,183] during experimental sepsis. As mentioned above, during sepsis melatonin prevents the recruitment of leucocytes to the affected organs [176,177]. When leucocytes infiltrate organs the enzyme myloperoxidase (MPO) is elevated resulting in the formation to a toxic chlorine-based species, hypochlorous acid. Since melatonin inhibits MPO activity and scavenges hypochlorous acid [184] even the reduced number of leucocytes within tissues are neutralized in terms of inflicting damage because of the presence of melatonin.

Sepsis is associated with marked changes in the physiology of mitochondria and these alterations obviously contribute significantly to the malfunction and death that septic animals/ /humans experience. Escames et al. [175,185] have examined melatonin's protective actions at the level of the mitochondria in LPS-treated rats. This group showed that melatonin administration reduces mitochondrial oxidative damage and inhibits mtNOS protein expression and enzyme activity [186] in both rat lungs and liver following LPS exposure. These observations may well explain some of the beneficial actions of melatonin during the septic response. This group has recently extended these findings by showing that melatonin similarly improves mitochondrial function in mice rendered septic by cecal ligation and intestinal puncture to induce severe peritonitis [187]. Importantly, this group showed that in addition to reducing oxidative/nitrosative damage at the mitochondrial level, melatonin also restored ATP production.

Given that animal studies have repeatedly confirmed the beneficial actions of melatonin in preventing sepsis-mediated molecular damage and death in animals [178,179,186,188], it is not surprising that Gitto and colleagues [189] utilized melatonin to treat premature newborn humans suffering from sepsis. The results of their study documented the high efficacy of melatonin in humans with septic shock. Ten neonates with sepsis were treated with melatonin in addition to receiving conventional therapy while ten infants received conventional therapy only. The melatonin dose was 20 mg given orally in two equal doses separated by 1 hour within the first 12 hours of the diagnose of sepsis. These neonates exhibited early beneficial signs of the melatonin therapy with levels of lipid peroxidation products in the blood being already reduced 1 hour after being given melatonin. Furthermore, melatonin caused a marked drop in circulating C-reactive protein (an inflammatory indicator) levels. Most important, however, was the prevention of death in the septic neonates treated with melatonin. It is common for up to 50% of these infants to die [189]. Of the 10 septic neonates who did not receive melatonin in the study of Gitto et al. [190], three infants died; conversely, none of the septic neonates treated with melatonin died.

These findings are compelling given the serious nature of sepsis and septic shock and the lack of an adequate therapy for this often fatal condition. Research related to use of melatonin as a treatment for sepsis should be aggressively pursued; along

***Figure 5*. Structure of melatonin in comparison to the two melatonin mimetics developed by the pharmaceutical industry. Ramelteon is currently being marketed while agomelatine is in phase III trials. Ramelteon is prescribed as a sleep aid whereas agomelatine will be sold to reduce depression**



these lines Buonocore and Groenendaal [191] have suggested that melatonin be thoroughly tested in clinical trials to overcome oxidative/nitrosative toxicity in clinical conditions such as bacterial sepsis.

Patented melatonin minetics

Since the molecule melatonin is not *per se* patentable, the pharmaceutical industry has pursued the development of melatonin analogues which can be patented. One of these drugs, ramelteon (Roserum™) developed by Takeda Pharmaceuticals has been issued a US patent (US6034239) [192] and is currently being marketed. The second drug, agomelatine (commercial name Valdoxan™, US5318994) [193] is currently in phase III trials and is expected to be on the market soon. This drug will be sold by Servier and Norvatis Pharmaceuticals. The structures of ramelteon and agomelatine relative to that of melatonin are shown in *Fig. 5*.

Melatonin itself has been widely reported to have sleep promoting activity [77,194-196] although this has also been disputed in recent years, at least in the case of some sleep disorders [65]. It is of interest that among mammals, melatonin is uniquely elevated during the night, yet many species are night active and actually sleep during the day when melatonin levels are at their lowest. This implies that melatonin is not a direct hypnotic or soporific but rather influences sleep propensity via other means.

Ramelteon. Ramelteon, which is marketed as a sleep aid, is an indenofuran derivative of melatonin which binds to MT1 and MT2 melatonin receptors [197]. The major interest seems to be its binding to the melatonin receptors in the biological clock, the suprachiasmatic nuclei (SCN); these nuclei are believed to be involved in mediating the effects of melatonin on circadian rhythms and on sleep.

Ramelteon inhibits forskolin-stimulated cAMP production in neonatal rat pituitary glands with an IC_{50} of 20.8 pM suggesting it is a potent agonist of the MT1 receptor [198]. In reference to its MT2 receptor activity, ramelteon binds to Chinese hamster ovarian cells with a binding affinity is roughly 3-fold lower than for the MT1 receptor (Ki=45pm vs Ki=14pm, respectively). Compared to melatonin, the binding affinity of ramelteon for the MT3 hamster brain binding sites is weak. Ramelteon does not bind to a variety of other ligand binding sites; some of those that have been tested include benzodiazepines, dopamine, ion channels and transporters and opiates [199,200]. This melatonin mimetic has a longer half life than melatonin [201,202] and promotes sleep. Chronobiologically, the drug accelerates re-entrainment of running wheel activity in rats [203] to the same degree as does melatonin.

Ramelteon was initially designed as a drug to treat patients with insomnia and related circadian rhythm disorders. In particular, the drug is proported to advance sleep onset in individuals who have difficulty falling asleep, i.e., to reduce sleep latency, but it does not have generalized depressive actions on the electrical activity of the central nervous system [81,204,205]. Ramelteon has not exhibited evidence for abuse or psychological or physical dependency [206]. When used as a sleep aid, the usual recommended dose is 8 mg orally at 30 minutes before desired sleep onset [204].

Agomelatine. Agomelatine was synthesized by replacing the indole scaffold of melatonin with a naphthalene ring [198]. Agomelatine is an agonist of the MT1 and MT2 melatonin receptor [207]. The affinity of agomelatine for cloned human MT1 and MT2 receptors (Ki=6.15 x 10^{-11} and 2.68 x 10^{-10} M respectively) is similar to the binding affinities of melatonin to the same receptor subtypes which are Ki=8.52 x 10^{-11} and 2.63 x 10^{-10} M, respectively.

Agomelatine is designed as a once-daily treatment of major depressive disorders particularly when anxiety and sleep problems are features of the condition [208]. Its efficacy in reference for depression was originally tested in rats and mice and in transgenic murine models [209]. Under these conditions, agomelatine at a dose of 10 mg/kg was as effective as imipramine or fluoxetine at the same doses; the latter two drugs are commonly used antidepressant molecules.

In clinical trials, agomelatine was found to clinically relief depressive symptoms and to be well tolerated [210,211]. It has been found effective in a range of mild to severely depressed patients with its efficacy in relieving symptoms increasing in more severely depressed subjects [210].

Agomelatine has fewer side effects than the other classes of antidepressive drugs, i.e., the selective serotonin reuptake inhibitors and the serotonin and norepinephrine reuptake inhib-

itors [210,212]. Additionally, it has been reported to relief some sleep disturbances and improved daytime alertness [213]. After discontinuing the use of agomelatine, no symptoms associated with ending the treatment have been observed [214]. For antidepressant efficacy, agomelatine is recommended at a dose of 25 mg once daily in the evening.

Concluding remarks

Functionally, melatonin is a remarkably diverse molecule that has been identified in species throughout the animal kingdom and, in the last decade, it has also been found in plants as well. It seems possible that melatonin exists in all life forms.

For decades it was assumed that melatonin's effects at the cellular level are mediated exclusively via membrane receptors on the target cells. These receptors were first definitively identified slightly over a decade ago. A feature of the membrane melatonin receptors which was probably a surprise to most scientists is the distribution of these receptors. Whereas they were initially found in and thought to be confined to a small number of nuclear groups in the brain (especially in the suprachrasmatic nuclei of the hypothalamus) they have subsequently been uncovered not only in many parts of the central nervous system but in numerous sites throughout multicellular organisms. This widespread distribution of melatonin membrane receptors portends numerous functions for melatonin, certainly more diverse actions than originally envisaged.

