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[REVIEW]

Receptor-Mediated and Receptor-Independent Actions of Melatonin in Vertebrates

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Melatonin (*N*-acetyl-5-methoxytryptamine) is an indolamine that is synthesized from tryptophan in the pineal glands of vertebrates through four enzymatic reactions. Melatonin is a quite unique bioactive substance, characterized by a combination of both receptor-mediated and receptor-independent actions, which promote the diverse effects of melatonin. One of the main functions of melatonin, via its membrane receptors, is to regulate the circadian or seasonal rhythm. In mammals, light information, which controls melatonin synthesis, is received in the eye, and transmitted to the pineal gland, via the suprachiasmatic nucleus, where the central clock is located. Alternatively, in many vertebrates other than mammals, the pineal gland cells, which are involved in melatonin synthesis and secretion and in the circadian clock, directly receive light. Recently, it has been reported that melatonin possesses several metabolic functions, which involve bone and glucose, in addition to regulating the circadian rhythm. Melatonin improves bone strength by inhibiting osteoclast activity. It is also known to maintain brain activity during sleep by increasing glucose uptake at night, in an insulin-independent manner. Moreover, as a non-receptor-mediated action, melatonin has antioxidant properties. Melatonin has been proven to be a potent free radical scavenger and a broad-spectrum antioxidant, even protecting organisms against radiation from space. Melatonin is a ubiquitously distributed molecule and is found in bacteria, unicellular organisms, fungi, and plants. It is hypothesized that melatonin initially functioned as an antioxidant, then, in vertebrates, it combined this role with the ability to regulate rhythm and metabolism, via its receptors.

Key words: melatonin receptor, circadian and seasonal rhythms, bone metabolism, glucose metabolism, therapeutic drugs, free radical scavenger, evolutionary implications

INTRODUCTION

Melatonin was discovered as a substance that lightened skin color when extracts from bovine pineal glands were injected into tadpoles (McCord and Allen, 1917). The chemical structure of melatonin was determined in the bovine pineal gland in 1958, and it was shown to lighten the melanophores of the northern leopard frog, *Rana pipiens* (Lerner et al., 1958). Thereafter, the rates of melatonin urinary excretion and possibly secretion into the blood were found to be higher at night than during the day (Lynch et al., 1975). For instance, melatonin collected in urine samples from healthy adult volunteers between 11 p.m. and 7 a.m. was higher than that when collected between 7 a.m. and 3 p.m. or between 3 p.m. and 11 p.m. (Lynch et al., 1975). Since the release of that report, research into melatonin and circadian rhythms has gained popularity. One of the main functions of melatonin is to regulate circadian rhythms, which is performed through melatonin membrane receptors. In this review, we first introduce the regulation of circadian (Cassone, 1998; Schomerus and Korf, 2005; Bilu and Kronfeld-Schor, 2013) or seasonal (Reiter et al., 1980; Bartness et al., 1993; Malpaux et al., 1999; Foá and Bertolucci, 2003; viviD and Bentley, 2018) rhythms by melatonin. In addition, we describe the remarkable functions of melatonin in bones (Suzuki and Hattori, 2002; Ikegame et al., 2019; Hirayama et al., 2023) and in glucose metabolism (Karamitri and Jockers, 2019; Watanabe et al., 2023). Moreover, melatonin is a ubiquitously distributed molecule and is found in bacteria (Manchester et al., 1995), unicellular organisms (Pöggeler et al., 1991), fungi (Hattori et al., 1995), and plants (Hattori et al., 1995; Kolar and Machackova, 2005), where it has been proven to be a potent free radical scavenger and broad-spectrum antioxidant (Tan et al., 2002; Reiter et al., 2014; Slominski et al., 2018). Melatonin performs antioxidant functions in all organisms through a receptor-independent mechanism. We hypothesized that

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the original function of melatonin was the detoxification of free radicals, resulting in numerous applications in physiology and medicine (Reiter et al., 2014). This review also addresses the evolution of melatonin functions from the perspective of organismal evolution.

MELATONIN RECEPTORS

Melatonin receptors are G protein-coupled receptors that bind melatonin (Li et al., 2013). Three types of melatonin membrane receptors have been identified: Mel1a (or MT1, ML1a, ML1, MTNR1a) and Mel1b (or MT2, ML1b, MTNR1b) receptor subtypes, which are present in humans and other mammals, while an additional melatonin receptor subtype, Mel1c (MTNR1C), has been identified in fishes, amphibians, and birds (Emet et al., 2016). Mel1a and Mel1b as GPCR bonded with Gαs to decrease cAMP, while Mel1c coupled with Gαq to activate the membrane-associated phospholipase C and protein kinase C pathways (Ning et al., 2019).

Melatonin receptors are located in several central nervous and peripheral tissues in vertebrates. Mammalian melatonin receptors (Mel1a and Mel1b) have been detected in the suprachiasmatic nuclei (SCN) of the hypothalamus, the pars tuberalis (PT) of the pituitary gland (Fig. 1), and additional brain areas, such as the paraventricular nucleus, periventricular nucleus, supraoptic nucleus, and hippocampus, as well as in peripheral organs, such as retina, bone, pancreas, liver, kidneys, adrenal gland, intestine, stomach, heart, lungs, skin, testis, and ovary (Hardeland et al., 2011; Li et al., 2013; Kumar et al., 2015; Hirayama et al., 2023). The Mel1a subtype is present within the SCN of the hypothalamus, the PT of the pituitary gland, and retinas, while the Mel1b subtype is mainly expressed in the retinas (Sugden et al., 2004). Mel1c is also expressed in various areas of the brain in many non-mammalian vertebrates, such as in the SCN and PT (Kameda et al., 2002; Sugden et al., 2004; Turkowska et al., 2014). Since SCN is known to be a circadian pacemaker (Hardeland et al., 2011; Li et al., 2013), the main function of melatonin is to regulate the circadian rhythm (Fig. 1). The sensitivity of the SCN to melatonin occurs primarily during the transition from day to night (dusk) and night to day (dawn). During these times, melatonin modulates circadian rhythms by advancing the phase of neurally active rhythms produced within the SCN. The activation of Mel1a inhibits the neuronal firing rate in the SCN, while activation of Mel1b phase shifts the circadian rhythms generated within the SCN (Dubocovich et al., 2003).

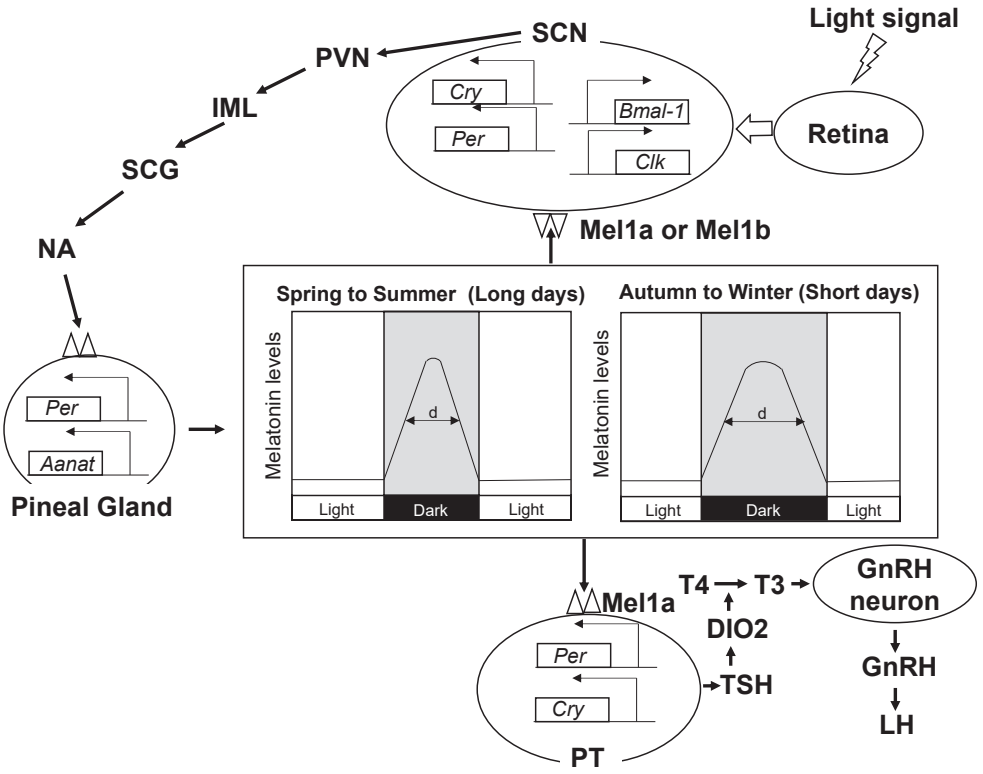

Fig. 1. Summary of seasonal and circadian regulatory mechanisms by melatonin. A light signal is received in the retina and transmitted to the pineal gland via the suprachiasmatic nucleus (SCN), which is mainly the circadian clock, the paraventricular nucleus (PVN), and intermediolateral nucleus of the spinal cord (IML) connecting the superior cervical ganglion (SCG), in order. The duration of time that blood melatonin levels are high differs between long and short days. Then, melatonin functions in the pars tuberalis (PT) and regulates reproduction via gonadotropins, such as luteinizing hormone (LH). Melatonin regulation of TSH in PT is limited to mammals. Abbreviations: *Aanat*: *arylalkylamine N-acetyltransferase*; *Bmal-1*: *Brain and muscle Arnt-like protein-1*; *Clk*: *Clock*; *Cry*: *Cryptochrome*; d: duration of secreted melatonin; DIO2: deiodinase 2; GnRH: gonadotropin-releasing hormone; IML: intermediolateral nucleus of the spinal cord; LH: luteinizing hormone; Mel1a: melatonin receptor subtype 1; Mel1b: melatonin receptor subtype 2; NA: noradrenaline (norepinephrine); *Per*: *Period*; PT: pars tuberalis; TSH: thyroid-stimulating hormone; T3: triiodothyronine; T4: thyroxine.

