Introduction

Melatonin is a somewhat mysterious substance. In animals, melatonin is secreted from the pineal gland during the night. It acts as a hormone, functioning as a circadian mediator for time information over the course of each day, and is also able to eliminate free radicals (reactive oxygen species). Melatonin also exists in higher plants (edible plants), and is inadvertently obtained from daily meals. This substance was isolated by chance from the pineal gland, an endocrine organ, and is therefore named a hormone.

Regarding the effect of melatonin in inducing synchronization of circadian rhythms, which is generally regarded as a sleep-promoting effect, melatonin administration lowers deep body temperatures not only in those with rhythm disorders but also in healthy individuals, from children to elderly people; shortens the time required to fall asleep; and improves sleep. In addition, melatonin functions as an antioxidative substance (Review: Reiter, 2000) and acts on bone metabolism. Melatonin thus has a variety of activities. In a rat model, melatonin inhibited age-related visceral adiposity. These findings may indicate that in addition to meals and physical exercise, sleep is deeply involved in metabolic syndrome, which has become of increasing concern in recent years.

In the Current Concept Session “Melatonin” at the 9th Scientific Meeting of the Japanese Society of Anti-Aging Medicine in 2009, three speakers presented basic data and their interpretations and applications in clinical settings from the viewpoints of brain functions, sleep medicine, and reproductive functions, namely “Melatonin and Brain Functions” by Kazuyoshi Tsutsui, “Sleep Medicine and Melatonin” by Masako Okawa, and “Melatonin and Reproductive Functions” by Bunpei Ishizuka. This Session was co-held with the Society of Anti-Aging Endocrinology. In the future, we intend to introduce new basic and clinical research studies on melatonin and propose a lifestyle that will not decrease but rather lead to an increase in melatonin.

Synthesis and Distribution of Melatonin

Melatonin is an amine of molecular weight 232 that is synthesized from tryptophan, an essential amino acid, via serotonin. It has been regarded as a specific hormone of the pineal gland, but is actually produced in the retina, brain (cerebral cortex, raphe nuclei, striate body, etc.), gastrointestinal tract (stomach, small intestine, etc.), testes, ovaries, spinal cord, lymphocytes, lens, cochlea, and skin. Melatonin is widely distributed not only in both vertebrate and invertebrate animals but also in plants such as rice, barley, and wheat. Figure 1 illustrates the pathway of melatonin synthesis in the human pineal gland. Light information received by the retina passes primarily through the retino-hypothalamic pathway and is transmitted to the suprachiasmatic nucleus (SCN), where a circadian clock (for most organisms and plants, a body that oscillates over a 24±4-hour cycle; for human beings, about a 25-hour cycle) exists, thus enabling synchronization of the phases of the circadian clock with the light/dark cycle (over a 24-hour cycle) of the outside world. The time information at the SCN passes through a new nerve to reach the superior cervical ganglion and is then transmitted to the pineal gland. This pathway is actually activated during the night without light stimuli, as the nervous activities of the superior cervical ganglion are inhibited by light stimulation. Noradrenaline is secreted by nerve terminals derived from the superior cervical ganglion and stimulates the pineal cells, primarily via ß-receptors, thereby accelerating the synthesis of cAMP, the second messenger, to activate aryalkylamine N-acetyltransferase activity (AANAT), a rate-limiting enzyme of melatonin synthesis. During the daytime, AANAT is only weakly activated. Melatonin levels in the pineal gland and blood show a circadian variation, being high during the nighttime and low during the daytime. In human beings, melatonin secretion is highest at the age of 1 to 3 years, starts to decrease from puberty onwards, and reduces to 1/10 of the peak value at age 70 years or older. The circadian rhythm of melatonin secretion is noted not only in blood but also in almost every type of bodily fluid, including

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cerebrospinal fluid, saliva, aqueous humor of the anterior chamber, follicular fluid, and breast milk. Melatonin receptors are distributed over a variety of tissues and organs, and accordingly, the time information based on melatonin concentration is transmitted to tissues throughout the entire body. The presence of melatonin receptors has so far been confirmed in the brain (including the SCN), spinal cord, pituitary gland, retina, spleen, thymus, adrenal gland, liver, kidney, heart, lungs, testes, ovaries, blood vessels, lymphocytes, and osteoblasts\(^{11,12}\). It is therefore assumed that in addition to the melatonin-related synchronization of the circadian clock at the SCN, synchronization with sleep phase may occur at the entire body level, bringing better rest to the body.