In addition to the typical membrane receptors that have been identified for melatonin, nuclear binding sites/receptors also have been unveiled [215] and functions have been attributed to them. These receptors belong to the RZR/ROR subfamily and include the products of three genes, i.e., splicing variants of RORα1, RORα2, RORα3, RZRα), which differ in the N-terminal domain, and RZRβ and RORγ [216]. How widespread the nuclear receptors are in the body remains to be established. Thusfar these receptors have been linked to melatonin's actions on the immune system [217-220] and possibly to the stimulation of antioxidative enzymes [130]. In the latter case, they may cooperate with membrane melatonin receptors in promoting the activities of the radical detoxifying enzymes. Finally, some of melatonin's actions involve its binding to intracellular calmodulin [221].

Melatonin's ability to vanquish free radicals and related reactants, an action that is independent of any receptor or binding site, is a rather recent discovery [118]. When this function was uncovered, a dynamic new field of investigation was launched, i.e., melatonin as an antioxidant. This area of study has flourished within the last decade and melatonin has been shown, in more than a thousand reports, to reduce oxidative damage due to the direct free radical scavenging capacity of not only melatonin itself [118,222,223], but also of its metabolites [120,122,126,224]. Free radicals and related reactants create a highly inhopsital environment with cells; to preserve the functional integrity of essential molecules and to protect them from the destruction of toxic reactants, cells utilized a large number of antioxidants including melatonin.

This review summarizes only a very few of the areas where melatonin's receptor-mediated and its receptor-independent actions have been described. The reader should be reminded that, although they are categorized according to the presumed modes of action of the indole, it also seems likely that melatonin may, in many circumstances use both processes to achieve its effects. For example, while melatonin may work via membrane and/or nuclear receptors, when it is in a cell it will also always function of a free radical scavenger. This only requires that melatonin be in the vicinity of the radical when it is generated.

Some of the most critical actions of melatonin may be those in the mitochondria were the indole has been shown to improve the transfer of electrons through the electron transport chain [137]. While doing so, melatonin limits electron leakage and the subsequent formation of free radicals; this has been referred to as melatonin's ability to avoid free radical generation [120], which supplements its ability to scavenge radicals that are formed. Also in the mitochondria, melatonin enhances ATP production [225,226]. The generation of energy in the form of ATP is critical to optimal cell function, including aiding in repairing any cellular damage that has occurred, and in improving survivability of the cell, of the tissue and of the organism.

Many important areas where melatonin may have clinical applicability are not discussed in this brief review. For example, melatonin's ability to prevent initiation [227-229] and progression [230-233] of many cancer cell types have been well documented. Also, in models of Alzheimer's [234-237] and Parkinson`s disease [238-241] the indole has proven beneficial effects. Likewise, free radical mediated ischemia/reperfusion injury has yielded to melatonin therapy; benefits of melatonin for this condition have been shown for every organ where it has been tested including in models of heart attack [242-244] and stroke [245-247]. Melatonin has been found to reduce the toxicity of a variety of prescription drugs [248-250] and the damaging effects of heavy metals [251-253]. Also, hyperglycemia associated with animal models of diabetes is highly destructive, usually via free radical processes, to the cardiovascular system as well as to other organs; this damage has been shown to be attenuated by melatonin treatment [254-256].

Clearly, the pharmaceutical industry has been attentive to the potential utility of melatonin in clinical medicine. In lieu of using melatonin, however, which is inexpensive and nonpatentable, they have developed patentable melatonin receptor agonists that are either currently on the market or soon will be. At this point the data are incomplete in terms of whether these analogues will be more or be less efficacious than melatonin in their specific use paradigm. Likewise, their long-term safety is unknown.

Overall, melatonin would appear to be a highly beneficial molecule with unexploited clinical potential. It could prove to be an important drug, especially in countries where financial resources are limited. Even in the well-developed countries, melatonin should be considered as a treatment for a number of conditions because of its multiple beneficial actions and uncommonly low toxicity.

Considering the increased life span of humans in many countries, a condition typically associated with increased prescription drug usage, the availability of an inexpensive agent such as melatonin could potentially save individuals and government agencies millions of dollars annually.

