Melatonin as a Hormone: New Physiological and Clinical Insights

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ABSTRACT Melatonin is a ubiquitous molecule present in almost every live being from bacteria to humans. In vertebrates, besides being produced in peripheral tissues and acting as an autocrine and paracrine signal, melatonin is centrally synthetized by a neuroendocrine organ, the pineal gland. Independently of the considered species, pineal hormone melatonin is always produced during the night and its production and secretory episode duration are directly dependent on the length of the night. As its production is tightly linked to the light/dark cycle, melatonin main hormonal systemic integrative action is to coordinate behavioral and physiological adaptations to the environmental geophysical day and season. The circadian signal is dependent on its daily production regularity, on the contrast between day and night concentrations, and on specially developed ways of action. During its daily secretory episode, melatonin coordinates the night adaptive physiology through immediate effects and primes the day adaptive responses through prospective effects that will only appear at daytime, when melatonin is absent. Similarly, the annual history of the daily melatonin secretory episode duration primes the central nervous/endocrine system to the seasons to come. Remarkably, maternal melatonin programs the fetuses' behavior and physiology to cope with the environmental light/dark cycle and season after birth. These unique ways of action turn melatonin into a biological time-domain–acting molecule. The present review focuses on the above considerations, proposes a putative classification of clinical melatonin dysfunctions, and discusses general guidelines to the therapeutic use of melatonin. ***(Endocrine Reviews 39: 990 – 1028, 2018)***

> *"We wish to report isolation from beef pineal glands of the active factor that can lighten skin color and inhibit MSH. It is suggested that this substance be called 'melatonin'. "* (1)

It was 1958 and the hard-working team of researchers led by Aaron Lerner finally managed to isolate the pineal active factor that had been studied for 40 years and was named for its ability to aggregate melanin granules within the melanocytes. Sixty years later, those researchers' work (1), along with more than 23,000 other published research articles, have shown that melatonin has myriad functions that encompass every live organism in which its presence was attested. From bacteria to humans, this rather simple molecule has proven its skill to be involved in the sustainment of life. This review is dedicated to the study of melatonin as a mammalian pineal hormone and focuses on human physiology and pathophysiology.

It is noteworthy that in spite of the impressive amount of data on melatonin and pineal research, a standard and systematic theoretical framework of analysis is lacking, among researchers and clinicians, that would lead to appropriate interpretation of the obtained data and adequate understanding of the role played by the hormone melatonin in human physiology and pathophysiology. To contribute to this discussion, it is the authors' intention to propose a framework of analysis that would help researchers and health professionals to analyze, understand, and interpret the effects of melatonin and its putative role in several pathologies. The first point is the proposal of a classification of melatonin's well-known ways of action and ensuing effects based on the present experimental and clinical knowledge. It intends to clarify that the hormonal effects of melatonin cannot be deduced and

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ESSENTIAL POINTS

- Melatonin is considered a pineal hormone
- Melatonin is a biological time–domain molecule acting on the circadian, seasonal, and transgenerational timescales
- Melatonin developed special ways of action
- The ways of action determine immediate effects expressed during the night and prospective effects expressed during the following day
- The prospective effects are classified as proximal effects, expressed immediately after melatonin ceases in the early morning, and distal lengthy effects, expressed throughout the following day
- The following melatonin-related syndromes are described: hypomelatoninemia, hypermelatoninemia, circadian displacement, and inappropriate melatonin receptor–mediated responses
- General guidelines about therapeutic uses of melatonin are discussed

interpreted exclusively, as is usually done for other classic hormones, as a result of its ongoing immediate interaction with its molecular effectors. As presented here, several of its effects are primed by this interaction but will appear only several hours afterward, provided melatonin is not present anymore. Additionally, several of its hormonal effects directly depend on the circadian and seasonal characteristics of pineal melatonin synthesis and secretion, which are dependent on its daily repetition, daily duration of nocturnal signal, and seasonal direction of changing (increasing or decreasing period of synthesis), resulting in the timing of the physiological phenomena in the circadian and annual timescale. The second point, based on the understanding of melatonin's ways of action, is to propose a classification of melatonin-related putative clinical syndromes, considering the classic hypoproduction and hyperproduction or function, and introducing the dysfunctions that directly depend on melatonin temporal signal organization and melatonin timing action. Finally, as a third point, the authors introduce a discussion about the therapeutic use of melatonin, taking into consideration its characteristic ways of action.