### Antioxidative Effect

Melatonin has an antioxidative effect\(^5\). The first mechanism of this effect is to function as a free-radical scavenging antioxidant that removes hydroxy radicals (HO\(^•\))\(^{13}\), peroxyl radicals\(^{14}\), and in addition extremely highly toxic peroxynitrite\(^{15}\). Melatonin also inhibits lipid peroxidation and blocks the production of isoprostanes\(^{16}\). The second mechanism of action is to activate endogenous enzymes that scavenge free-radicals. Administration of melatonin to pregnant rats increased the activities of superoxide dismutase (SOD) or glutathione peroxidase in fetal brain tissues\(^{17}\). Melatonin has its own antioxidative effect and also intensifies the activity of endogenous antioxidative enzymes, which together exert a powerful antioxidative effect. Considering that melatonin is secreted during the nighttime and passes through the blood-brain barrier, it may play a preventive role against oxidation disorders of cerebral nerve cells during nocturnal sleep. A clinical study performed by the Anti-Aging Medical Research Center, Doshisha University, demonstrated that when the quality of sleep was upgraded by the use of comfortable bedding, oxidative stress disorders as evaluated by an indicator of urinary 8-hydroxydeoxyguanosine (8-OHdG) were improved\(^{18}\). This may be explained by the exertion of an antioxidative effect resulting from increased melatonin secretion.

### Melatonin and Neurosteroids

Recently, the Laboratory of Integrative Brain Sciences, Faculty of Education and Integrated Arts and Sciences, Waseda University, has found that the central nerve system synthesizes a variety of neurosteroids from cholesterol\(^{19}\). Many neurosteroids act on ionotropic receptors present in the cell membranes to change the inflow of ions and thus regulate the transmission of information at synapses (a non-genomic effect). Some neurosteroids accelerate brain cell development and synapse formation via intranuclear receptors (a genomic effect)\(^{19-21}\). In the brain, the Purkinje cell, located in the cerebellar cortex, is the representative cell class of synthesizing neurosteroids\(^{19-21}\). Purkinje cells constitute the memory storage neurons responsible for the learning of locomotor activity. The process of learning and memory in the cerebellum primarily rely on the change of synaptic neurotransmission in neuronal circuits. Neurosteroids control the establishment of neuronal circuits involved in the process of learning and memory and the neurotransmission at the synapses\(^{19-21}\). More recently, the Laboratory of Integrative Brain Sciences identified 7α-hydroxyprogrenolone, a newly-discovered neurosteroid, in the brain of newts\(^{22}\) and quail\(^{23}\). The presence of this new neurosteroid increased the amount of spontaneous locomotor activity in these types of animals.
The circadian change in spontaneous locomotor activity was analyzed in relation to 7α-hydroxypregnenolone synthesis and melatonin secretion. Quail is active during the daytime; the amount of spontaneous locomotor activity is circadian, being high during the light period and low during the dark period. Melatonin is secreted during the dark period and inhibits the synthesis of 7α-hydroxypregnenolone: the amount of spontaneous locomotor activity in quail decreases during the dark period. In newts, a nocturnal animal, melatonin secreted during the dark period accelerates the synthesis of 7α-hydroxypregnenolone, which increases the amount of spontaneous locomotor activity during the dark period. It is thus evident that the regulation of 7α-hydroxypregnenolone synthesis by melatonin plays a central role in the circadian rhythms of animal activity.