References

1. Tan DX, Manchester LC, Reiter RJ, Qi W, Karbownik M, Calvo JR. Significance of melatonin in antioxidative defense system: reactions and products. Biol Signals Receptors, 2000; 9: 137-59.
2. Tan DX, Manchester LC, Hardeland R, Lopez Burillo S, Mayo JC, Sainz RM, Reiter RJ. Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. J Pineal Res, 2003; 34: 75-8.
3. Reiter RJ, Hester RJ. Interrelationships of the pineal gland, the superior cervical ganglia and the photoperiod in the regulation of the endocrine systems of hamsters. Endocrinology, 1966; 79: 1168-70.
4. Mess B, Ruzsas C. Central nervous innervation of the pineal gland. Pineal Res Rev, 1987; 5: 191-216.
5. Reiter RJ, Trakulrungsi WK, Trakulrungsi C, Vriend et al. Pineal melatonin production: endocrine and age effects. In: Melatonin Rhythm Generating System, Klein DC, editor. Basel: Karger; 1982, p. 143-54.
6. Binkley SA. Pineal biochemistry: comparative aspects and circadian rhythms. In: The Pineal Gland, Vol. 1, Anatomy and Biochemistry, Reiter RJ, editor, CRC Press, Boca Raton, 1981, p. 155-72.
7. Hardeland R, Poeggeler B. Non-vertebrate melatonin. J Pineal Res, 2003; 34: 233-41.
8. Kolar J, Machackova I. Melatonin in higher plants: occurrence and possible functions. J Pineal Res, 2005; 39: 333-41.
9. Reiter RJ, Tan DX, Manchester LC, Simopoulos AP, Maldonado MD, Flores LJ, Terron MP. Melatonin in edible plants (phytomelatonin): identification, concentrations, bioavailability and proposed functions. World Rev Nutr Diet, 2007; 97: 211-30.
10. Pang SF, Allen AE. Extra-pineal melatonin in the retina: its regulation and physiological function. Pineal Res Rev, 1986; 4: 55-96.
11. Bubenik GA. Localization of melatonin in the gastrointestinal tract of the rat: effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Horm Res, 1980; 12: 313-23.
12. Tan DX, Manchester LC, Reiter RJ, Qui WB, Zhang M, Weintraub ST, Cabrera J, Sainz RM, Mayo JC. Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. Biochim Biophys Acta, 1999; 1472: 206-14.
13. Conti A, Conconi S, Hertens E, Skwarlo-Sonta K, Markowska M, Maestrani JM. Evidence for melatonin synthesis in mouse and human bone marrow. J Pineal Res, 2000; 28: 193-202.
14. Reiter RJ, Richardson BA, Matthews SA, Lane SJ, Ferguson BN. Rhythms in immunoreactive melatonin in the retina and Harderian glands in the Syrian hamster. Life Sci, 1983; 32: 1229-36.
15. Slominski A, Pisarchik A, Semak I, Sweatman T, Wortsman J, Szczesniewski A, Slugocki G, McNulty J, Kauser S, Tobin DJ, Jing C, Johansson O. Serotoninergic and melatonininergic systems are fully expressed in human skin. FASEB J, 2002; 16: 896-8.
16. Carrillo-Vico A, Calvo JR, Abreu P, Lardone PJ, Garcia-Mauriño S, Reiter RJ, Guerrero JM. Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine and/or paracrine substance. FASEB J, 2004; 18: 537-9.
17. Itoh MT, Takahashi N, Abe M, Shimizu K. Expression and cellular localization of melatonin-synthesizing enzymes in the rat lens. J Pineal Res, 2007; 42: 92-6.
18. Stefulj J, Hörtner M, Ghosh M, Schauenstein K, Rinner I, Wölfler A, Semmler J, Liebmann PM. Gene expression of the key enzymes of melatonin synthesis in extrapineal tissues of the rat. J Pineal Res 2001; 30: 243-7.
19. Dubocovich ML. Melatonin is a potent modulator of dopamine release in the retina. Nature, 1983; 306: 782-4.
20. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors step into the light: cloning and classification of subtypes. Trends Pharmacol Sci, 1996; 17: 100-2.
21. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. Endocrine, 2005; 27: 101-10.
22. Reppert SM, Weaver DR, Godson C. Melatonin receptors step into the light: cloning and classification of subtypes. Trends Pharmacol Sci, 1996; 17: 100-2.
23. Dubocovich ML, Masana MI, Iacob S, Sauri DM. Melatonin receptor antagonists that differentiate between the human Mel1a and Mel1b recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML1 presynaptic heteroreceptor. Naunyn Schmiedebergs Arch Pharmacol, 1997; 355: 365-75.
24. Slaugenhaupt SA, Roca AL, Liebert CB, Altherr MR, Gusella JF, Reppert SM. Mapping of the gene for the Mel1-melatonin receptor to humans chromosome 4 (MTNR1A) and mouse chromosome 8 (Mtnr1a). Genomics, 1995; 27: 355-7.
25. Gilman AG. Nobel lecture. G proteins and regulation of adenylyl cyclase. Biosci Rep, 1995; 15: 65-97.
26. Vanecek J. Cellular mechanisms of melatonin action. Physiol Rev, 1998; 78: 687-721.
27. Dubocovich ML, Rivera-Bermudez MA, Gerdin MJ, Masana MI. Molecular pharmacology, regulation and function of mammalian melatonin receptors. Front Biosci, 2003; 8: d1093-108.
28. New DC, Tsim ST, Wong YH. G-protein linked effector and second messenger systems involved in signal transduction. Neurosignals, 2003; 12: 59-70.
29. Reiter RJ, Fraschini F. Endocrine aspects of the mammalian pineal gland: a review: Nueroendocrinology, 1969; 2: 219-55.
30. Reiter RJ. Circannual reproductive rhythms in mammals related to photoperiod and pineal function: a review. Chronobiology, 1974, 1: 365-95.
31. Hoffman RA, Reiter RJ. Pineal gland: influence of gonads on male hamsters. Science, 1965; 148: 1609-11.
32. Hoffman RA, Reiter RJ. Influence of compensatory mechanisms and the pineal gland on dark-induced gonadal atrophy in male hamsters. Nature, 1965; 203: 658-9.
33. Gaston S, Menaker M. Photoperiodic control of hamster testis. Science, 1967; 167: 925-8.
34. Hoffman RA, Reiter RJ. Responses of some endocrine organs of female hamsters to pinealectomy and light. Life Sci, 1966; 5: 1147-51.
35. Reiter RJ, Hoffman RA, Hester RJ. The effects of thiourea, photoperiod and the pineal gland on the thyroid, adrenal and reproductive organs of female hamsters. J Exp Zool, 1966; 162: 263-8.
36. Reiter RJ. The effect of pinealectomy, pineal grafts and denervation of the pineal gland on the reproductive organs of male hamsters. Neuroendocrinology, 1967; 2: 138-46.
37. Reiter RJ. Pineal control of a seasonal reproductive rhythm in male golden hamsters exposed to natural daylight and temperature. Endocrinology, 1973; 92: 422-30.
38. Reiter RJ. Influence of pinealectomy on the breeding capability of hamsters maintained under natural photoperiodic and temperature conditions. Neuroendocrinology, 1973/1974; 13: 366-70.
39. Rollag MD, Panke ES, Reiter RJ. Pineal melatonin content in male hamsters throughout the seasonal reproductive cycle. Proc Soc Exp Biol Med, 1980; 165: 330-4.
40. Brainard GC, Petterborg LJ, Richardson BA, Reiter RJ. Pineal melatonin in Syrian hamsters: circadian and seasonal rhythms in animals maintained under laboratory and natural conditions. Neuroendocrinology, 1982; 35: 342-8.
41. Carter DS, Goldman BD. Antigonadal effects of timed melatonin infusion in pinealectomized male Djungarian hamsters (Phodopus sungorus sungorus): pulse duration is the critical parameter. Endocrinology, 1983; 13: 1261-7.
42. Stetson MH, Tay DE. Time course of sensitivity of golden hamsters to melatonin injections throughout the day. Biol Reprod, 1983; 29: 432-8.
43. Hoffmann K. The role of the pineal gland in the photoperiodic control of seasonal cycles in a hamster (Phodopus sungous). In: Clocks in Seasonal Reproductive Cycles, Follett BK, Follet DE, editors. Bristol: John Wright and Sons; 1981, p. 237-50.
44. Grocock CA, Clarke JR. Photoperiodic control of testis activity in the vole, Microtus agrestis. J Reprod Fertil, 1974; 43: 461-5.
45. Heath HW, Lynch GR. Interspecific differences for melatonin-induced reproductive regression and seasonal molt in Peromycus leucopus. Gen Comp Endocrinol, 1982; 48: 289-95.
46. Thorpe PA, Herbert J. The role of the pineal gland in the response of ferrets to artificially restricted photoperiods. J Endocrinol, 1974; 63: 56-73.
47. Turek FW. Antigonadal effect of melatonin in pinealectomized and intact male hamsters. Fed Proc, 1977; 155: 31-4.
48. Reiter RJ, Vaughan MK, Blask DE, Johnson LY. Melatonin: its inhibition of pineal antigonadotrophic activity in male hamsters. Science, 1974; 185: 1169-71.
49. Lincoln GA. Neuroendocrine regulation of seasonal gonadotropin and prolactin rhythms: lessons from the Sony ram model. Repord Suppl, 2002; 59: 131-47.

50. Malpaux B, Tricoire H, Mailliet F, Daveau A, Migaud M, Skinner DC, Pelletier J, Chemineau P. Melatonin and seasonal reproduction: understanding the neuroendocrine mechanisms using the sheep as a model. Reprod Suppl, 2002; 59: 167-79.

51. Schulte BA, Seal US, Plotka ED, Letellier MA, Verme LJ, Ozoga JJ, Parsons JA. The effect of pinealectomy on seasonal changes in prolactin secretion in the white-tailed deer (Odocoileus borealis). Endocrinology, 1981; 108: 173-8.

52. Voordouw BC, Euser P, Verdonk RE, Alberda BT, de Jong FH, Drogendijk AC, Fauser BC, Cohen M. Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. J Clin Endocrinol Metab, 1992; 74: 108-17.

53. Thiery JC, Chemineau P, Hernandez X, Migaud M, Malpaux B. Neuroendocrine interactions and seasonality. Domest Anim Endocrinol 2002; 23: 87-1000.

54. Lincoln GA. Melatonin entrainment of circannual rhythms. Chronobiol Int, 2006; 23: 301-6.

55. Lincoln GA. Decoding the nightly melatonin signal through circadian clockwork. Mol Cell Endocrinol, 2006; 252: 69-73.

56. Recabarren SE, Lobos A, Cendoyya E, Correa C, Rudolph I. Pituitary responsiveness to diurnal and nocturnal GnRH in melatonin-treated ewe lambs. Reprod Fertil Dev, 2000; 12: 45-50.

57. Wagner GC, Johnston JD, Thournier BB, Ebling EJ, Hazelerigg DG. Melatonin induces gene-specific effects on rhythmic mRNA expression in the pars tuberalis. Eur J Neurosci, 2007; 25: 485-90.

58. Barrett P, Schuster C, Mercer J, Morgan PJ. Sensitization: a mechanism for melatonin action in the pars tuberalis. J Neuroendocrinol, 2003; 15: 415-21.

59. Dijk DJ, Duffy JF, Riel E, Shanahan TL, Czeisler CA. Aging and the circadian and homeostatic regulation of human sleep during forced desynchrony rest, melatonin and temperature rhythms. J Physiology 1999; 516: 611-27.

60. Brown EN, Choe Y, Shanahan TL, Czeisler CA. A mathematical model of diurnal variations in human plasma melatonin levels. Am J Physiol, 1997; 272: E506-16.

61. Czeisler CA, Dijik DJ. Human circadian physiology and sleep-wake regulation. In: Handbook of Behavioral Neurobiology: Circadian Clocks, Takahashi JS, Turek FW, Moore RY, editors. New York: Klumer; 2001, p. 531-68.

62. Monk TH, Buysee DJ, Reynolds CF, Berga SL, Jarrett DB, Begley AE, Kupfer DJ. Circadian rhythms in human performance and mood under constant conditions. J Sleep Res, 1997; 6: 9-18.

63. Campbell SS. Intrinsic disruption of normal sleep and circadian patterns. In: Regulation of sleep and circadian rhythms, Turek RW, Zee PC, editors. New York: Marcel Dekker; 1999, p. 465-86.

64. Brzezinski A, Vangel MG, Wurtman RJ, Norrie G, Zhdanova I, Ben-Shushan A, Ford I. Effects of melatonin on sleep: a meta-analysis. Sleep Med Rev, 2005; 9: 41-50.

65. Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Vohra S, Klassen TP, Baker G. Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: metaanalysis. BMJ, 2006; 332: 385-93.

66. Zaidan R, Geoffriau M, Brun J, Taillard J, Bureau C, Chazot G, Claustrat B. Melatonin is able to influence its secretion in humans: description of a phase-response curve. Neuroendocrinology, 1994; 60: 105-12.

67. Lewy AJ, Bauer VK, Ahmed S, Thomas KH, Cutler NL, Singer CM, Moffit MT, Sack RL. The human phase response curve (PCR) to melatonin is about 12 hours out of phase with the PCR to light. Chronobiol Int, 1998; 15: 71-83.

68. Arendt J, Deacon S. Treatment of circadian rhythm disorders. Chronobiol Int, 1997; 14: 185-204.

69. American Academy of Sleep Medicine. The International Classification of Sleep Disorders: Diagnostic Manuel, 2nd ed. Westchester, Illinois: American Academy of Sleep Medicine; 1995.

70. Dahlitz M, Alvarez B, Vignau I, English J, Arendt J, Parkes JD. Delayed sleep phase syndrome response to melatonin. Lancet, 1991; 337: 1121-4.

71. Nagtegaal JE, Kerkhof GA, Smits MG, Swart AC, Vander Meer YG. Delayed sleep phase syndrome: a placebo-controlled cross over study on the effects of melatonin administered 5 hours before the individual dim light melatonin onset. J Sleep Res, 1998; 7: 135-43.

72. Mundey K, Benclоucif S, Harsanyi K, Duboeovich ML, Zee PC. Phase dependent treatment of delayed sleep phase syndrome with melatonin. Sleep, 2005, 28: 1271-8.

73. Revell VL, Burgess HJ, Gozda CJ, Smith MR, Fogg LF, Eastman CI. Advancing human circadian rhythms with afternoon melatonin and morning intermittent bright light. J Clin Endocrinol Metab, 2006; 91: 54-9.

74. Dodge NN, Wilson GA. Melatonin for treatment of sleep disorders in children with developmental disabilities. J Child Neurol, 2001; 16: 581-4.

75. Smits MG, van Stel HF, van der Heijden K, Meijer AM, Coenen AM, Kerkhof GA. Melatonin improved health status and sleep in children with idiopathic chronic sleep-onset insomnia: a randomized placebo-controlled study. J Am Acad Child Adolesc Psychiatry, 2003; 42: 1286-93.

76. Jan JE, Freeman RD. Melatonin therapy for circadian rhythm sleep disorders in children with multiple disabilities. Dev Med Child Neurol, 2004; 46: 772-6.

77. Jan JE, Freeman RD. Melatonin therapy for circadian rhythm sleep disorders in children with multiple disabilities. What have we learned in the last decade? Dev Med Child Neurol, 2004; 46: 776-82.

78. Zhdanova IV. Melatonin as a hypnotic. Proc Sleep Med Dev, 2005; 9: 51-65.

79. Ancoli-Israel S. Sleep and aging: prevalence of disturbed sleep and treatment considerations in older adults. J Clin Psychiatry, 2005; 9: 24-30.

80. Haimov I. Melatonin rhythm abnormalities and sleep disorders in the elderly. CNS Spectr, 2001; 6: 502-6.

81. Bellon A. Searching for new options for treating insomnia: are melatonin and ramelteon beneficial? J Psychiatr Pract, 2006; 12: 229-43.

82. Zisapel N. Circadian rhythm sleep disorders: pathophysiology and potential approaches to management. CNS Drugs, 2001; 15: 311-22.

83. Valtonen M, Nishanen, Kangas AP, Koskinen T. Effect of melatonin-rich night-time milk on sleep and activity in elderly institutionalized subjects. Nord J Psychiatry, 2005; 59: 217-21.

84. Sandyk R, Anastassiades PG, Anninos PA, Tsogas N. In postmenopausal osteoporosis related to pineal gland function? Int J Neurosci, 1992; 62: 215-25.

85. Wespes E, Schulman CC. Male andropause: myth, reality and treatment. Int J Impot Res, 2002; 14 (Suppl 1): S93-8.

86. Lunenfeld B. Endocrinology of the aging male. Minerva Ginecol, 2006; 58: 153-70.

87. Cardinali DP, Ladizesky MG, Boggie V, Cutera RA, Mautalen C. Melatonin effects on bone: experimental facts and clinical perspectives. J Pineal Res, 2003; 34: 81-7.

88. Machida M, Dubousset J, Imamura Y, Iwaya T, Yamada T, Kimura J. An experimental study in chickens for the pathogenesis of idiopathic scoliosis. Spine, 1993; 18: 1609-15.

89. Machida M, Dubousset J, Imamura Y, Iwaya T, Yamada T, Kimura J. Role of melatonin deficiency in the development of scoliosis in pinealectomized chickens. J Bone Joint Surg Br, 1995; 77: 134-8.

90. Kanemura T, Kawakami N, Deguchi M, Mimatsu K, Iwata H. Natural course of experimental scoliosis in pinealectomized chickens. Spine, 1997; 22: 1563-7.

91. Wang X, Moreau M, Raso J, Moreau M, Mahood J, Zhao J, Bagnall K. Changes in serum melatonin levels in response to pinealectomy in the chicken and its correlation with development of scoliosis. Spine, 1998; 23: 2377-81.

92. Turgut M, Kaplan S, Turgut AT, Aslan H, Güvenc T, Cullu E, Erdogan S. Morphological, stereological and radiological changes in pinealectomized chicken cervical vertebrae. J Pineal Res, 2005; 39: 392-9.

93. Machida M, Murai I, Miyashita Y, Dubousset J, Yamada T, Kimura J. Pathogenesis of idiopathic scoliosis. Experimental study in rats. Spine, 1999, 24: 1985-9.

94. Machida M, Dubousset J, Yamada T, Kimura J, Saito M, Shiraishi T, Yamagishi M. Experimental scoliosis in melatonin-deficient C57BL/6J mice without pinealectomy. J Pineal Res, 2006; 41: 1-7.

95. Ebihara S, Hudson DJ, Marks T, Menaker M. Pineal indole metabolism in the mouse. Brain Res, 1987; 416: 136-40.

96. Goto M, Oshima T, Tomita T, Ebihara S. Melatonin content of the pineal gland in different mouse strains. J Pineal Res, 1989; 7: 195-204.

97. Oyama J, Maurai I, Kanazawa K, Machida M. Bipedal ambulation induces experimental scoliosis in C57BL/6J mice with reduced plasma and pineal melatonin levels. J Pineal Res, 2006; 40: 219-24.

98. Cheung KM, Wang T, Poon AM, Carl A, Tranmer B, Hu Y, Luk KD, Leong JC. The effect of pinealectomy on scoliosis development in young nonhuman primates. Spine, 2005; 30: 2009-13.

99. Ladizesky MG, Cutrera RA, Boggio V, Somoza J, Centrella JM, Mautalen C, Cardinali DP. Effect of melatonin in bone metabolism in ovariectomized rats. Life Sci, 2001; 70: 557-65.

100. Ladizesky MG, Boggio V, Albornoz LE, Castrillon PO, Mautalin C, Cardinali DP. Melatonin increases oestradiol-induced bone formation in ovariectomized rats. J Pineal Res, 2003; 34: 143-51.

101. Ostrowska Z, Kos-Kudla B, Nowak M, Swietochowska E, Marek B, Gorski J, Kajdaniuk D, Wolkowska K. The relationship between bone metabolism, melatonin and other hormones in sham-operated and pinealectomized rats. Endocr Regul, 2003; 37: 211-24.

102. Ostrowska Z, Kos-Kudla B, Swietochowska E, Marek B, Kajdaniuk D, Nowak M. Influence of pinealectomy and long-term melatonin administration on bone metabolism in orchidectomized rats. Endokrynol Pol, 2006; 57: 7-14.

103. Ladizesky MG, Boggio V, Cutrera RA, Mondelo N, Mastaglia S, Somoza J, Cardinali DP. Melatonin effect on bone metabolism in rats treated with methylprednisolone. J Pineal Res, 2006; 40: 297-304.

104. Roth JA, Kim BG, Lin WL, Cho MI. Melatonin promotes osteoblast differentiation and bone formation. J Biol Chem, 1999; 274: 22041-7.