MELATONIN-REGULATED CIRCADIAN RHYTHM IN VERTEBRATES

In mammals, light information, which controls melatonin synthesis, is received in the retina and transmitted to the pineal gland via the SCN, where central clock genes such as *Period*, *Cryptochrome*, *Brain and muscle Arnt-like protein-1*, and *Clock* are located (Hardeland et al., 2006; Zisapel, 2018; Brzezinski et al., 2021) (Fig. 1). Signals from the SCN are transmitted to the pineal gland via a complex neural chain involving the paraventricular nucleus of the hypothalamus and the intermediate lateral nucleus of the spinal cord (Korf, 1994). Conversely, in many vertebrates, other than mammals, the pineal gland cells (pinealocytes), which are involved in melatonin synthesis and secretion and the circadian clock, directly receive light (Shedpure and Pati, 1995; Ekström and Meissl, 2003). Melatonin is synthesized from tryptophan through four enzymatic reactions in the pineal glands of vertebrates (Fig. 2). At night, noradrenaline regulates the activity of arylalkylamine *N*-acetyltransferase (AANAT; the rate-limiting enzyme for melatonin biosynthesis) through β-adrenergic receptors and enhances cAMP levels in the pineal gland, which stimulates mRNA expression of AANAT in vertebrates (Brzezinski et al., 2021; Ubuka, 2021). This signal pathway promotes melatonin synthesis (Fig. 2). The noradrenergic signal for melatonin synthesis is inhibited by light exposure (Schomerus and Korf, 2005), meaning that these light signals regulate the rhythms of day/night melatonin synthesis.

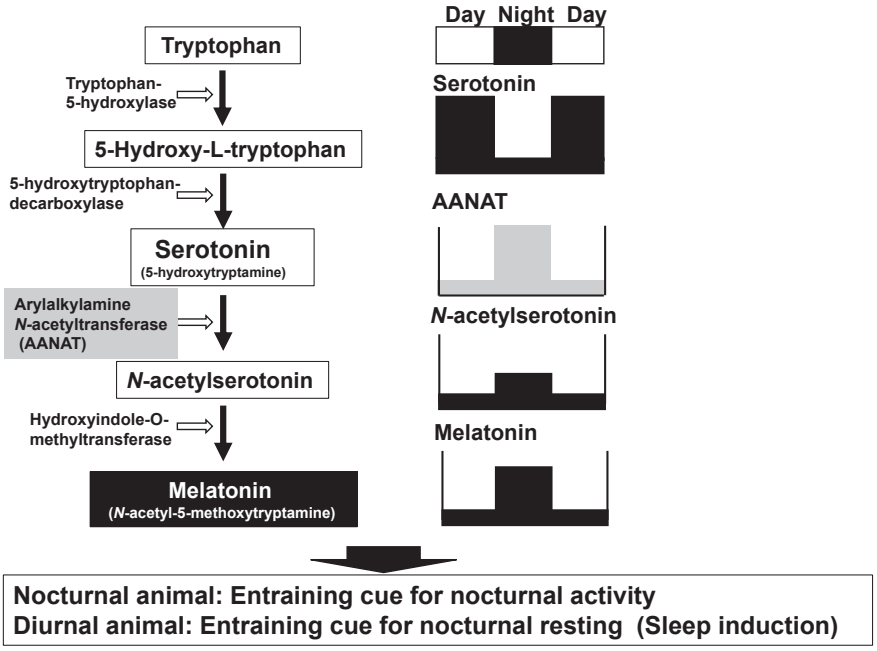

Fig. 2. Metabolic pathway from tryptophan to melatonin. Melatonin is synthesized at night by the increased expression of arylalkylamine *N*-acetyltransferase (AANAT) in the pineal gland. Both diurnal and nocturnal animals synthesize melatonin only at night. However, the effects of melatonin on these animals are different. Both diurnal and nocturnal animals could use melatonin as an entraining cue.

Circadian rhythm in diurnal and nocturnal animals, especially mammals

Elevated blood melatonin functions differently in diurnal and nocturnal animals, although in both of them, melatonin secretion in the pineal glands occurs only at night (Schomerus and Korf, 2005; Bilu and Kronfeld-Schor, 2013). Nocturnal melatonin secretion induces the resting phase in diurnal animals, including human (Lewy et al., 1992; McArthur et al., 1996). Melatonin exerts its action on a timing mechanism controlling the switch from behavioral activity to quiescence in diurnal animals. On the other hand, in nocturnal animals, melatonin injections can influence the daily activity cycle of rats, and daily injections are followed by the onset of activity in constant darkness (Redman et al., 1983). When melatonin injection was stopped in rats, activity rhythms started free-running again (Redman et al., 1983). The circadian timing system controlling the rhythm of wheel-running activity in the C3H/HeN mouse was responsive to both light and melatonin (Benloucif and Dubocovich, 1996). Wild mice (nocturnal) produce melatonin, but they lost the ability to produce melatonin when they were laboratory animalized (Kasahara et al., 2010). Only recently have they lost the ability to produce melatonin, but they have maintained their genetically nocturnal nature. Thus, in nocturnal animals, melatonin acts as an entraining agent, as does light. The mechanisms by which melatonin regulates behavior in diurnal and nocturnal animals remain unclear.

Circadian rhythm in nonmammalian vertebrates

Melatonin secreted into the blood performs significant roles in the circadian organizations of many nonmammalian vertebrates, compared to mammals (Cassone, 1998). The removal of the pineal gland (pinealectomy) in the house sparrow, *Passer domesticus*, altered the free-running locomotor period and abolished their circadian rhythms (Chabot and Menaker, 1992). Similarly, in male European seabass, *Dicentrarchus labrax*, locomotor activity revealed a change from light-synchronization to free-running rhythmic patterns following pinealectomy (Cowan et al., 2017). Therefore, the melatonin secreted from the pineal gland likely influences behavior and physiology.

The significance of retinal melatonin synthesis is well known (Wiechmann, 1986; Zawilska and Nowak, 1992; Wiechmann and Sherry, 2013). In the retinas of nonmammalian species, such as the goldfish, *Carassius auratus*, frog *Xenopus laevis*, lizard *Anolis carolinensis*, and chicken, *Gallus gallus domesticus*, melatonin and the capacity for its synthesis and metabolism have been detected (Cahill and Besharse, 1989; Nowak et al., 1990; Grace et al., 1991). Diurnal variations in melatonin levels and AANAT activity in the retina have also been

observed in *Xenopus laevis* and *Gallus gallus domesticus* (Hamm and Menaker, 1980; Besharse and Iuvone, 1983). Melatonin secreted from the retina may regulate the function of the retina, although there is no evidence that retinal melatonin regulates their behavior.

MELATONIN-REGULATED SEASONAL RHYTHM IN VERTEBRATES

Seasonal changes in daylight duration (day length) play an important role in creating reproductive rhythms in many seasonally breeding animals, whereby changes in day length trigger reproductive statuses in photoperiodic species (Fig. 1). Small mammals, such as hamsters, and birds, including quails, are considered long-day breeders because they breed from spring to summer, during the longer daylight seasons (Brandstätter et al., 2003). Conversely, sheep are short-day breeders because they breed from fall to winter, during the short daylight seasons (Malpaux et al., 1999).

Photoperiodic information is translated into a duration of melatonin secretion: high at night and low during the day. The length of the nocturnal melatonin secretion reflects the secretion of the gonadotropin-releasing hormone (GnRH) from the hypothalamus (Bartness et al., 1993; Malpaux et al., 1999). GnRH release induces the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which are responsible for the alternating presence or absence of ovulation in the female and varying sperm production in the male (Bartness et al., 1993; Malpaux et al., 1999). Thus, melatonin plays a major role in a series of seasonal reproductive strategies (Fig. 1).

Seasonal rhythm regulation of melatonin in mammals

Pinealectomy in the male hamster causes an inability to sense changes in day length and the loss of photoperiodic responses, resulting in atrophy of the gonads (Hoffman and Reiter, 1965). Therefore, melatonin has an important function in regulating seasonal reproductive physiology in mammals. Long-day breeding animals (e.g., hamsters) are sexually depressed from autumn to winter and active from spring to summer, while short-day breeders, such as sheep and deer are actually sexually most active and capable during the shortest days of the year (autumn to winter). The elevated melatonin levels regulate sexual maturation in these animals (Fig. 1). Long-day shortens the duration of melatonin secretion in both long-day and short-day breeders. In addition, the changing duration of nocturnal melatonin secretion determines reproductive capability in seasonal breeders through the hypothalamic-pituitary-gonadal axis (Reiter et al., 2009) (Fig. 1). In mammals, kisspeptin (*Kiss*) and RFamide-related peptide (RFRP, the mammalian homologs of gonadotropin-inhibitory hormone: GnIH), are hypothalamic neuropeptides that stimulate and inhibit the synthesis and release of GnRH, respectively (viviD and Bentley, 2018; Ubuka, 2021). Administration of peripheral KISS to short-day-housed Syrian hamsters with regressed testes significantly increased testicular volume and testosterone secretion, while this response was blocked by the GnRH receptor antagonist acyline (Ansel et al., 2011). Melatonin injections reduce *Kiss1* expression, and pinealectomy prevents short-day inhibition of *Kiss1* in the arc of the Syrian hamster hypothalamus (Revel et al., 2007; Simonneaux et al., 2009). Thus, melatonin affects *Kiss1* expression and mediates a reproductive signal via a pathway responsive to GnRH (viviD and Bentley, 2018).