The definition of a common intellectual framework is essential to guide the planning of experimental and clinical research and data interpretation, allowing the construction of a solid foundation to properly understand the melatonin functional role as a hormone in human physiology and consequently to interpret and deal with its dysfunction in human pathology.

Melatonin—the Basics

Melatonin, chemically characterized in 1959 (2), is an amphiphilic tryptophan-derived indoleamine (232.3 molecular weight) proficient in free radical scavenging with noteworthy antioxidant properties due to its additional ability to stimulate antioxidant enzymes in different tissues. This ancient role has been proposed as melatonin's primary function that is conserved throughout evolution, as melatonin has been found in numerous live organisms from different taxa, including cyanobacteria, dinoflagellates, fungi, flatworms, molluscs, starfish, insects, yeast, plants, fish, amphibians and reptiles, and birds and mammals (3–14).

Melatonin synthesis has been described in all of the above-mentioned organisms, presenting autocrine and paracrine actions. It is also valid for several tissues and organs of multicellular organisms that present local melatonin production with specific intracrine, autocrine, and paracrine effects, such as retina, gastrointestinal tract, bone marrow, lymphocytes, and skin (15, 16). Vertebrates, in addition to that, present a specialized gland, the pineal gland, that synthesizes melatonin to serve as a hormone with endocrine actions.

Pineal gland melatonin synthesis in mammals is timed by the hypothalamic suprachiasmatic nucleus (SCN) master clock and synchronized to the light/dark cycle by the retinal intrinsic photosensitive ganglion cells whose projections to the SCN convey the environment photoperiodic information so that melatonin production is confined to the dark phase of the night (17). Importantly, note that melatonin synthesis is blocked by light at night, an effect mediated by the retinal melanopsinergic system and a complex neural system (18–21) that culminates in inhibition of the sympathetic projection to the pineal gland. In humans this photoinhibition phenomenon is determined by light preferably in the blue range (460 to 480 nm) and in intensities starting at $<$200 lux (60 to 130 lux) (22–24). These two facts (synchronization by the light/dark cycle and nocturnal photoinhibition) are imperative for the role of melatonin as a time domain molecule that synchronizes the organism's internal temporal order to the external daily and seasonal light/dark environment, as presented here (see "Chronobiotic effects" and "Seasonal effects").

Pineal melatonin synthesis timing by the SCN is achieved by its projections to the paraventricular hypothalamic nucleus, which communicates with the higher thoracic segments of the intermediolateral spinal column, conveying information to the superior cervical ganglion from where sympathetic postsynaptic fibers reach the pineal gland, releasing norepinephrine exclusively during the dark phase of the night, triggering the enzymatic conversion of tryptophan to melatonin (25, 26). Tryptophan hydroxylase converts tryptophan to 5-hydroxytryptophan, which is converted to serotonin, which, in turn, is acetylated to *N*-acetylserotonin by arylalkylamine *N*-acetyltransferase (AANAT), and *N*-acetylserotonin is converted to melatonin by acetylserotonin *O*-methyltransferase. Norepinephrine activates β_1 and α_{1b} adrenergic receptors that increase cAMP and protein kinase A (PKA) activity, ultimately increasing cAMP response element binding protein (CREB) phosphorylation and AANAT activity, among other mechanisms, leading to melatonin synthesis activation (27, 28). The above-mentioned neural control of melatonin production is also seen in humans, as neurologic patients showing tetraplegia or lesions on the cervical spinal cord or superior cervical ganglion and its sympathetic trunks and patients submitted to surgical sympathectomy or who are under treatment with beta-blockers show very low levels of melatonin production, losing the expected nocturnal increase and, consequently, its circadian rhythm (29–33). Additionally, note that this particular area of sympathetic innervation is not activated by the well-known characteristic mass mobilization of the sympathetic system, both in humans and in experimental animals (34).