105. Nakade O, Koyama H, Ariji H, Yajima A, Kaku T. Melatonin stimulates proliferation of type 1 collagen synthesis in human bone cells in vitro. J Pineal Res, 1999; 27: 106-10.

106. Satomura K, Tobiume S, Tokuyama R, Yamasaki Y, Kudoh K, Maeda E, Nakayama M. Melatonin at pharmacological doses enhances human osteoblastic differentiation in vitro and promotes mouse cortical bone formation in vivo. J Pineal Res, 2007; 42: 231-9.

107. Sanchez-Hidalgo M, Lu Z, Tan DX, Maldonado MD, Reiter RJ, Gregerman RI. Melatonin inhibits fatty acid-induced triglyceride accumulation in ROS17/2.8 cells: implications for osteoblast differentiation and osteoporosis. Am J Physiol Regul Integr Comp Physiol, 2007; 292, R2208-15.

108. Gimble JM, Zvonic S, Floyd ZE, Kassen M, Nuttall ME. Playing with bone and fat. J Cell Biochem, 2006; 98: 251-66.

109. Reiter RJ, Richardson BA, Johnson LY, Ferguson BN, Rink DT. Pineal melatonin rhythm: reduction in aging Syrian hamsters. Science, 1980; 19: 1372-3.

110. Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM. Human melatonin production decreases with age. J Pineal Res, 1986; 3: 379-88.

111. Reiter RJ. The pineal gland and melatonin in relation to aging: a summary of the theories and of the data. Exp Gerontol, 1995; 30: 199-212.

112. Suzuki N, Hattori A. Melatonin suppresses osteoclastic and osteoblastic activities in the scales of goldfish. J Pineal Res, 2002; 33: 253-8.

113. Koyama A, Nakade O, Takada Y, Koku T, Lou KH. Melatonin at pharmacological doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediated osteoclast formation and activation. J Bone Miner Res, 2002; 17: 1219-29.

114. Suzuki A, Palmer G, Bonjour JP, Caverzasio J. Regulation of alkaline phosphatase activity by p38 MAP kinase in response to activation of Gi protein-coupled receptors by epinephrine in osteoblast-like cells. Endocrinology, 1999; 140: 3177-82.

115. Moreau A, Wang DS, Forget S, Azeddine B, Angeloni D, Fraschini F, Labelle H, Poitras B, Rivard CH, Grimard G. Melatonin signaling dysfunction in adolescent idiopathic scoliosis. Spine, 2004; 29: 1772-81.

116. Radio NM, Doctor JS, Witt-Enderley PA. Melatonin enhances alkaline phosphatase in differentiating human adult mesenchymal stem cells grown in osteogenic medium via MT2 melatonin receptors and the MEK/ERK (1/2) signaling cascade. J Pineal Res, 2006; 40: 332-42.

117. Witt-Enderby PA, Radio NM, Doctor JS, Davis VL. Therapeutic treatments potentially mediated by melatonin receptors: potential clinical uses in the prevention of osteoporosis cancer and as an adjuvant therapy. J Pineal Res, 2006; 41: 297-305.

118. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent endogenous hydroxyl radical scavenger. Endocrine J 1993; 1: 57-60.

119. Reiter RJ. Lowering the high price of free radicals. News Physiol Sci, 2000; 15: 246-50.

120. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine, 2005; 27: 119-30.

121. Tan DX, Manchester LC, Burkhardt S, Sainz RM, Mayo JC, Kohen R, Shonami E, Huo YS, Hardeland R, Reiter RJ. N1-acetyl-N2--formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. FASEB J, 2001; 15: 2294-6.

122. Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, Mayo JC, Kohen R, Allegra M, Hardeland R. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem, 2002; 2: 181-97.

123. Ressmeyer AR, Mayo JC, Zelosko V, Sainz RM, Tan DX, Poeggeler B, Antolin I, Zsizsik BK, Reiter RJ, Hardeland R. Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. Redox Rep, 2003; 8: 205-13.

124. Guenther AL, Schmidt SI, Laatsch H, Fotsos S, Ness H, Ressmeyer AR, Poeggeler B, Hardeland R. Reactions of the melatonin metabolite (N1-acetyl-5-methoxykynuramine) with reactive nitrogen species: formation of novel compounds, 3-acetoamidomethyl-6-methoxycinnolinone and 3-nitro-AMK. J Pineal Res, 2005; 39: 251-60.

125. Onuki J, Almeida EA, Medeiros MHG, Mascio PD. Inhibition of 5-aminolevulenic acid-induced DNA damage by melatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, quercetin and resveratrol. J Pineal Res, 2005; 38: 107-15.

126. Rosen J, Than NN, Koch D, Poeggeler B, Laatsch H, Hardeland R. Interactions of melatonin and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. J Pineal Res, 2006; 41: 374-81.

127. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. J Biomed Res, 2000; 7: 444-58.

128. Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Rodriquez C. Melatonin regulation of antioxidant enzyme gene expression. Cell Mol Life Sci, 2002; 59: 1706-23.

129. Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res, 2004; 36: 1-9.

130. Tomas-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulatory effect of melatonin an antioxidative enzymes. J Pineal Res, 2005; 39: 99-104.

131. León J, Escames G, Rodriguez MI, Lopez LC, Tapias V, Entrena A, Camacho E, Carrión MD, Gallo MA, Espinosa A, Tan DX, Reiter RJ, Acuña-Castroviejo D. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. J Neurochem, 2006; 98: 2023-33.

132. Urata Y, Honma S, Goto S, Todoroki S, Iida T, Cho S, Honma K, Kondo T. Melatonin induces gamma-glutamylcysteine synthase mediated by activator protein-1 in human vascular endothelial cells. Free Radic Biol Med, 1999; 27: 838-47.

133. Winiarska K, Fraczyk T, Malinska D, Drozak J, Bryla J. Melatonin attenuates diabetes induced oxidative stress in rabbits. J Pineal Res, 2006; 40: 168-76.

134. Gitto E, Tan DX, Reiter RJ, Karbowsnik M, Manchester LC, Cuzzocrea S, Fulia F, Barberi I. Individual and synergistic actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferoxamine in liver homogenates. J Pharm Pharmacol, 2001; 53: 1393-401.

135. Lopez-Burillo S, Tan DX, Mayo JC, Sainz RM, Manchester LC, Reiter RJ. Melatonin, xanthurenic acid, resveratrol, EGCG, vitamin C and alpha-lipoic acid differentially reduce oxidative DNA damage induced by Fenton reagents: a study of their individual and synergistic actions. J Pineal Res, 2003; 34: 269-77.

136. Acuña-Castroviejo D, Escames G, Carazo A, León J, Khaldy H, Reiter RJ. Melatonin, mitochondrial homeostasis and mitochondrial related diseases. Curr Top Med Chem, 2002; 2: 133-52.

137. León J, Acuña-Castroviejo D, Escames E, Tan DX, Reiter RJ. Melatonin mitigates mitochondrial malfunction. J Pineal Res, 2005; 38: 1-9.

138. Jou MJ, Peng TI, Reiter RJ, Jou SB, Wu HY, Wen ST. Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. J Pineal Res, 2004; 37: 55-70.

139. Martensson J, Meister A. Glutathione deficiency decreases tissue ascorbate levels in newborn rats: ascorbate spares glutathione and protects. Proc Nat Acad Sci USA, 1991; 88: 4656-60.

140. Calvin HI, Hagen SV, Hess JL, Patel SA, Fu SCJ. Lens depletion and electrolyte changes preceding cataracts induced by buthionine sulfoximine in suckling mice. Exp Eye Res, 1992; 54: 621-6.

141. Laver NM, Robison WG Jr, Calvin HI, Fu SCJ. Early epithelial lesions in cataracts of GSH-depleted mouse pups. Exp Eye Res, 1993; 57: 493-8.

142. Martensson J, Steinharz R, Jain A, Meister A. Glutathione ester prevents buthionine sulfoximine-induced cataracts and lens epithelial cell damage. Proc Nat Acad Sci USA, 1989; 86: 8727-31.

143. Reddy VN. Glutathione and its function in the lens – an overview. Exp Eye Res, 1990; 50: 771-8.

144. Abe M, Reiter RJ, Orhii PB, Hara M, Poeggeler B. Inhibitory effect of melatonin on cataract formation in newborn rats: evidence for an antioxidative role for melatonin. J Pineal Res, 1994; 17: 94-100.