On the other hand, the melatonin binding site in the PT is altered by the photoperiod. Namely, in the PT, the *Per* gene expression peaks during the daytime, while the *Cry* gene expression peaks early in the nighttime. Thus, the phase relationship between *Per* and *Cry* changes during the different photoperiods, and the PER/CRY protein/protein interactions due to the photoperiodic differences are potential mechanisms for producing photoperiodic responses (Ikegami and Yoshimura, 2012) (Fig. 1). As the thyroid hormone–stimulating hormone is secreted in the PT, thyroid hormones are also involved in seasonal reproduction (Watanabe et al., 2004; Ono et al., 2008; viviD and Bentley, 2018) (Fig. 1). Local thyroid hormone catabolism in the mediobasal hypothalamus by the deiodinase DIO2 and DIO3 enzymes that activate or inactivate thyroid hormones regulates the seasonal reproduction in birds and mammals (Ono et al., 2008). DIO3 inactivates the thyroid hormones triiodothyronine (T3, active form) and thyroxin (T4, prohormone of T3), while DIO2 converts T4 into T3. Long days induce thyroid-stimulating hormone production in the PT of the pituitary gland, which triggers DIO2 expression in the ependymal cells of the mediobasal hypothalamus (Fig. 1). Melatonin is indirectly involved in the regulation of *Dio2* expression via thyroid-stimulating hormones. Furthermore, the *Dio2* expression levels in the Djungarian hamster (*Phodopus sungorus*) are high under long-day conditions and low under short-day conditions and can be reduced further by melatonin injections (Watanabe et al., 2004). Therefore, melatonin regulates *Dio2* and *Dio3* expression and reproductive physiology through the thyroid hormone (Fig. 1).

Considering the above information, studies using long-day and short-day rearing animals have significantly contributed to our understanding of the mechanisms through which day length and melatonin can regulate seasonal reproduction (Fig. 1). As a result of these findings, melatonin has been successfully used as a pharmacological agent to accelerate the reproductive season in sheep, induce estrous cycles at a time when they would normally be in seasonal anestrus, and increase lambing (Haresign et al., 1990; Haresign, 1992; Abecia et al., 2007). Furthermore, daily afternoon injections of melatonin in Syrian hamsters under long-day conditions caused genital atrophy and hormonal changes similar to those induced by short-day exposures (Reiter et al., 1980). Thus, melatonin is an effective agent for regulating maturation in seasonally breeding animals.

Seasonal rhythm regulation of melatonin in non-mammals

Among birds, quail (*Coturnix japonica*) are the most sensitive to GnRH and LH alterations—changes occur from the first day—when transferred from short-day to long-day conditions. Further, GnRH and LH levels increase from the first day when birds are moved from short-day to long-day conditions, causing the testis weight to change dramatically in a short period of time (Perera and Follett, 1992; Yoshimura, 2006). This is why it has been continuously used in photoperiodic experiments. PT-derived TSH may be the key factor in regulating seasonal reproduction in birds as well as mam-

mals (Nakane and Yoshimura, 2019), as shown in Fig. 1. TSH secreted from the PT influences ependymal cells and increases the expression of *dio2*. T3, augmented by Dio2, regulates the seasonal morphological change in neuroglial interactions. This morphological change photoperiodically regulates GnRH secretion. Melatonin regulates GnIH secretion and is involved in seasonal reproductive physiology (Chowdhury et al., 2013; Tsutsui and Ubuka, 2021). Melatonin appears to act on GnIH neurons by stimulating GnIH synthesis and release, and then inhibiting plasma LH concentration in birds (Chowdhury et al., 2013).

In the ruin lizard, *Podarcis sicula*, melatonin may play an important role in the seasonal reorganization of the circadian system (Bertolucci et al., 2002; Foà et al., 2002; Foà and Bertolucci, 2003). Pinealectomy in ruin lizards did not abolish circadian locomotor rhythmicity; instead, it induced marked changes in both the free-running period and circadian activity time (Foà, 1991). Either pinealectomy or melatonin implants caused an immediate transition from the typical summer circadian locomotor pattern to the typical spring or autumn circadian locomotor pattern, respectively (Foà et al., 1997). In addition, the degrees of melatonin and pineal involvement in the circadian organization are highest in summer compared to the other three seasons (Bertolucci et al., 2002; Foà et al., 2002; Foà and Bertolucci, 2003).

In the female skipper frog, *Rana cyanophlyctis*, continuous light stimulates, and melatonin inhibits, reproductive function (Udaykumar and Joshi, 1997). Continuous light exposure for 30 days stimulated the gonad somatic index (GSI), while daily injections of melatonin prevented the increase in GSI caused by continuous light. Oviductal weights decreased only in the melatonin-injected group. In the male frog *Rana hexadactyla* melatonin also inhibits testicular function and spermatogenesis when administered at an optimum dose for a stipulated period (Kasinathan and Gregalatchoumi, 1988). The inhibition of spermatogenesis by melatonin has also been reported in toads (*Bufo melanostictus*), where it suppresses light-induced spermatogenesis (Biswas et al., 1978; Chanda and Biswas, 1982). In both female and male frogs, therefore, melatonin inhibits gonad functions and regulates reproductive physiology.

Melatonin is involved in the seasonal reproductive physiology in fish. For example, in the blue damselfish (Sapphire devil), the expression of *aanat2* was higher during short-day conditions than long-day, suggesting that there is a seasonal change in melatonin levels at night (Imamura et al., 2022). Therefore, *kiss1* and *kiss2*, *gonadotropin-releasing hormone 1* (*gnrh1*), and the β-subunit of gonadotropins (*fshβ* and *lhβ*) levels were examined in the brain. Feeding mature females melatonin-containing pellets lowered *kiss1*, *gnrh1*, and *lhβ* mRNA levels within 3 h. Continuous melatonin treatment for 1-week resulted in oocyte regression and decreased *kiss2*, *gnrh1*, *fshβ*, and *lhβ* mRNA expressions. In catfish (*Heteropneustes fossilis*) and blue damselfish, melatonin inhibited *kiss2* and *gnrh2* expression in the brain (Chaube et al., 2020).

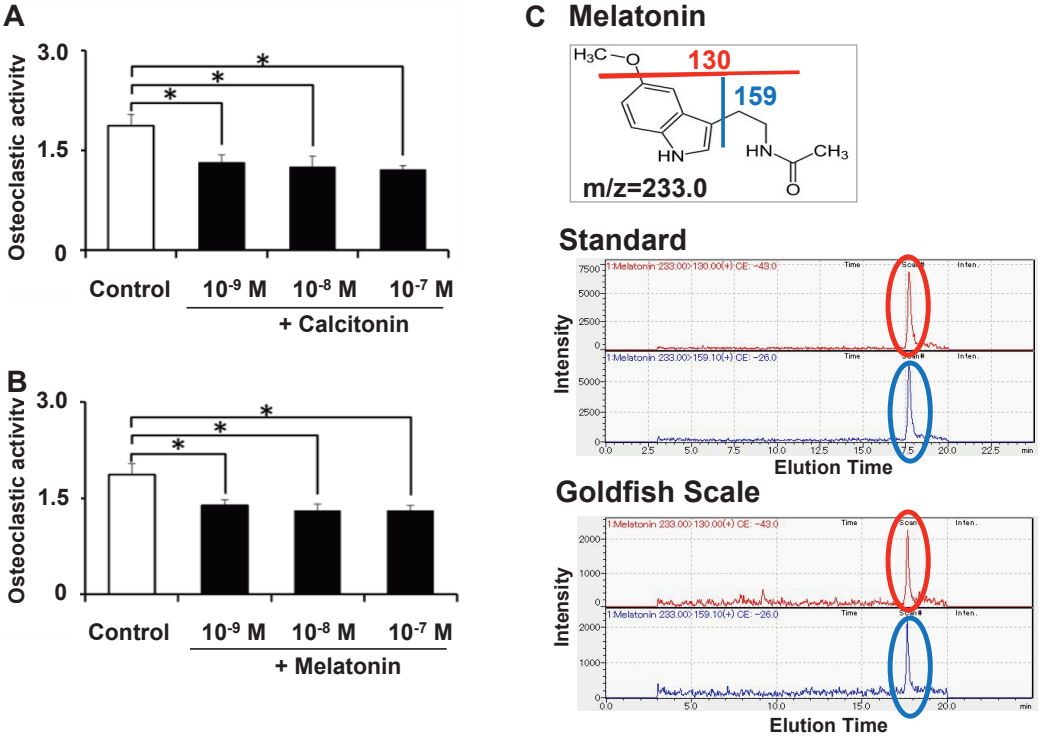

Fig. 3. Inhibitory action of calcitonin **(A)** and melatonin **(B)** on osteoclasts and detection of melatonin produced in goldfish scales **(C)**. **(A, B)** Both calcitonin and melatonin significantly reduce osteoclastic activity (*$P < 0.05$), starting in the nanomolar range (Ikegame et al., 2019). One-way repeated measures analysis of variance followed by Dunnett's test was used to examine differences in the values of the experimental groups. **(C)** Melatonin produced in the goldfish scales was analyzed by liquid chromatography–tandem mass spectrometry (LCMS-8050; Shimadzu Co., Kyoto, Japan). Analysis of the extract from goldfish scales revealed the presence of melatonin product ions (red and blue) at the same elution time as the melatonin standard (Ikegame et al., 2019).