Pineal melatonin is not stored, being readily released to the bloodstream (where it is bound to albumin) and to the cerebrospinal fluid (CSF), reaching several areas of the central nervous system (CNS) and all peripheral organs, where it will trigger different effects by various mechanisms of action that are pointed out below. Melatonin half-life in the blood is ~40 minutes in humans, as it is converted to 6-hydroxymelatonin by cytochrome P450 isoforms (mainly CYP1A2) and conjugated to 6-sulfatoxymelatonin in the liver and kidneys for subsequent urinary excretion (35). In the CNS, melatonin is metabolized to N^1-acetyl-N^2-formyl-5-methoxykynuramine that is deformylated to *N*-acetyl-5-methoxykynuramine (36, 37).

Maternal melatonin is the only source of this hormone both to the mammalian fetus (via placental circulation) and to the mammalian newborn (via breastfeeding), as their pineal glands do not produce melatonin until later after birth (38–40). The ontogeny of human melatonin production was well studied (41–43) and pineal melatonin was found in 3- to 4-month-old full-term infants, reaching its peak in prepubertal children, reducing after puberty and reaching the young adult level (39, 40). After 25 years of age according to some researchers (44) and after 40 years of age according to others (45), pineal melatonin production declines to 60% of the young adult level. From there on, there is a continuous decline to values as low as 20% of the young adult level in people ≥90 years of age (46). Importantly, note that pineal melatonin production is always higher in women at all ages after puberty (43).

Melatonin, Mechanisms of Action, Ways of Action, and Effects

Melatonin mechanisms of action

As for its amphiphilicity, melatonin is able to cross the cell, organelles, and nuclear membranes and directly interact with intracellular molecules in the so-called non–receptor-mediated actions. In addition to that, melatonin also presents receptor-mediated actions that result from the interaction of this hormone with both membrane and nuclear receptors.

Non–receptor-mediated actions

Melatonin is a well-known effective antioxidant, as it is both a proficient direct free radical scavenger [and so are its metabolites (47)] and an activator of a series of scavenging mechanisms such as stimulation of the transcription and activity of antioxidative enzymes (48, 49) and binding to transition metals that inhibits the formation of the hydroxyl radical (50). Besides that, melatonin protects lipids, protein, and DNA from oxidative damage (51, 52), being highly concentrated in the mitochondria (53). The mechanisms of melatonin antioxidant actions are extensively reviewed elsewhere (54, 55).

The antioxidant properties of melatonin are of crucial importance for the mitochondrial functions, a site were free radicals are naturally formed as a result of cellular respiration [reviewed in (56)]. Indeed, melatonin plays critical roles in mitochondrial function besides the antioxidant protection such as regulation of respiratory chain complexes I and IV activities (57) and protection of mitochondrial DNA from mutations and deletions (58). It was recently demonstrated that melatonin is synthetized in mice brain mitochondria and acts through the mitochondrial external membrane melatonin receptor 1 (MT_1), preventing cytochrome *c* leakage and subsequent apoptosis (an action defined as automitocrine) (59).

Some of the above-mentioned effects are usually a consequence of melatonin–protein direct interaction. It is also notable that melatonin plays a role in the regulation of the ubiquitin–proteasome system that ultimately controls protein degradation. Melatonin was reported to inhibit Ca^{2+}/calmodulin-dependent protein kinase II activity and autophosphorylation by a direct interaction with Ca^{2+}-activated calmodulin, acting as an antagonist (60). It has also been suggested that melatonin influences clock genes expression (see

"Prospective effects") by acting as a direct proteasome inhibitor (61).