Relation to Glucose and Lipid Metabolism

Regarding the effects of melatonin on glucose metabolism, some conflicting results have been reported, reflecting differences in the dosage, treatment period, and experimental animals used in these studies. Melatonin exhibits crosstalk with insulin in intracellular signal transmission. Phosphorylation of IRS-1 under insulin and melatonin stimulation triggers chains of reactions promoting glucose transport, glycogen synthesis, and inhibition of lipolysis, controlling body weight and glucose metabolism. Membrane-bound melatonin receptors MT1/MT2 may reduce intracellular cAMP via Gi-proteins, and control the activity of cAMP sensitive phosphotyrosine phosphatase, accelerating phosphorylation of insulin receptors.

Administration of melatonin to an experimental rat model of diabetes improved blood levels of neutral fat, free fatty acid, and total cholesterol, and in addition reduced TNF-α by 50%. On the other hand, pinealectomy increased insulin resistance and induced hyperinsulinemia, accelerating disease progression in type 2 diabetic rats. These animal data indicate that melatonin improves insulin resistance.

Long-term administration of melatonin to humans and experimental animals reduces blood and liver cholesterol and LDL-cholesterol levels. These effects are remarkably noted in experimental animal models of hypercholesterolemia, middle-aged and older animals, and experimental animal models of diabetes, but rarely noted in young healthy rats. Treatment of peri- and postmenopausal women with melatonin increased HDL-cholesterol. Overall, melatonin may favorably affect lipid metabolism.

Relation to Bone Metabolism

Since pinealectomy in chicks immediately after hatching caused vertebral column deformities, the possibility that melatonin is involved in bone formation has long been considered. This phenomenon was confirmed by a subsequent study, which demonstrated that three to five days after hatching, specific melatonin receptors were expressed in the vertebrae of chicks.
More recently, newly discovered functions of melatonin involvement in regulation of bone metabolism have been reported. A joint study performed by the Department of Biology, Tokyo Medical and Dental University, and the Marine Laboratory, Kanazawa University, used fish scales as an experimental bone model and found that melatonin inhibits the activity of osteoclasts. Subsequently, the study group reported that melatonin derivatives enhanced bone mineral density and bone strength in ovariectomized rats (an experimental model of post-menopausal osteoporosis) and in rats fed with low-calcium feed. In addition, administration of melatonin to growing mice increased bone mineral density and bone mass, to which a reduction of RANKL-mediated osteoclast formation was reported to contribute. In ovariectomized rats with fewer osteoclasts and more osteoclasts, melatonin treatment returned the numbers of both osteoclasts and osteoclasts to the control levels. The presence of melatonin receptors was proven in osteoclasts derived from human jaw bones and ilia, and melatonin accelerated the proliferative differentiation of osteoclasts and increased collagen production.

On the basis of comprehensive evaluation of the above findings, it is expected that melatonin may prevent osteoporosis and accelerate fracture healing (bone regeneration).

**Melatonin and Reproductive Functions**

Melatonin affects reproductive function, including that of the ovaries. Although it has long been generally accepted that melatonin inhibits reproductive function in animals, a recent report noted that melatonin actually promotes these functions. Melatonin receptors are present in the granulose and theca cells, both of which occur in mature follicles, and in lutein cells, and these cells promote the production of sex steroid hormones. When oocytes were cultured in a medium containing melatonin and then subjected to in vitro fertilization, fertility and segmentation rate were increased. In oocytes, melatonin functions as a calmodulin antagonist. These findings indicate that melatonin directly acts on ovarian function.

It has also been shown that melatonin is synthesized in human ovaries and rat oocytes. Together with the previously reported findings, the Department of Obstetrics and Gynecology, St. Marianna University School of Medicine proposed the following hypotheses: (1) serotonin synthesized by the granulose cells is used to synthesize melatonin in an ovum; (2) melatonin functions as a calmodulin antagonist in an oocyte and is thus involved in the calmodulin-dependent regulation system; (3) in the oocyte, melatonin scavenges free-radicals and acts as an antioxidative substance; (4) and melatonin synthesized by the oocyte is released from the cell and then promotes production of sex steroid hormones by the granulosa cells. In other words, it is hypothesized that melatonin is directly involved in the growth and maturity of oocytes as well as in the inhibition of factors which might impair the quality of oocytes.