MELATONIN-REGULATED BONE AND GLUCOSE METABOLISM

Melatonin influences bone and glucose metabolisms in addition to the regulation of circadian and seasonal rhythms (Karamitri and Jocker, 2019; Hirayama et al., 2023). In this section, we describe the influence of melatonin on bone and glucose metabolisms owing to the potential of melatonin to cure bone diseases such as osteoporosis, and diabetes.

Bone metabolism regulated by melatonin

Spinal malformations have been reported in chickens (Machida et al., 1993), rats (Machida et al., 1999), and fish (Fjelldal et al., 2004), after surgical ablation of the pineal gland. Experimental scoliosis observed in pinealectomized chickens is similar to that in human idiopathic scoliosis. These results suggest that melatonin is involved in bone metabolism. Subsequently, in vitro studies have been performed to elucidate the direct influence of melatonin on bone metabolism. Melatonin has a strong inhibitory action on osteoclasts (Fig. 3A, B). Calcitonin is well known as a hypocalcemic hormone that inhibits osteoclasts and is used as a therapy for osteoporosis (Body, 2002; Suzuki, 2021). The ability of melatonin to exert an inhibitory action on osteoclasts is very similar to that exerted by calcitonin, as detected using a fish-scale (bone model) bioassay (Suzuki and Hattori, 2002; Suzuki et al., 2000). Melatonin (10^{-9}–10^{-7} M) suppressed osteoclastic activity (Fig. 3B). As Mel1b is detected in osteoclasts immunohistochemically (Igarashi-Migitaka et al., 2020), melatonin directly acts on osteoclasts. In addition, the indirect influence of melatonin on osteoclasts has been reported. For example, the receptor activators of the nuclear factor-κB (RANK)/receptor activators of the nuclear factor-κB ligand (RANKL) system regarding the interaction between osteoclasts and osteoblasts are involved in the inhibitory action of melatonin on osteoclasts (Koyama et al., 2002; Kim et al., 2017; Li et al., 2019). Since RANKL induces osteoclast activation, it appears to be an important factor in osteoclastic bone resorption (Maria and Witt-Enderby, 2014; Nagy and Penninger, 2015). By suppressing RANKL expression, melatonin inhibited osteoclast activation (Koyama et al., 2002; Ikegame et al., 2019). A novel pathway was recently identified for the inhibitory effect of melatonin on osteoclasts (Ikegame et al., 2019; Nakano et al., 2019). As shown in Fig. 4, melatonin prevents bone resorption by increasing the expression of *calcitonin* by osteoblasts in fish (Ikegame et al., 2019) and chickens (Nakano et al., 2019).

Physical stimuli play an essential role in the regulation of bone remodeling (bone resorption and formation), either directly or indirectly (Carmeliet et al., 2001; Civitelli, 2008). Astronauts are exposed to the microgravity of space, which is known to reduce bone density (Collet et al., 1997; Caillot-Augusseau et al., 2000). Melatonin synthesized in osteoblasts in goldfish scales (Fig. 3C) was found to be involved in the progressive bone density loss effect in space (Fig. 4). Using fish scale culture systems (Fig. 4), containing co-existing osteoblasts and osteoclasts, in vitro space experiments have been performed on the International Space Station (ISS) since 2010 to examine the effect of microgravity on bone metabolism (Ikegame et al., 2019; Yamamoto et al., 2020; Yamamoto et al., 2022). Moreover, *aanat* expression decreases in microgravity (Ikegame et al., 2019). Furthermore, bone resorption under microgravity was inhibited by melatonin treatments via the promotive action of calcitonin production in osteoblasts (Fig. 4), suggesting that

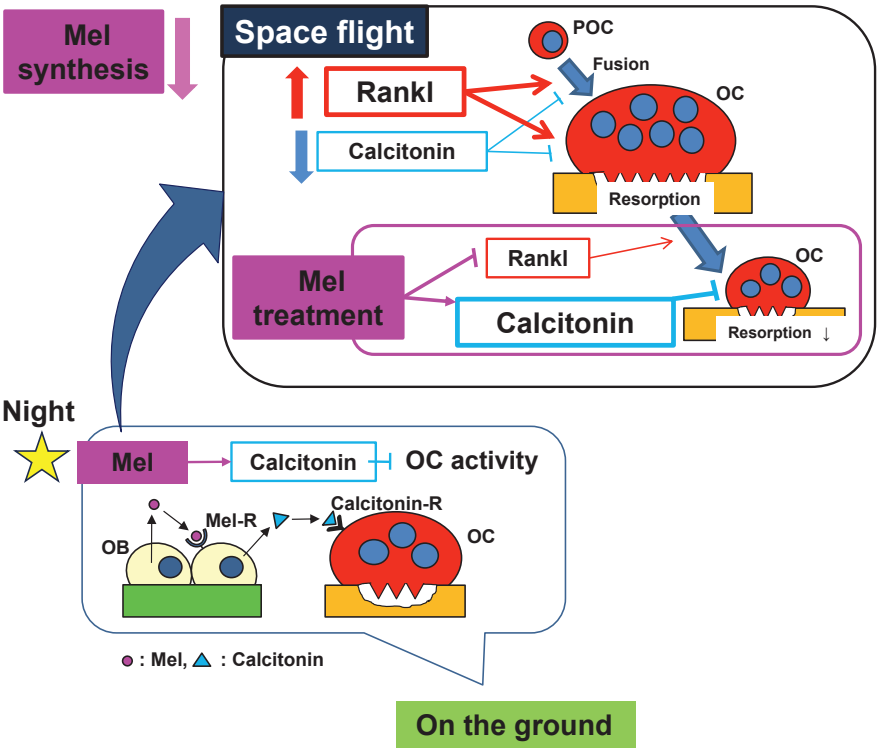

Fig. 4. Summary of the space experiment using goldfish scales. Lower frame: We observed the expression of the melatonin receptor, melatonin synthetic enzymes, and calcitonin (an osteoclast-inhibiting hormone) in osteoblasts of the regenerating scales. Melatonin stimulates calcitonin production in the scales, which has also been detected in mammalian bones. The melatonin-induced calcitonin suppresses osteoclast activity. Upper frame: Microgravity during space flight accelerated the multinucleation and resorption activity in scale osteoclasts, which was associated with a decrease in melatonin synthesis, the stimulated gene expression of *rankl* (a major factor for osteoclastogenesis), and the suppressed gene expression of *calcitonin*. Melatonin administration maintained the normal gene expression levels of the factors during space flight and suppressed microgravity-stimulated osteoclast activity. The results suggest that melatonin could be used as a prophylactic drug to prevent bone loss in astronauts during space flight. Abbreviations: OB: osteoblasts; OC: osteoclasts; POC: preosteoclasts; Rankl: receptor activator of nuclear factor κB ligand; Mel: melatonin; Mel-R: melatonin receptor; Calcitonin-R: calcitonin receptor.

melatonin can be used as a potential drug for bone disease in astronauts (Hirayama et al., 2023).

Glucose metabolism regulated by melatonin

Since melatonin receptors are found in human islet β-cells, cohort analysis of single-nucleotide polymorphisms (SNPs) in the melatonin receptor (Mel1b) and type 2 diabetes mellitus (T2DM) has shown a link between melatonin and glucose metabolism (Karamitri and Jockers, 2019).

It has been reported that the rs1387153 SNP, near *MTNR1B* (which encodes Mel1b), is associated with increased fasting plasma glucose levels and an elevated risk of T2DM (Bouatia-Naji et al., 2009). Furthermore, *MTNR1B* is expressed in human islet β-cells, while insulin release in INS-1 832/13 clonal β-cells, in response to stimulation with glucose, was inhibited in the presence of melatonin (Lyssenko et al., 2009). Since *MTNR1B* expression increases in individuals at risk of T2DM (Lyssenko et al., 2009), its pathological effects are likely exerted through the direct inhibition of insulin release from β-cells by melatonin.

Melatonin regulates basal glucose metabolism in fish. In fasted goldfish individuals, we found that plasma melatonin levels were significantly higher and insulin levels were significantly lower at night. Furthermore, glucose uptake in the brain, liver, and muscle tissues also significantly increases at night. After intraperitoneal administration of melatonin, glucose uptake by the brain and liver showed significantly greater increases than in the control group, although it failed to alter plasma insulin levels (Watanabe et al., 2023). Using an insulin-free medium, we further showed that melatonin treatment increased glucose uptake in primary cell cultures of goldfish brain and liver cells, in a dose-dependent manner (Watanabe et al., 2023). The administration of melatonin also significantly decreased plasma glucose levels in hyperglycemic goldfish but failed to alter *insulin* mRNA expression or plasma insulin levels, suggesting that melatonin has the same potency as a therapeutic drug as insulin does for diabetes.

ANTIOXIDANT ACTIVITY OF MELATONIN AS ITS RECEPTOR-INDEPENDENT ACTION

Melatonin has been shown to be a free radical scavenger (Allegra et al., 2003; Reiter et al., 2016). Melatonin has a redox effect because of the presence of an electron-rich aromatic ring system, and indoleamine easily functions as an electron donor. One melatonin molecule has the potential to scavenge up to four or more reactive species, such as $ONOO^-$, H_2O_2, $^{\cdot}OH$, and $O_2^{\cdot -}$ (Allegra et al., 2003). Under in vivo conditions, melatonin is often several times more potent at protecting tissues from oxidative injury than vitamins C and E, at equivalent dosages (Tan et al., 2002).