145. Hardeland R, Reiter RJ, Poeggeler B, Tan DX. The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. Neurosci Biobehav Rev, 1993; 17: 347-57.

146. Calvin HI, Medvedowsky C, Worgul BV. Near total depletion and age-specific cataracts induced by buthionine sulfoximine in mice. Science, 1986; 233: 553-5.

147. Yu HS, Yee RW, Howes KA, Reiter RJ. Diurnal rhythms in immunoreative melatonin in aqueous humor and serum of male pigmented rabbits. Neurosci Lett, 1990; 116: 309-14.

148. Li ZR, Reiter RJ, Fujimori O, Oh CS, Duan YP. Cataractogenesis and lipid peroxidation in newborn rats treated with buthionine sulfoximine: preventive actions of melatonin. J Pineal Res, 1997; 22: 117-23.

149. Bardak K, Ozertürk Y, Ozgüner F, Durmus M, Delibas N. Effect of melatonin against oxidative stress in ultraviolet-B exposed rat lens. Curr Eye Res, 2000; 20: 225-30.

150. Abe M, Itoh MT, Mijata M, Ishikawa S, Sumi Y. Detection of melatonin, its precursors and related enzyme activities in rabbit lens. Exp Eye Res, 1999; 68: 255-62.

151. Abe M, Itoh MT, Miyata M, Shimizu K, Sumi Y. Circadian rhythm in serotonin N-acetyltransferase activity in rat lens. Exp Eye Res, 2000; 70: 805-8.

152. Martin XD, Molina HZ, Brennan MC, Hendrickson PH, Lichter PR. The ciliary body – the third organ found to synthesize indoleamines in humans. Eur J Ophthalmol, 1992; 2: 67-72.

153. Wiechmann AF, Udin SB, Summers Rada JA. Localization of mel1b melatonin receptor-like immunoreactivity in ocular tissues of Xenopus laevis. Exp Eye Res, 2004; 79: 585-94.

154. Siu AW, Maldonado MD, Sanchez-Hidalgo M, Tan DX, Reiter RJ. Protective effects of melatonin in experimental free radical-related ocular diseases. J Pineal Res, 2006; 40: 101-9.

155. Feldmeier JJ. Hyperbaric oxygen: indications and results: the hyperbaric oxygen therapy committee report. In: The Hyperbaric Oxygen Therapy Committee Report, Feldmeier JJ, editor. Kensington, MD: Undersea and Hyperbaric Medical Society; 2003.

156. Jain KK. Physical, physiological and biochemical aspects of hyperbaric oxygenation. In: Textbook of Hyperbaric Medicine Part 1, Jain KK, editor. Cambridge: Hogrefe and Huber; 1996, p. 2-11.

157. Freeman B, Crapo JD. Hyperoxia increases oxygen radical production in rat lungs and mitochondria. J Biol Chem, 1981; 256: 10986-92.

158. Narkowicz CH, Vial JH, McCartney P. Hyperbaric oxygen therapy increases free radical levels in blood of humans. Free Radic Res Commun, 1993; 19: 71-80.

159. Pablos MI, Reiter RJ, Chuang JI, Ortiz GG, Guerrero JM, Sewerynek E, Agapito MT, Melchiorri D, Lawrence R, Deneke SM. Acutely administered melatonin reduces oxidative damage in lung and brain induced by hyperbaric oxygen. J Appl Physiol, 1997; 83: 354-8.

160. Topal T, Oter S, Korkmaz A, Sadir S, Metingurt G, Korkmazhan ET, Serdar MA, Bilgic H, Reiter RJ. Exogenously administered and endogenously produced melatonin reduce hyperbaric oxygen-induced oxidative stress in rat lung. Life Sci, 2004; 75: 461-7.

161. Dundar K, Topal T, Ay H, Oter S, Korkmaz A. Protective effects of exogenously administered or endogenously produced melatonin on hyperbaric oxygen-induced oxidative stress in rat brain. Clin Exp Pharmacol Physiol, 2005; 32: 926-30.

162. Mollaoglu H, Topal T, Ozler M, Vysal B, Reiter RJ, Korkmaz A, Oter S. Antioxidant effects of melatonin in rats during chronic exposure to hyperbaric oxygen. J Pineal Res, 2007; 42: 50-4.

163. Poeggeler BH, Barlow-Walden LR, Reiter RJ, Saarela S, Menendez-Pelaez A, Yaga K, Manchester LC, Chen LD, Tan DX. Red light-induced suppression of melatonin synthesis is mediated by N-methyl-D-aspartate receptor activation in retinally normal and retinally degenerate rats. J Neurobiol, 1995; 28: 1-8.

164. Asayama K, Dobashi K, Hayashibe H, Megata Y, Kato K. Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. Endocrinology, 1987; 121: 2112-9.

165. Venditti P, Balestrieri M, Di Meo S, De Leo T. Effect of thyroid state on lipid peroxidation, antioxidant defenses, and susceptibility to oxidative stress in rat tissues. J Endocrinol, 1997; 155: 151-7.

166. Chien KR, Zhu H, Knowlton KV, Miller-Hance W, van-Bilsen M, O'Brien TX, Evans SM. Transcriptional regulation during cardiac growth and development. Annu Rev Physiol, 1993; 55: 77-95.

167. Ghosh G, De Sangeeta Marty K, Bandyopadhyay D, Bhattacharya S, Reiter RJ, Bandyopadhyay A. Melatonin protects against oxidative damage and restores expression of GLUT4 gene in the hyperthyroid rat. J Pineal Res, 2007; 42: 71-82.

168. Baker CC, Huynh T. Sepsis in the critically ill patient. Curr Prob Surg, 1995; 32: 1013-92.

169. Sands KE, Bates DW, Lanken PN, Graman PS, Hibberd PL, Kahn KL, Parsonnet J, Panzer R, Orav EJ, Snydman DR, Black E, Schwartz JS, Moore R, Johnson BL Jr, Platt R, Academic Medical Center Consortium Sepsis Project Working Group. Epidemiology of sepsis syndrome in 8 academic medical centers. J Am Med Assoc, 1997; 278: 234-40.

170. Sriskandan S, Cohen J. The pathogenesis of septic shock. J Infect, 1995; 30: 201-6.

171. Peters K, Unger RE, Brunner J, Kirkpatrick J. Molecular basis of endothelial dysfunction in sepsis. Cardiovasc Res, 2003; 60: 49-57.

172. Angus DC, Wax RS. Epidemiology of sepsis: an update. Crit Care Med, 2001; 29: S109-16.

173. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in United States 1979 through 2000. N Engl J Med, 2003; 348: 1546-54.

174. Iskit AB, Guc O. A new therapeutic approach for the treatment of sepsis. Med Hypothesis, 2004; 62: 342-5.

175. Escames G, López LC, Tapias V, Utrilla P, Reiter JR, Hitos AB, León J, Rodriquez MI, Acuña-Castroviejo D. Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. J Pineal Res, 2006; 40: 71-8.

176. Sewerynek E, Abe M, Reiter RJ, Barlow-Walden LR, Chen L, McCabe TJ, Roman LJ, Diaz-Loez B. Melatonin administration prevents lipopolysacharide-induced oxidative damage in phenobarbital-treated animals. J Cell Biochem, 1995; 58: 436-44.

177. Sewerynek E, Melchiorri D, Chen LD, Reiter RJ. Melatonin reduces both basal and bacterial lipopolysaccharide-induced lipid peroxidation in vitro. Free Radic Biol Med, 1995; 19: 903-9.

178. Crespo E, Macias M, Pozo D, Escames G, Martin M, Vives F, Guerrero JM, Acuña-Castroviejo D. Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction in rats. FASEB J, 1999; 13: 1537-46.

179. Reynolds FD, Dauchy R, Blask DE, Dietz PA, Lynch D, Zuckerman R. The pineal hormone melatonin improves survival in a rat model of sepsis/shock induced by zymosan A. Surgery, 2003; 134: 474-9.

180. Wu CC, Chiao CW, Hsiaa G, Chen A, Yen MH. Melatonin prevents endotoxin-induced circulatory failure in rats. J Pineal Res, 2001; 30: 147-56.