It is considered that melatonin also has favorable effects on both the mother and her fetus during pregnancy. Melatonin readily passes through the placenta and easily penetrates the fetal brain tissue. Oxidative stress due to free radicals is substantially involved in the development of brain damage in the fetal and neonatal periods. Experimental studies have demonstrated that melatonin administration via the dam inhibits oxidative brain damage due to ischemia or reperfusion in offspring. A possible application of melatonin in clinical settings may be the prevention of brain damage during the fetal and neonatal periods.

**Relation to Cancer**

It is generally accepted that breast and endometrial carcinomas occur less frequently in the blind than in the nonhandicapped. This may be because the absence of light stimulation on the retina keeps blood melatonin at high levels. In addition, experimental studies have demonstrated the anti-tumor effects of melatonin in different types of tissues. Melatonin inhibited the formation of aoxymethane-induced aberrant crypt foci in rat colon. Although treatment of LNCaP cells derived from the prostate with dihydrotestosterone increased PSA production, melatonin inhibited this increase and induced apoptosis of LNCaP cells. Melatonin receptor 1a might be a tumor suppressor gene and it has been demonstrated that melatonin inhibits oral squamous-cell carcinoma via this receptor. In an estrogen-positive uterine body cancer cell line, melatonin significantly intensified the anti-tumor effect of paclitaxel, which is mediated by the melatonin receptor 1a and involves expression of the estrogen receptor. In breast cancer cells, melatonin inhibited the expression of estrogen-responsive cancer-related genes. It is expected that combined use of melatonin in a variety of anticancer therapies may bring additional efficacy.

**Sleep Medicine and Melatonin**

The quality of sleep is lower in the elderly than in the young, and sleep disorders therefore occur more frequently in the elderly. A Japanese survey on sleep has revealed that 18.1%, 18.9%, and 29.5% of those aged 20-39, 40-59, and over 60 years, respectively, complain of some types of sleep disorders. Among sleep disorders, those related to the biological clock are called circadian rhythm sleep disorders. The elderly suffer from the advanced sleep-phase type, in which the major sleep episode is advanced in relation to the desired clock time and patients fall asleep very early in the evening and wake up very early in the morning. They also suffer from the irregular sleep-wake type, in which patients wake frequently during the night and take naps during the day. The former type is regarded as the advancement of phases of biological rhythms, and the latter as the reduced amplitude of biological rhythms or the collapsed rhythms. These changes in biological rhythms are noted in the sleep/wake cycle and also in the autonomic nervous system such as in body temperature, as well as in the hormone secretion rhythms such as melatonin secretion. Aging reduces the amplitude of body temperature rhythms and melatonin secretion, as is indicated by the study result that distinction between day and night becomes less clear with aging.

The decrease in deep body temperature which occurs upon falling asleep is considered to be attributable to melatonin. Oral administration of melatonin increases heat radiation due to dilatation of peripheral blood vessels of the skin and decreases deep body temperatures during the nighttime, both of which cooperatively contribute to sleep. In the elderly, who have decreased melatonin secretion, deep body temperatures do not favorably decrease upon falling asleep.

The decreased melatonin secretion during the nighttime is caused by reduced exposure to light during the daytime through daily living activities. When elderly individuals complaining of sleep disorders were exposed to bright artificial light during the daytime, the quantity of melatonin secreted during the nighttime...
significantly increased and sleep was improved\textsuperscript{57}. Furthermore, a 4-year follow-up study demonstrated that in the group of subjects in whom melatonin secretion decreased, sleep was improved by melatonin treatment. Among patients with circadian rhythm sleep disorders, 30 with delayed sleep phase syndrome (DSPS) and 16 with non-24-hour sleep-wake syndrome (non-24h) received melatonin treatment. A total of 17 patients (12 with DSPS and 5 with non-24h) responded to melatonin, showing normalization of the time required to fall asleep and wake; and the duration of sleep became adequate \textsuperscript{58}. Those who responded to melatonin had a shorter duration of sleep and had developed the clinical symptoms at an older age than those who did not respond to the treatment.