Like melatonin, N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) and N^1-acetyl-5-methoxykynuramine (AMK), which are enzymatically and oxidatively generated from melatonin, are also radical-scavenging molecules (Tan et al., 2001). In this section, we describe the therapeutic effects of melatonin's radical scavenging action and its metabolites, such as AFMK and AMK (Fig. 5) in mammals such as rats and mice.

Therapeutic benefits of melatonin through antioxidative action

Therapeutic effects of melatonin have been reported for several disorders, such as different types of tumors (intestinal tumors, melanoma, breast cancer, etc.), caused by its antioxidative action because oxidative stress is involved in the initiation, promotion, and progression of carcinogenesis (Karbownik et al., 2001; Tordjman et al., 2017). The inhibitory action of melatonin by its oral administration, after being dissolved in tap water at 20 mg/L, has been reported in intestinal carcinogenesis in rats, induced by the carcinogen 1,2-dimethylhydrazine (Anisimov et al., 1997).

Melatonin is effective in cardiovascular diseases (Tordjman et al., 2017). Urinary noradrenaline increases at night in patients with coronary heart disease (Brugger et al., 1995). The increase in noradrenaline in these patients may be related to the suppression of sympathetic activity by melatonin. Therefore, melatonin levels in the blood of these patients (6.5–11.8 pg/mL) were significantly lower than those in 10 healthy controls (32.2–42.5 pg/mL) (Brugger et al., 1995). Additionally, the administration of melatonin (1 mg) significantly reduced blood pressure in comparison to the

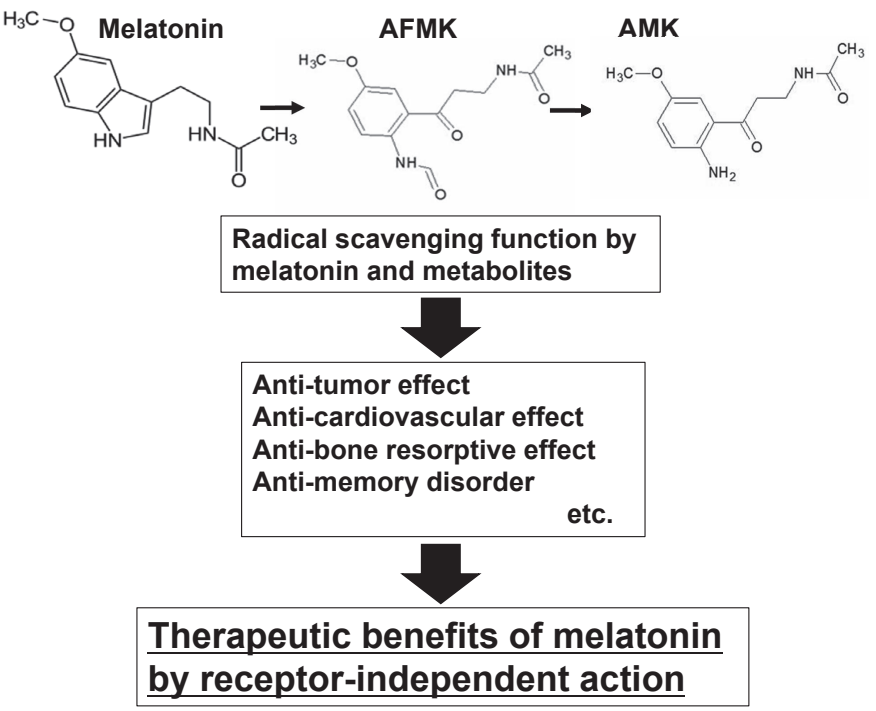

Fig. 5. Schematic diagram of therapeutic benefits of melatonin by receptor-independent action. Melatonin and its metabolites (AFMK: N^1-acetyl-N^2-formyl-5-methoxykynuramine; AMK: N^1-acetyl-5-methoxykynuramine) have some therapeutic effects, such as anti-tumor, anti-cardiovascular disease, anti-bone resorption, and anti-memory disorder by radical scavenging function.

placebo (Arangino et al., 1999).

On the other hand, as described above, melatonin inhibits osteoclastic activity via the membrane receptor Mel1b. Since reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, promote bone resorption (Cardinali et al., 2003), melatonin suppresses osteoclastic activity through its free radical scavenging function. Therefore, we hypothesize that melatonin can provide a therapeutic effect on osteoporosis by inhibiting bone resorption through both receptor-mediated and receptor-independent actions.

Protection against space radiation by melatonin

During space flight, astronauts are constantly exposed to radiation, including high linear energy transfer radiation, which differs from the radiation on Earth (Takahashi et al., 2018; Hirayama et al., 2023). The radioprotective effect of melatonin is well known, whereby many in vitro and in vivo studies have shown that melatonin protects mammalian cells from the toxic effects of ionizing radiation (Vijayalaxmi, 2004; Farhood et al., 2019; Nuszkiewicz et al., 2020). Here, we consider that melatonin provides a potent protective effect against space radiation. However, there have been no previous studies on this topic. Therefore, we performed a space experiment using goldfish scales (Fig. 4) with the ISS to determine the radioprotective potential of melatonin during space flight (Furusawa et al., 2020). After returning to Earth, RNA-sequencing analysis with subsequent de novo transcriptome assembly and computational gene expression analysis were performed. Genes coding for heat shock proteins, DNA repair markers, and those involved in the oxidative stress response comprised the majority of the identified upregulated genes in response to space radiation (Furusawa et al., 2020). Fish scales treated with melatonin counteracted the upregulation of those genes in response to space radiation. The effects of melatonin on gene expression are expected to enhance cell survival. In our space experiment, melatonin improved the expression of genes that support cell survival in response to space radiation, such as the anti-apoptotic *bcl-2* gene (Furusawa et al., 2020).

Novel functions of melatonin derivatives

AFMK is endogenously generated from melatonin by indoleamine 2,3-dioxygenase or metabolized from melatonin by free-radical scavenging action (Tan et al., 2002). This metabolized AFMK possesses antioxidative ability in calf thymus, rat liver, and HT22 cells, a subclone of the HT4 hippocampal cell line (Tan et al., 2001; Manda et al., 2008). Moreover, Tan et al. (2001) showed that AFMK inhibited the formation of 8-hydroxydeoxyguanosine induced by the incubation of DNA with oxidants in calf thymus. Lipid peroxidation resulting from free radical damage in rat liver homogenates was also inhibited by the addition of AFMK (Tan et al., 2001). In radiation-induced oxidation, AFMK also suppressed the loss of proliferating and immature neurons in the dentate gyrus of the brain by inhibiting oxidative stress in mice irradiated with high-LET ^{56}Fe particles (Manda et al., 2008). We recently found a novel action of AFMK and AMK regarding the enhancement of learning and memory in mice (Iwashita et al., 2021). Both AFMK and AMK enhanced long-term memory in the object recognition test. Moreover, melatonin metabolites serve the same physiological functions as melatonin, which may contribute to the endurance of its effects.

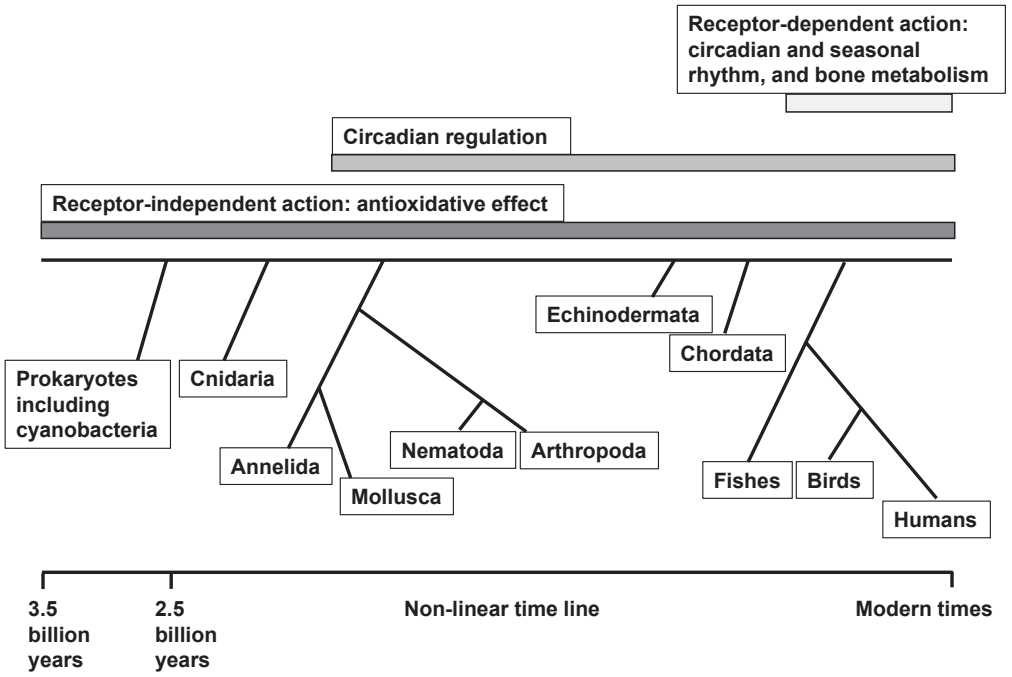

Fig. 6. Evolutionary implications of melatonin. The antioxidant properties of melatonin indicate that this action is extremely important for aerobic organisms to remove harmful reactive oxygen species. In Annelida, melatonin signaling regulates circadian swimming by rhythmically activating cholinergic neurons (Schippers and Nichols, 2014). As a result, circadian regulation occurs in invertebrates. In vertebrates, melatonin is mainly secreted from the pineal gland and regulates the circadian and seasonal rhythms systematically via melatonin receptors. Due to rhythmical regulation, we believe that several processes of metabolism must be controlled by melatonin in vertebrates.