Protection against DNA damage is fundamental and melatonin has shown to be efficient in doing so due to its antioxidant properties, once elevated reactive oxygen species (ROS) levels are a major cause of DNA damage. Additional mechanisms include the decrease of ATM (a phosphoinositide 3-kinase–related kinase) expression and of the histone H2AX phosphorylation, a step involved in the DNA damage response, among others [reviewed in (62)].

Receptor-mediated actions

The discovery, cloning, and characterization of melatonin membrane receptors were performed in the late 1980s and early 1990s (63–67). MT_1 and melatonin receptor 2 (MT_2), formerly named Mel1a and Mel1b, are high-affinity specific G protein–coupled receptors encoded by the *MTNR1A* (human chromosome 4q35.1) and *MTNR1B* (human chromosome 11q21–q22) genes. Human MT_1 is a 350–amino acid protein, and human MT_2 is a 362–amino acid protein with predicted molecular masses of 39,374 and 40,188 Da, respectively, that were found in several areas of the CNS, including the SCN, mediobasal hypothalamus, thalamus, temporal, parietal, and frontal cortex, hippocampus, the preoptic area, basal ganglia, area postrema, retina, cerebellum, and *pars tuberalis* (PT) [reviewed in (68, 69)]. Peripheral organs such as adipose tissue (70), kidney (71), pancreatic islets (72), parotid glands (73), adrenal glands (74), liver (75), bone (76), skin (16), reproductive tract (77–80), immune cells (81), and cardiovascular system (CVS) [reviewed in (82)], among others, also present MT_1 and MT_2 melatonin receptors.

MT_1 and MT_2 melatonin receptors are heterotrimetric G_i/G_o and $G_{q/11}$ protein–coupled receptors that interact with downstream messengers such as adenylyl cyclase, phospholipase A_2, phospholipase C, and calcium and potassium channels, generally decreasing cAMP and cGMP production and/or activating phospholipase C. MT_1 and MT_2 usually dimerize, forming homodimers or heterodimers that keep both melatonin binding sites functional and with the respective selectivity (83). GPR50 is another G protein–coupled receptor that may dimerize to MT_1, reducing its affinity to melatonin and to melatonin agonists, being a potential regulatory step of this signaling mechanism (84, 85).

MT_1 signaling pathways involve, for example, (1) activation of Kir3.1/3.2 potassium ion channels that mediate the inhibition of neuronal firing in the SCN (86, 87); (2) modulation of protein kinase C (PKC) and phospholipase A_2 (88); (3) modulation of specific ion channels by MT_1 coupling to $G_{q/11}$ proteins (89); (4) mitogen-activated protein kinase kinase 1/2–ERK1/2 pathway stimulation in nonneuronal cells (90, 91); and (5) vasoconstriction (92, 93).

Complementary MT_2 signaling pathways involve, for example, (1) inhibition of cGMP formation and stimulation of PKC activity in SCN (94); (2) regulation of uterus contractility (95); and (3) vasodilatation (96). It was recently suggested that MT_2 melatonin receptors in the SCN might correspond to a G protein–coupled inwardly rectifying potassium channel (97).

MT_3 (previously named ML2) is a third characterized mammalian melatonin binding site (not considered a receptor) that is a form of quinone reductase 2, a detoxifying enzyme (98, 99), and was reported to be involved, for example, in the melatonin-derived increase of chemotherapeutic-induced cytotoxicity and apoptosis in tumor cell lines (100).

Melatonin may also interact with nuclear receptors of the retinoic acid–related orphan receptor (ROR)/retinoid Z receptor group, although that is still controversial in the literature (101–104). Melatonin-induced decrease of 5-lipoxygenase gene transcription was shown to involve ROR/retinoid Z receptor melatonin signaling (105).

Melatonin ways of action and effects

In view of the aforementioned general characteristics of pineal melatonin production and its several specific mechanisms of action, it is noteworthy that to accomplish its physiological role, melatonin presents several ways of action that will determine different time-allocated effects. It is important to note that melatonin's ways of action and ensuing effects might vary according to the considered physiological system; however, they should be broadly taken into account to fully understand and interpret melatonin physiology and pathophysiology.