181. Cuzzocrea S, Costantino G, Caputi A. Protective effect of melatonin on cellular energy depletion mediated by peroxynitrite and poly (ADP) ribose synthetase activation in a non-septic shock model induced by zymosan in the rat. J Pineal Res, 1998; 25: 78-85.

182. El-Sokkary GH, Reiter RJ, Cuzzocrea S, Caputi AP, Hossanein AF, Tan DX. Role of melatonin in reduction of lipid peroxidation and peroxynitrite formation in non-septic shock induced by zymosan. Shock, 1999; 12: 402-8.

183. Sener G, Toklu H, Kapucu C, Ercan F, Erkanli G, Kacmaz

A, Tilki M, Yegen BC. Melatonin protects against organ injury in rat model of sepsis. Surg Today, 2005; 35: 52-9.

184. Zavodnik IB, Lapshina EA, Zavodnik LB, Labieniec M, Bryszewska M, Reiter RJ. Hypochlorous acid-induced oxidative stress in Chinese hamster B14 cells: viability, DNA and protein damage and the protective action of melatonin. Mutat Res, 2004; 559: 39-48.

185. Escames G, León J, Macias M, Khaldy H, Acuña-Castroviejo D. Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. FASEB J, 2003; 17: 932-4.

186. Escames G, Acuña-Castroviejo D, Lopez LC, Tan DX, Maldonado MD, Sanchez-Hidalgo M, León J, Reiter RJ. Pharmacological utility of melatonin in the treatment of septic shock: experimental and clinical evidence. J Pharm Pharmacol, 2006; 58: 1153-65.

187. Escames G, Lopez LC, Ortiz F, Lopez A, Garcia JA, Ros E, Acuña-Castroviejo D. Attentuation of cardiac mitochondrial dysfunction by melatonin in septic mice. FEBS J, 2007; 274: 2135-47.

188. Wakatsuki A, Okatani Y, Shinohara K, Ikenoue N, Kaneda C, Fukaya T. Melatonin protects fetal rat brain against mitochondrial oxidative damage. J Pineal Res, 2001; 30: 22-8.

189. Perez EM, Weisman LE. Novel approaches to the prevention and therapy of neonatal bacterial sepsis. Clin Perinatol, 1997; 24: 213-25.

190. Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, Cardaro S, Corona G, Trimarchi G, Barberi I. Effects of melatonin treatment in septic newborns. Pediatr Res, 2001; 50: 756-60.

191. Buonocore G, Groenendaal F. Antioxidant strategies. Semin Fetal Neonatal Med 2007; 12: 287-95.

192. Ohkawa S, Uchikawa O, Fakatsu K, Mujamoto. Patent # US 603A239, 2000.

193. Andrieux J, Houssin R, Yous S, Gardiola B, Lesieur D, Patent # US 5318944 1994.

194. Brzezinski A. Melatonin in humans. N Engl J Med, 1997; 336: 186-95.

195. Zhdanova IV, Wurtman RJ. Efficacy of melatonin as a sleep-promoting agent. J Biol Rhythms, 1998; 12: 644-50.

196. Zisapel N. The use of melatonin for treatment of insomnia. Biol Signals Recept, 1999; 8: 84-9.

197. Hirai K, Koto K, Nishiyama, Ota H, Nishikawa H, Fujiwara H, Miyamoto IM. TAK-375 and its metabolites are selective agonists at ML-1 receptors. Sleep, 2003; 26: A79.

198. Zlotos DP. Recent advances in melatonin receptor ligands. Arch Pharm Chem Life Sci, 2005; 338: 229-47.

199. Kato K, Hirai K, Nishiyama K, Uchikawa O, Fukatsu K, Ohkawa S, Kawamata Y, Hinuma S, Miyamoto M. Neurochemical properties of ramelteon (TAK-375), a selective MT1/MT2 receptor agonist. Neuropharmacology, 2005; 48: 301-10.

200. Cajochen C. TAK-375. Curr Opin Invest Drugs, 2005; 6: 114-21.

201. Stubbs CM, Karim A. A safety, tolerance and pharmacokinetic study of five single doses of TAK-375 in healthy adults. Sleep, 2003; 26: 76-7.

202. Karim A, Tolbert D, Cao C. Disposition kinetics and tolerance of escalating single doses of ramelteon, a high affinity MT1 and MT2 melatonin receptor agonist indicated for treatment in insomnia. J Clin Pharmacol, 2006; 46: 140-8.

203. Hirai K, Kita M, Ohta H, Nishikawa H, Fujiwara Y, Ohkawa S, Miyamoto IM. Ramelteon (TAK-375) accelerates reentrainment of circadian rhythms after a phase advance of the light-dark cycle in rats. J Biol Rhythms, 2005; 20: 27-37.

204. Barja NL, Daniel KL. Ramelteon for the treatment of insomnia. Clin Ther, 2006; 28: 1540-55.

205. Roth T, Seeden D, Sainati S, Wang-Weigand S, Zhang J, Zee P. Effects of ramelteon on patient-reported sleep latency in older adults with chronic insomnia. Sleep Med, 2006; 7: 312-8.

206. Wurtman RJ. Ramelteon: a novel treatment for insomnia. Expert Rev Neurother, 2006; 6: 957-64.

207. Yous S, Andrieux J, Howell HE, Morgan PJ, Renard P, Pfeiffer B, Lesieur D, Guardiola-Lamaitre B. Novel naphthalene ligands with high affinity for the melatonin receptor. J Med Chem, 1992; 35: 1484-6.

208. Dubocovich ML. Agomelatine targets a range of major depressive disorder symptoms. Curr Opin Invest Drugs, 2006; 7: 670-80.

209. Guardiola-Lemaitre BB. Agonistes et antagonists de recepteurs melatoninergiques: effects pharmacologiques et perspectives therapeutiques. Ann Pharm France, 2005; 63: 385-400.

210. Montogomery SA. Major depressive disorders: clinical efficacy and tolerability of agomelatine, a new melatonergic agonist. Neuropsychopharmacology, 2006; 16: 5633-8.

211. Pjrek E, Winkler D, Konstantinidies D, Willeit M, Praschak-Rieder N, Kasper S. Agomelatine in the treatment of seasonal affective disorder. Psychopharmacology, 2007; 190: 575-9.

212. Ebmeier KP, Donaghey C, Steele JD. Recent developments and current controversies in depression. Lancet, 2006; 367: 153-67.

213. Kupfer DJ. Depression and associated sleep disturbances: patient benefits with agomelatine. Eur Neuropychopharmacol, 2006; 16: 5639-43.

214. Montgomery SA, Kennedy SH, Burrows GD, Lejoyeux M, Hindmarch I. Absence of discontinuation symptoms with agomelatine and occurrence of discontinuation symptoms with paroxetine: a randomized, double-blind, placebo-controlled discontinuation study. Int Clin Psychopharmacol, 2004; 19: 271-80.

215. Acuña-Castroviejo D, Reiter RJ, Menendez-Pelaez A, Pablos MI, Burgos A. Characterization of high-affinity binding sites inpurified nuclei of rat liver. J Pineal Res, 1994; 16: 100-12.

216. Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin in the immune system. Endocrine, 2005; 27: 189-200.

217. Poon AM, Pang SF. 2[125I] iodomelatonin binding sites in spleen of guinea pups. Life Sci, 1992; 50: 1719-26.

218. Garcia-Maurino S, Pozo D, Calvo JR, Guerrero JM. Correlation between nuclear melatonin receptor expression and enhanced cytokine production in human lymphocytes and monocytic cell lines. J Pineal Res, 2000; 29: 129-37.

219. Carrillo-Vico A, Garcia-Pergañeda A, Naji L, Calvo JR, Romero MP, Guerrero JM. Expression of membrane and nuclear melatonin receptor mRNA and protein in mouse immune system. Cell Mol Life Sci, 2003; 60: 2272-8.

220. Pozo D, Garcia-Mauriño S, Guerrero JM, Calvo J. mRNA expression of nuclear receptor RZR/RORα, melatonin membrane receptor MT1, and hydroxindole-O-methyltransferase in different populations of human immune cells. J Pineal Res, 2004; 37: 48-54.

221. Benitez-King G. Melatonin as a cytoskeletal modulator: implications for cell physiology and disease. J Pineal Res, 2006; 40: 1-9.

222. Reiter RJ, Tan DX, Manchester LC, Qi W. Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence. Cell Biochem Biophys, 2001; 34: 237-56.

223. Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin's interaction with reactive species. J Pineal Res, 2003; 34: 1-10.

224. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species. J Pineal Res, 2007; 42: 28-42.

225. López LC, Escames G, Ortiz F, Ros E, Acuña-Castroviejo D. Melatonin restores the mitochondrial production of ATP in septic mice. Neuroendocrinol Lett, 2006; 27: 623-30.

226. Rodriguez MI, Carretero M, Escames G, Lopez LC, Maldonado MD, Tan DX, Reiter RJ, Acuña-Castroviejo D. Chronic melatonin treatment prevents age-dependent cardiac mitochondrial dysfunction in senescene-accelerated mice. Free Radic Res, 2007; 41: 15-24.

227. Vijayalaxmi, Meltz ML, Reiter RJ, Herman TS, Kumar KS. Melatonin and protection from genetic damage in blood and bone marrow: whole-body radiation studies in mice. J Pineal Res, 1999; 27: 221-5.

228. Qi, W, Reiter RJ, Tan DX, Manchester LC, Siu AW, Garcia JJ. Increased levels of oxidatively damaged DNA induced by chromium (III) and H_2O_2: protection by melatonin and related molecules. J Pineal Res, 2000; 54-61.

229. Majeterek I, Gloc E, Blasiak J, Reiter RJ. A comparison of the action of amifostine and melatonin on DNA-damaging effects and apoptosis induced by idarubicin in normal and cancer cells. J Pineal Res, 2005; 38: 254-63.

230. Blask DE, Dauchy RT, Sauer LA, Krause JA, Brainard GC. Light during darkness, melatonin suppression and cancer progression. Neuroendocrinol Lett, 2002; Suppl 2: 52-6.

231. Blask DE, Brainard GC, Dauchy RT, Hanifin JP, Davidson LK,

Krause JA, Sauer LA, Riviera-Bermudez MA, Dubocovich ML, Jasser SA, Lynch DT, Rollaq MD, Zalatan F. Melatonin-depleted blood from premenopausal women exposed to light at night stimulates growth of human breast cancer xenografts in nude rats. Cancer Res, 2005; 65: 11174-84.

232. Martinez-Campa C, Alonso-González C, Mediavilla MD, Cos S, González A, Ramos S, Sánchez-Barceló EJ. Melatonin inhibits both ER alpha activation and breast cancer cell proliferation induced by the metalloestrogen, cadmium. J Pineal Res, 2006; 40: 291-6.

233. Yang QH, Xu JN, Xu RK, Pang SF. Antiproliferative effects of melatonin on the growth of rat pituitary prolactin-secreting tumor cells in vitro. J Pineal Res, 2007; 42: 172-9.

234. Pappolla MA, Chyan YJ, Poeggeler B, Frangione B, Wilson G, Ghiso J, Reiter RJ. An assessment of the antioxidant and the antiamyloidogenic properties of melatonin: implications for Alzheimer's disease. J Neural Transm, 2000; 107: 203-31.

235. Matsubara E, Bryant-Thomas T, Pacheco-Quinto J, Henry TL, Poeggeler B, Herbert D, Cruz-Sanchez F, Chyan YJ, Smith MA, Perry G, Shoi M, Abe K, Leone A, Grundke-Ikbal I, Wilson GL, Ghiso J, Williams C, Refolo LM, Pappolla MA, Chain DG, Neria E. Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease. J Neurochem, 2003; 85: 1101-8.

236 Wu YH, Swaab D. The human pineal gland and melatonin in aging and Alzheimer's disease. J Pineal Res, 2005; 145-52.

237. Cheng Y, Feng Z, Zhang QZ, Zhang JT. Beneficial effects of melatonin in experimental models of Alzheimer's disease. Acta Pharmacol Sin, 2006; 27: 129-39.

238. Antolin I, Mayo JC, Sainz RM, del Brio Mde L, Herrera F, Martin V, Rodriquez C. Protective effect of melatonin in a chronic model of Parkinson's disease. Brain Res, 2002; 943: 163-73.

239. Mayo JC, Sainz RM, Tan DX, Antolin I, Rodriguez C, Reiter RJ. Melatonin and Parkinson's disease. Endocrine, 2005; 27: 169-178.

240. Chen LJ, Cao YQ, Li XJ, Shen DH, Sun FY. Melatonin protects against MPTP/MPP+- induced mitochondrial DNA damage in vivo and in vitro. J Pineal Res, 2005; 39: 34-42.

241. Alvira D, Tajes M, Verdoguer E, Acuña-Castroviejo D, Folch J, Camins A, Pallas M. Inhibition of the cdk5/p25 fragment formation may explain the antiapoptotic effects of melatonin in an experimental model of Parkinson's disease. J Pineal Res, 2006; 40: 251-8.

242. Dobsak P, Siegelova J, Eicher JC, Jancik J, Svacinova H, Vascu J, Kuchtickova S, Horky M, Wolf JE. Melatonin protects against ischemia-reperfusion injury and inhibits apoptosis in isolated working rat heart. Pathophysiology, 2003; 9: 179-87.

243. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic-reperfused heart. Cardiovasc Res, 2003; 58: 10-9.

244. Petrosillo G, Di Venosa N, Pistolese M, Casanova G, Tiravanti E, Colantuono G, Federici A, Paradies G, Ruggiero FM. Protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia-reperfusion: role of cardiolipin. FASEB J, 2006; 20: 269-76.

245. Reiter RJ, Tan DX, León J, Kilic U, Kilic E. When melatonin gets on your nerves: its beneficial actions in experimental models of stroke. Exp Biol Med, 2005; 230: 104-17.

246. Zou LY, Chueng RT, Liu S, Li G, Huang L. Melatonin reduces infarction volume in a photothrombotic stroke model in the wild-type but not cycoxygenase-1-gene knowkout mice. J Pineal Res, 2006; 41: 150-6.

247. Lee MY, Kuan YH, Chen HY, Chen TY, Chen ST, Huang CC, Yang IP, Hsu YS, Wu TS, Lee EY. Intravenous administration of melatonin reduces the intracerebral cellular inflammatory response following transient focal cerebral ischemia in rats. J Pineal Res, 2007; 42: 297-309.

248. Reiter RJ, Tan DX, Sainz RM, Mayo JC, Lopez-Burrillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol, 2002; 54: 1299-321.

249. Kim C, Kim N, Joo H, Youm JB, Parkk WS, Cuong DV, Park YS, Kim E, Min CK, Han J. Modulation by melatonin of the cardiotoxic and antitumor activities of adriamycin. J. Cardiovasc Pharmacol, 2005; 46: 200-10.

250. Yildirim Z, Kotuk M, Erdogan H, Iraz M, Yagmurca M, Kuku I, Fadillioglu E. Preventive effect of melatonin on bleomycin-induced lung fibrosis in rats. J Pineal Res, 2006; 40: 27-33.

251. El-Sokkary GH, Kamel ES, Reiter RJ. Prophylactic effect of melatonin in reducing lead-induced neurotoxicity in the rat. Cell Mol Biol Lett, 2003; 8: 460-70.

252. Sener G, Sehirli AD, Ayanoglu-Dulger G. Melatonin protects against mercury (II)-induced oxidative tissue damage in rats. Pharmacol Toxicol, 2003; 93: 290-6.

253. Esparza JL, Gomez M, Rosa Nogues M, Patemain JL, Mallol J, Domingo JL. Melatonin reduces oxidative stress and increases gene expression in the cerebral cortex and cerebellum of aluminum-exposed rats. J Pineal Res, 2005; 39: 129-36.

254. Nishida S. Metabolic effects of melatonin on oxidative stress and diabetes mellitus. Endocrine, 2005; 27: 131-6.

255. Kadhim HM, Ismail SH, Hussein KI, Bakir IH, Sahib AS, Khalaf BH, Hussain SA, et al. Effects of melatonin and zinc on lipid profile and renal function in type 2 diabetic patients poorly controlled with metformin. J Pineal Res, 2006; 41: 189-93.

256. Derlacz RA, Sliwinski M, Piekutowska, Winiarska K, Drozak J, Bryla J. Melatonin is more effective than taurine and 5-hydroxytryptophan against hyperglycemia-induced kidney cortex tubules injury. J Pineal Res, 2007; 42: 203-4.