EVOLUTIONARY SIGNIFICANCE OF WIDELY DISTRIBUTED MELATONIN

Molecular oxygen (O_2), a key player in biological evolution, began rising in the Earth's atmosphere (the Great Oxygenation Event) about 2.5 billion years ago, as a result of its continuous release from photosynthetic bacteria, which evolved an estimated billion years ago (Luo et al., 2016). The rise in atmospheric oxygen has accelerated the evolution of organisms based on advancements in metabolism using O_2 (Zhao et al., 2019). Organisms undergoing aerobic metabolism use oxygen as an electron recipient, whereby it accepts four electrons and is then reduced to water (H_2O). In this process, electrons leak from the electron transfer chain, leading to the formation of ROS (Tan et al., 2014). It is assumed that up to 4–5% of the oxygen consumed by organisms in aerobic metabolism is eventually reduced to ROS (Tan et al., 2014). High levels of ROS are harmful to cells and organisms (Reiter et al., 2017). Melatonin is speculated to have emerged as an antioxidant and free radical scavenger in early photosynthetic prokaryotes to reduce the oxidative stress induced by ROS (Tan et al., 2014; Zhao et al., 2019) (Fig. 6).

In modern organisms, including plants, invertebrates, and vertebrates, melatonin is widely distributed (Hattori et al., 1995; Schippers and Nichols, 2014; Tan et al., 2014; Reiter et al., 2017; Zhao et al., 2019), as shown in Fig. 6. In the dinoflagellate *Gonyaulax polyedra*, the existence of melatonin has been demonstrated using two different methods: reverse-phase high-performance liquid chromatography and radioimmunoassay (Pöggeler et al., 1991). Reports of the presence of melatonin in dinoflagellates led to its discovery in several organisms in the plant kingdom (primitive photosynthetic bacteria (cyanobacteria), fungi, and land plants) (Dubbels et al., 1995; Hardeland et al., 1995; Hattori et al., 1995; Manchester et al., 1995; Tilden et al., 1997). The chemical structure of melatonin has not changed over the course of evolution, and the melatonin present in humans is identical to that found in cyanobacteria, which have existed on Earth for billions of years (Reiter et al., 2017) (Fig. 6). The antioxidant properties of melatonin indicate that it is extremely important for organisms undergoing aerobic metabolism. In Annelida, melatonin signaling regulates circadian swimming by rhythmically activating cholinergic neurons (Schippers and Nichols, 2014), meaning that circadian regulation also occurs in invertebrates (Fig. 6). In vertebrates, melatonin is secreted from the pineal gland and regulates circadian and seasonal rhythms systematically via melatonin receptors. Therefore, we believe that melatonin must regulate multiple metabolisms in vertebrates because of its rhythmic regulation.

CONCLUSION

As oxygen concentrations on Earth increased, organisms with aerobic metabolisms emerged. Melatonin is thought to have evolved to allow these aerobic organisms to eliminate harmful ROS. Therefore, the receptor-independent action preceded the receptor-mediated action. Subsequently, organisms acquired circadian rhythms, and in vertebrates, pineal gland-secreted melatonin could regulate clear diurnal and circadian rhythms of some metabolisms. Overall, we conclude that melatonin is an essential hormone for the survival of organisms.

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COMPETING INTERESTS

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

AH and NS are responsible for the conception of this review, data interpretation, and discussion, and manuscript writing. Both authors have read and approved the final manuscript.

REFERENCES

Abecia JA, Valares JA, Forcada F, Palacín I, Martín S, Martino A (2007) The effect of melatonin on the reproductive performance of three sheep breeds in Spain. Small Rumin Res 69: 10–16

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

Ansel L, Bentsen AH, Ancel C, Bolborea M, Klosen P, Mikkelsen JD, et al. (2011) Peripheral kisspeptin reverses short photoperiod-induced gonadal regression in Syrian hamsters by promoting GNRH release. Reproduction 142: 417–425

Anisimov VN, Popovich IG, Zabezhinski MA (1997) Melatonin and colon carcinogenesis: I. Inhibitory effect of melatonin on development of intestinal tumors induced by 1,2-dimethylhydrazine in rats. Carcinogenesis 18: 1549–1553

Arangino S, Cagnacci A, Angiolucci M, Vacca AMB, Longu G, Volpe A, et al. (1999) Effects of melatonin on vascular reactivity, catecholamine levels, and blood pressure in healthy men. Am J Cardiol 83: 1417–1419

Bartness TJ, Powers JB, Hastings MH, Bittman EL, Goldman BD (1993) The timed infusion paradigm for melatonin delivery: What has it taught us about the melatonin signal, its reception, and the photoperiodic control of seasonal responses? J Pineal Res 15: 161–190

Benloucif S, Dubocovich ML (1996) Melatonin and light induce phase shifts of circadian activity rhythms in the C3H/HeN mouse. J Biol Rhythms11: 113–125

Bertolucci C, Foà A, Van't Hof TJ (2002) Seasonal variations in circadian rhythms of plasma melatonin in ruin lizards. Horm Behav 41: 414–419

Besharse JC, Iuvone PM (1983) Circadian clock in *Xenopus* eye controlling retinal serotonin N-acetyltransferase. Nature 305: 133–135

Bilu C, Kronfeld-Schor N (2013) Effects of circadian phase and melatonin injection on anxiety-like behavior in nocturnal and diurnal rodents. Chronobiol Int 30: 828–836

Biswas NM, Chakraborty J, Chanda S, Sanyal S (1978) A basic experimental approach in perspective of pineal and melatonin involvement in photoperiod-induced alteration of spermatogenesis in toad (*Bufo melanostictus*). Endokrinologie 71: 143–148

Body JJ (2002) Calcitonin for the long-term prevention and treatment of postmenopausal osteoporosis. Bone 30(5 Suppl): 75S–79S

Bouatia-Naji N, Bonnefond A, Cavalcanti-Proença C, Sparsø T, Holmkvist J, Marchand M, et al. (2009) A variant near *MTNR1B*