In light of the above studies, melatonin may affect sleep homeostasis or the clock system related to biological rhythms, as it acts on the autonomous nerve system and endocrine system to affect the sleep regulating system. Its application to medical use has been expanded.

### Alzheimer’s Disease and Melatonin

Patients with dementia such as Alzheimer’s disease develop circadian rhythm disorders, and in addition nocturnal delirium, in which they do not sleep but rather experience excitement, hallucination, or delirium during the nighttime; and sundowning syndrome, in which they show poriomania or excitement in late afternoon, evening, and/or night. The sleep of such patients is characterized by continued disturbance of sleep (increased frequency of arousal during sleep) as well as decreased amounts of deep sleep and REM sleep\textsuperscript{59,60}. Consequently, the proportion of daytime sleep in a single day increases and that of deep sleep and REM sleep in the daytime sleep becomes greater. In addition, together with disturbed sleep/wake rhythms, the circadian body temperature rhythms become flattened and their phases are delayed\textsuperscript{61,62}. With regard to the circadian rhythm of melatonin secretion in blood, the phases tend to advance and their amplitudes to reduce\textsuperscript{63,64}.

It is expected that bright light therapy or melatonin therapy be effective in improving sleep disturbances resulting from Alzheimer’s disease\textsuperscript{65,66}. Some researchers have reported that music therapy increases melatonin secretion\textsuperscript{57}. Bright light therapy stimulates melatonin secretion in patients with Alzheimer’s disease\textsuperscript{68}. Clinical investigations of melatonin therapy in elderly patients have demonstrated a relaxation effect\textsuperscript{69} and the improvement of depressed mood and memory\textsuperscript{70}. On the other hand, in patients with sleep disturbance resulting from dementia, a variety of changes are noted in circadian rhythms due to aging and nerve degeneration, and accordingly, melatonin replenishment in some patients does not favorably achieve synchronization of circadian rhythms\textsuperscript{71,72}.

In experimental animal models, age-related reductions in melatonin secretion may induce hyperphosphorylation of nerve fiber proteins and thus contribute to the development of nerve fiber degeneration. One study reported that melatonin administration exerted an antioxidative effect, and in addition inhibited β-amyloid accumulation and β-amyloid fiber formation\textsuperscript{73}. The nerve-protecting actions of melatonin such as its antioxidative activities and anti-amyloid effects indicate the possibility that melatonin therapy may be administered as a preventive measure for Alzheimer’s disease and dementia in the future.

### Conclusion

Age-related changes in hormone secretion include somatopause, which is the decline in secretion of the growth hormone/IGF-I system; adrenopause, which is the decline in DHEA (−s) level; and menopause/andropause, which is the decline in secretion of sex hormones\textsuperscript{74}. Since melatonin secretion by the pineal gland substantially decreases with aging, we would like to propose the term “pinealpause” to characterize this condition. In the elderly, endocrine diseases are characterized by the fact that reduced secretion of a single hormone alone among the above does not cause symptoms, but rather that declining levels of multiple hormones induce composite symptoms. Deepening our understanding of interactions among hormones from the viewpoint of Anti-Aging Medicine is important to the realization of hormone replacement therapies. Hormone replacement therapy has two main aspects: to replenish a hormone that is diminishing, and to promote health. How much a given hormone will be replenished should not be determined according to a one-size-fits-all criterion but needs to be adequately quantified on the basis of an individual’s morphometry, intensity of activity, desired lifestyle patterns, and blood hormone levels. For melatonin, however, no methods have been established to make an accurate measurement of its secretion. The key to understanding how to administer these hormones safely and effectively while minimizing adverse events and achieving maximum benefit first depends on the accumulation of data from many medical institutions over a long period of time.
Effects of Melatonin

References


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