is associated with increased fasting plasma glucose levels and type 2 diabetes risk. Nat Genet 41: 89–94
Brandstätter R (2003) Encoding time of day and time of year by the avian circadian system. J Neuroendocrinol 15: 398–404
Brugger P, Marktl W, Herold M (1995) Impaired nocturnal secretion of melatonin in coronary heart disease. Lancet 345: 1408
Brzezinski A, Rai S, Purohit A, Pandi-Perumal SR (2021) Melatonin, clock genes, and mammalian reproduction: What is the link? Int J Mol Sci 22: 13240
Cahill GM, Besharse JC (1989) Retinal melatonin is metabolized within the eye of *Xenopus laevis*. Proc Natl Acad Sci U S A 86: 1098–1102
Caillot-Augusseau A, Vico L, Heer M, Voroviev D, Souberbielle JC, Zitterman A, et al. (2000) Space flight is associated with rapid decreases of undercarboxylated osteocalcin and increases of markers of bone resorption without changes in their circadian variation: Observations in two cosmonauts. Clin Chem 46: 1136–1143
Cardinali DP, Ladizesky MG, Boggio V, Cutrera RA, Mautalen C (2003) Melatonin effects on bone: Experimental facts and clinical perspectives. J Pineal Res 34: 81–87
Carmeliet G, Vico L, Bouillon R (2001) Space flight: A challenge for normal bone homeostasis. Crit Rev Eukaryot Gene Expr 11: 131–144
Cassone VM (1998) Melatonin's role in vertebrate circadian rhythms. Chronobiol Int 15: 457–473
Chabot CC, Menaker M (1992) Circadian feeding and locomotor rhythms in pigeons and house sparrows. J Biol Rhythms 7: 287–299
Chanda S, Biswas NM (1982) Effect of morning and evening injections of melatonin on the testis of toad (*Bufo melanostictus*). Endocrinol Jpn 29: 483–485
Chaube R, Sharma S, Senthilkumaran B, Bhat SG, Joy KP (2020) Expression profile of kisspeptin2 and gonadotropin-releasing hormone2 mRNA during photo-thermal and melatonin treatments in the female air-breathing catfish *Heteropneustes fossilis*. Fish Physiol Biochem 46: 2403–2419
Chowdhury VS, Ubuka T, Tsutsui K (2013) Review: Melatonin stimulates the synthesis and release of gonadotropin-inhibitory hormone in birds. Gen Comp Endocrinol 181: 175–178
Civitelli R (2008) Cell-cell communication in the osteoblast/osteocyte lineage. Arch Biochem Biophys 473: 188–192
Collet P, Uebelhart D, Vico L, Moro L, Hartmann D, Roth M, et al. (1997) Effects of 1- and 6-month spaceflight on bone mass and biochemistry in two humans. Bone 20: 547–551
Cowan M, Paullada-Salmerón JA, López-Olmeda JF, Sánchez-Vázquez FJ, Muñoz-Cueto JA (2017) Effects of pinealectomy on the neuroendocrine reproductive system and locomotor activity in male European sea bass, *Dicentrarchus labrax*. Comp Biochem Physiol A 207: 1–12
Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C, et al. (1995) Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. J Pineal Res 18: 28–31
Dubocovich ML, Rivera-Bermudez MA, Gerdin MJ, Masana MI (2003) Molecular pharmacology, regulation and function of mammalian melatonin receptors. Front Biosci 8: d1093–1108
Ekström P, Meissl H (2003) Evolution of photosensory pineal organs in new light: The fate of neuroendocrine photoreceptors. Phil Trans R Soc Lond B 358: 1679–1700
Emet M, Ozcan H, Ozel L, Yayla M, Halici Z, Hacimuftuoglu A (2016) A review of melatonin, its receptors and drugs. Eurasian J Med 48: 135–141
Farhood B, Goradel NH, Mortezaee K, Khanlarkhani N, Salehi E, Nashtaei MS, et al. (2019) Melatonin as an adjuvant in radiotherapy for radioprotection and radiosensitization. Clin Transl Oncol 21: 268–279
Fjelldal PG, Grotmol S, Kryvi H, Gjerdet NR, Taranger GL, Hansen T, et al. (2004) Pinealectomy induces malformation of the spine and reduces the mechanical strength of the vertebrae in Atlantic salmon, *Salmo salar*. J Pineal Res 36: 132–139
Foà A (1991) The role of the pineal and the retinae in the expression of circadian locomotor rhythmicity in the ruin lizard, *Podarcis sicula*. J Comp Physiol A 169: 201–207
Foá A, Bertolucci C (2003) Toward a seasonal model of the circadian system: The case of ruin lizards. Front Biosci 8: s236–242
Foà A, Bertolucci C, Marsanich A, Innocenti A (1997) Pineal transplantation to the brain of pinealectomized lizards: Effects on circadian rhythms of locomotor activity. Behav Neurosci 111: 1123–1132
Foà A, Magnone MC, Bertolucci C (2002) Circadian organization in ruin lizards: Phase response curve for melatonin changes with season. J Comp Physiol A 188: 141–145
Furusawa Y, Yamamoto T, Hattori A, Suzuki N, Hirayama J, Sekiguchi T, et al. (2020) De novo transcriptome analysis and gene expression profiling of fish scales isolated from *Carassius auratus* during space flight: Impact of melatonin on gene expression in response to space radiation. Mol Med Rep 22: 2627–2636
Grace MS, Cahill GM, Besharse JC (1991) Melatonin deacetylation: Retinal vertebrate class distribution and *Xenopus laevis* tissue distribution. Brain Res 559: 56–63
Hamm HE, Menaker M (1980) Retinal rhythms in chicks: Circadian variation in melatonin and serotonin *N*-acetyltransferase activity. Proc Natl Acad Sci U S A 77: 4998–5002
Hardeland R, Balzer I, Poeggeler B, Fuhrberg B, Uria H, Behrmann G, et al. (1995) On the primary functions of melatonin in evolution: Mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. J Pineal Res 18: 104–111
Hardeland R, Pandi-Perumal SR, Cardinali DP (2006) Melatonin. Int J Biochem Cell Biol 38: 313–316
Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR (2011) Melatonin – a pleiotropic, orchestrating regulator molecule. Prog Neurobiol 93: 350–384
Haresign W (1992) Manipulation of reproduction in sheep. J Reprod Fertil Suppl 45: 127–139
Haresign W, Peters AR, Staples LD (1990) The effect of melatonin implants on breeding activity and litter size in commercial sheep flocks in the UK. Anim Product 50: 111–121
Hattori A, Migitaka H, Iigo M, Itoh M, Yamamoto K, Ohtani-Kaneko R, et al. (1995) Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem Mol Biol Int 35: 627–634
Hirayama J, Hattori A, Takahashi A, Furusawa Y, Tabuchi Y, Shibata M, et al. (2023) Physiological consequences of space flight, including abnormal bone metabolism, space radiation injury, and circadian clock dysregulation: Implications of melatonin use and regulation as a countermeasure. J Pineal Res 74: e12834
Hoffman RA, Reiter RJ (1965) Pineal gland: Influence on gonads of male hamsters. Science 148: 1609–1611
Igarashi-Migitaka J, Seki A, Ikegame M, Honda M, Sekiguchi T, Mishima H, et al. (2020) Oral administration of melatonin contained in drinking water increased bone strength in naturally aged mice. Acta Histochem 122: 151596
Ikegami K, Yoshimura T (2012) Circadian clocks and the measurement of daylength in seasonal reproduction. Mol Cell Endocrinol 349: 76–81
Ikegame M, Hattori A, Tabata MJ, Kitamura K, Tabuchi Y, Furusawa Y, et al. (2019) Melatonin is a potential drug for the prevention of bone loss during space flight. J Pineal Res 67: e12594
Imamura S, Hur SP, Takeuchi Y, Badruzzaman M, Mahardini A, Rizky D, et al. (2022) Effect of short- and long-term melatonin

treatments on the reproductive activity of the tropical damselfish *Chrysiptera cyanea*. Fish Physiol Biochem 48: 253–262
Iwashita H, Matsumoto Y, Maruyama Y, Watanabe K, Chiba A, Hattori A (2021) The melatonin metabolite N1-acetyl-5-methoxykynuramine facilitates long-term object memory in young and aging mice. J Pineal Res 70: e12703
Kameda Y, Miura M, Maruyama S (2002) Effect of pinealectomy on the photoperiod-dependent changes of the specific secretory cells and α-subunit mRNA level in the chicken pars tuberalis. Cell Tissue Res 308: 121–130
Karamitri A, Jockers R (2019) Melatonin in type 2 diabetes mellitus and obesity. Nat Rev Endocrinol 15: 105–125
Karbownik M, Lewinski A, Reiter RJ (2001) Anticarcinogenic actions of melatonin which involve antioxidative processes: Comparison with other antioxidants. Int J Biochem Cell Biol 33: 735–753
Kasahara T, Abe K, Mekada K, Yoshiki A, Kato T (2010) Genetic variation of melatonin productivity in laboratory mice under domestication. Proc Natl Acad Sci USA 107: 6412–6417
Kasinathan S, Gregalatchoumi S (1988) Effect of melatonin on the spermatogenesis of *Rana hexadactyla* (Lesson). Endocrinol Jpn 35: 255–260
Kim HJ, Kim HJ, Bae MK, Kim YD (2017) Suppression of osteoclastogenesis by melatonin: A melatonin receptor-independent action. Int J Mol Sci 18: 1142
Kolář J, Macháčková I (2005) Melatonin in higher plants: Occurrence and possible functions. J Pineal Res 39: 333–341
Kolf HW (1994) The pineal organ as a component of the biological clock. Phylogenetic and ontogenetic considerations. Ann N Y Acad Sci 719: 13–42
Koyama H, Nakade O, Takada Y, Kaku T, Lau KH (2002) Melatonin at pharmacologic doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediated osteoclast formation and activation. J Bone Miner Res 17: 1219–1229
Kumar Jha P, Challet E, Kalsbeek A (2015) Circadian rhythms in glucose and lipid metabolism in nocturnal and diurnal mammals. Mol Cell Endocrinol 418: 74–88
Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. J Am Chem Soc 80: 2587
Lewy AJ, Ahmed S, Jackson JM, Sack RL (1992) Melatonin shifts human circadian rhythms according to a phase-response curve. Chronobiol Int 9: 380–392
Li DY, Smith DG, Hardeland R, Yang MY, Xu HL, Zhang L, et al. (2013) Melatonin receptor genes in vertebrates. Int J Mol Sci 14: 11208–11223
Li T, Jiang S, Lu C, Yang W, Yang Z, Hu W, et al. (2019) Melatonin: Another avenue for treating osteoporosis? J Pineal Res 66: e12548
Luo G, Ono S, Beukes NJ, Wang DT, Xie S, Summons RE (2016) Rapid oxygenation of Earth's atmosphere 2.33 billion years ago. Sci Adv 2: e1600134
Lynch HJ, Wurtman RJ, Moskowitz MA, Archer MC, Ho MH (1975) Daily rhythm in human urinary melatonin. Science 187: 169–171
Lyssenko V, Nagorny CLF, Erdos MR, Wierup N, Jonsson A, Spégel P, et al. (2009) Common variant in *MTNR1B* associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet 41: 82–88
Machida M, Dubousset J, Imamura Y, Iwaya T, Yamada T, Kimura J (1993) An experimental study in chickens for the pathogenesis of idiopathic scoliosis. Spine 18: 1609–1615
Machida M, Murai I, Miyashita Y, Dubousset J, Yamada T, Kimura J (1999) Pathogenesis of idiopathic scoliosis: Experimental study in rats. Spine 24: 1985–1989
Malpaux B, Thiéry JC, Chemineau P (1999) Melatonin and the seasonal control of reproduction. Reprod Nutr Dev 39: 355–366
Manchester LC, Pöeggeler B, Alvares FL, Ogden GB, Reiter RJ (1995) Melatonin immunoreactivity in the photosynthetic prokaryote *Rhodospirillum rubrum*: Implications for an ancient antioxidant system. Cell Mol Biol Res 41: 391–395
Manda K, Ueno M, Anzai K (2008) Space radiation-induced inhibition of neurogenesis in the hippocampal dentate gyrus and memory impairment in mice: Ameliorative potential of the melatonin metabolite, AFMK. J Pineal Res 45: 430–438
Maria S, Witt-Enderby PA (2014) Melatonin effects on bone: Potential use for the prevention and treatment for osteopenia, osteoporosis, and periodontal disease and for use in bone-grafting procedures. J Pineal Res 56: 115–125
McArthur AJ, Lewy AJ, Sack RL (1996) Non-24-hour sleep-wake syndrome in a sighted man: Circadian rhythm studies and efficacy of melatonin treatment. Sleep 19: 544–553
McCord CP, Allen FP (1917) Evidences associating pineal gland function with alterations in pigmentation. J Exp Zool 23: 207–224
Nagy V, Penninger JM (2015) The RANKL-RANK story. Gerontology 61: 534–542
Nakane Y, Yoshimura T (2019) Photoperiodic regulation of reproduction in vertebrates. Annu Rev Anim Biosci 7: 173–194
Nakano M, Ikegame M, Igarashi-Migitaka J, Maruyama Y, Suzuki N, Hattori A (2019) Suppressive effect of melatonin on osteoclast function via osteocyte calcitonin. J Endocrinol 242: 13–23
Ning S, Wang Z, Cao J, Dong Y, Chen Y (2019) Mel1c mediated monochromatic light-stimulated IGF-I synthesis through the intracellular Gαq/PKC/ERK signaling pathway. Int J Mol Sci 20: 1682
Nowak JZ, Sek B, Zurawska E (1990) Activation of D2 dopamine receptors in hen retina decreases forskolin-stimulated cyclic AMP accumulation and serotonin *N*-acetyltransferase (NAT) activity. Neurochem Int 16: 73–80
Nuszkiewicz J, Woźniak A, Szewczyk-Golec K (2020) Ionizing radiation as a source of oxidative stress: The protective role of melatonin and vitamin D. Int J Mol Sci 21: 5804
Ono H, Hoshino Y, Yasuo S, Watanabe M, Nakane Y, Murai A, et al. (2008) Involvement of thyrotropin in photoperiodic signal transduction in mice. Proc Natl Acad Sci U S A 105: 18238–18242
Perera AD, Follett BK (1992) Photoperiodic induction in vitro: The dynamics of gonadotropin-releasing hormone release from hypothalamic explants of the Japanese quail. Endocrinology 131: 2898–2908
Pöggeler B, Balzer I, Hardeland R, Lerchl A (1991) Pineal hormone melatonin oscillates also in the dinoflagellate *Gonyaulax polyedra*. Naturwissenschaften 78: 268–269
Redman J, Armstrong S, Ng KT (1983) Free-running activity rhythms in the rat: entrainment by melatonin. Science 219: 1089–1091
Reiter RJ, Richardson BA, Johnson LY, Ferguson BN, Dinh DT (1980) Pineal melatonin rhythm: Reduction in aging Syrian hamsters. Science 210: 1372–1373
Reiter RJ, Tan DX, Manchester LC, Paredes SD, Mayo JC, Sainz RM (2009) Melatonin and reproduction revisited. Biol Reprod 81: 445–456
Reiter RJ, Tan DX, Galano A (2014) Melatonin: Exceeding expectations. Physiology (Bethesda) 29: 325–333
Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L (2016) Melatonin as an antioxidant: Under promises but over delivers. J Pineal Res 61: 253–278
Reiter RJ, Rosales-Corral S, Tan DX, Jou MJ, Galano A, Xu B (2017) Melatonin as a mitochondria-targeted antioxidant: One of evolution's best ideas. Cell Mol Life Sci 74: 3863–3881
Revel FG, Ansel L, Klosen P, Saboureau M, Pévet P, Mikkelsen JD, et al. (2007) Kisspeptin: A key link to seasonal breeding. Rev Endocr Metab Disord 8: 57–65

Schippers KJ, Nichols SA (2014) Deep, dark secrets of melatonin in animal evolution. Cell 159: 9–10

Schomerus C, Korf HW (2005) Mechanisms regulating melatonin synthesis in the mammalian pineal organ. Ann N Y Acad Sci 1057: 372–383

Shedpure M, Pati AK (1995) The pineal gland: Structural and functional diversity. Indian J Exp Biol 33: 625–640

Simonneaux V, Ansel L, Revel FG, Klosen P, Pévet P, Mikkelsen JD (2009) Kisspeptin and the seasonal control of reproduction in hamsters. Peptides 30: 146–153

Slominski AT, Hardeland R, Zmijewski MA, Slominski RM, Reiter RJ, Paus R (2018) Melatonin: A cutaneous perspective on its production, metabolism, and functions. J Invest Dermatol 138: 490–499

Sugden D, Davidson K, Hough KA, Teh MT (2004) Melatonin, melatonin receptors and melanophores: A moving story. Pigment Cell Res 17: 454–460

Suzuki N, Suzuki T, Kurokawa T (2000) Suppression of osteoclastic activities by calcitonin in the scales of goldfish (freshwater teleost) and nibbler fish (seawater teleost). Peptides 21: 115–124

Suzuki N, Hattori A (2002) Melatonin suppresses osteoclastic and osteoblastic activities in the scales of goldfish. J Pineal Res 33: 253–258

Suzuki N (2021) Calcitonin. In "Handbook of Hormones. Comparative Endocrinology for Basic and Clinical Research. Vol. 1. 2nd ed" Ed by H Ando, K Ukena, S Nagata, Academic Press, Cambridge, MA, pp 405–408

Takahashi A, Ikeda H, Yoshida Y (2018) Role of high-linear energy transfer radiobiology in space radiation exposure risks. Int J Part Ther 5: 151–159

Tan DX, Manchester LC, Burkhardt S, Sainz RM, Mayo JC, Kohen R, et al. (2001) N^1-acetyl-N^2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. FASEB J 15: 2294–2296

Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, et al. (2002) Chemical and physical properties and potential mechanisms: Melatonin as a broad spectrum antioxidant and free radical scavenger. Curr Top Med Chem 2: 181–197

Tan DX, Zheng X, Kong J, Manchester LC, Hardeland R, Kim SJ, et al. (2014) Fundamental issues related to the origin of melatonin and melatonin isomers during evolution: Relation to their biological functions. Int J Mol Sci 15: 15858–15890

Tilden AR, Becker MA, Amma LL, Arciniega J, McGaw AK (1997) Melatonin production in an aerobic photosynthetic bacterium: An evolutionarily early association with darkness. J Pineal Res 22: 102–106

Tordjman S, Chokron S, Delorme R, Charrier A, Bellissant E, Jaafari N, et al. (2017) Melatonin: Pharmacology, functions and therapeutic benefits. Curr Neuropharmacol 15: 434–443

Tsutsui K, Ubuka T (2021) Gonadotropin-inhibitory hormone (GnIH): A new key neurohormone controlling reproductive physiology and behavior. Front Neuroendocrinol 61: 100900

Turkowska E, Majewski PM, Rai S, Skwarlo-Sonta K (2014) Pineal oscillator functioning in the chicken: Effect of photoperiod and melatonin. Chronobiol Int 31: 134–143

Ubuka T (2021) Melatonin. In "Handbook of Hormones. Comparative Endocrinology for Basic and Clinical Research. Vol 2. 2nd ed" Ed by H Ando, K Ukena, S Nagata, Academic Press, Cambridge, MA, pp 1053–1055

Udaykumar K, Joshi BN (1997) Effect of exposure to continuous light and melatonin on ovarian follicular kinetics in the skipper frog, *Rana cyanophlyctis*. Biol Signals 6: 62–66

Vijayalaxmi, Reiter RJ, Tan DX, Herman TS, Thomas CR Jr. (2004) Melatonin as a radioprotective agent: A review. Int J Radiat Oncol Biol Phys 59: 639–653

viviD D, Bentley GE (2018) Seasonal reproduction in vertebrates: Melatonin synthesis, binding, and functionality using Tinbergen's four questions. Molecules 23: 652

Watanabe K, Nakano M, Maruyama Y, Hirayama J, Suzuki N, Hattori A (2023) Nocturnal melatonin increases glucose uptake via insulin-independent action in the goldfish brain. Front Endocrinol 14: 1173113

Watanabe M, Yasuo S, Watanabe T, Yamamura T, Nakao N, Ebihara S, et al. (2004) Photoperiodic regulation of type 2 deiodinase gene in Djungarian hamster: Possible homologies between avian and mammalian photoperiodic regulation of reproduction. Endocrinology145: 1546–1549

Wiechmann AF (1986) Melatonin: Parallels in pineal gland and retina. Exp Eye Res 42: 507–527

Wiechmann AF, Sherry DM (2013) Role of melatonin and its receptors in the vertebrate retina. Int Rev Cell Mol Biol 300: 211–242

Yamamoto T, Ikegame M, Hirayama J, Kitamura K, Tabuchi Y, Furusawa Y, et al. (2020) Expression of sclerostin in the regenerating scales of goldfish and its increase under microgravity during space flight. Biomed Res (Tokyo) 41: 279–288

Yamamoto T, Ikegame M, Furusawa Y, Tabuchi Y, Hatano K, Watanabe K, et al. (2022) Osteoclastic and osteoblastic responses to hypergravity and microgravity: Analysis using goldfish scales as a bone model. Zool Sci 39: 388–396

Yoshimura T (2006) Molecular mechanism of the photoperiodic response of gonads in birds and mammals. Comp Biochem Physiol A 144: 345–350

Zawilska JB, Nowak JZ (1992) Regulatory mechanisms in melatonin biosynthesis in retina. Neurochem Int 20: 23–36

Zhao D, Yu Y, Shen Y, Liu Q, Zhao Z, Sharma R, et al. (2019) Melatonin synthesis and function: Evolutionary history in animals and plants. Front Endocrinol 10: 249

Zisapel N (2018) New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol 175: 3190–3199

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