Immediate effects

Immediate effects are the consequence of what can be called the classic hormonal way of action and are related to melatonin being present in biological fluids and its instant interaction with corresponding molecular effectors. Therefore, these effects are expressed during the night when melatonin is released by the pineal gland and is present in blood and CSF.

Examples of these immediate effects are described in the previous section and are mediated or not by melatonin receptors. The receptor-mediated immediate effects are expected to be quantitatively and/or qualitatively different depending on the target organs, local concentration of the hormone, the type of cellular receptors and signaling system, the duration of the signal, and the affinity and desensitization of the different receptor types. Therefore, the immediate effects of melatonin depend on, among other factors, the phase of the melatonin circadian production cycle (the rising, evening phase; the phase of nocturnal daily peak; and the dawn falling phase at the end of the night) that will determine the concentration of extracellular melatonin and the duration of its interaction with its targets and their sensitivity (106–111).

Prospective effects

Prospective effects are dependent on a special melatonin's way of action, as they are primed during the night (112), through the immediate effects, but are expressed only during the following day when melatonin is no longer present. In other words, the nocturnal action of melatonin triggers cellular and molecular mechanisms that will determine effects that are expressed only after cessation of the melatonin signal and, as a rule, the absence of melatonin during the following day is a necessary condition for their occurrence.

Two types of prospective effects should be considered (Fig. 1), as presently discussed.

Proximal or consecutive effects. Proximal or consecutive effects are expressed immediately after cessation of melatonin signal. Assuming an adequate synchronization to the light/dark cycle, these effects are seen in the beginning of the following day and might last for hours, given that melatonin is not present.

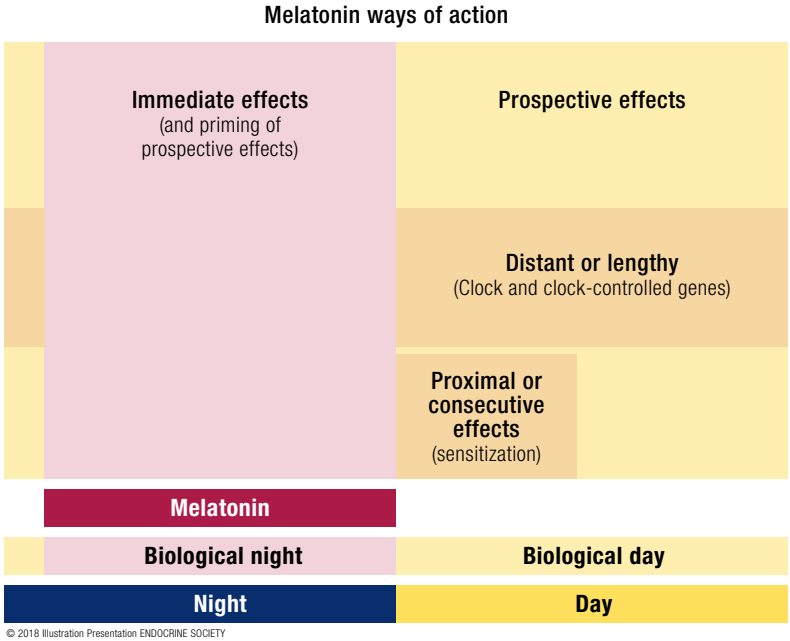


Figure 1. Melatonin ways of action. The daily temporal allocation of immediate, prospective, distant/lengthy, and proximal/consecutive melatonin effects is shown. Using these two different of ways of action (immediate and prospective), melatonin can control cell function distribution during night and day. Immediate effects are all measurable effects occurring during the ongoing melatonin interaction with its effectors. These effects are seen during the night. At the same time and still dependent on these immediate effects, melatonin primes other effects that will only be seen during the following day, if and when melatonin is not present anymore. These prospective effects are divided into two categories depending on the time of occurrence and the primed mechanisms. Proximal effects are dependent on the duration of the nocturnal melatonin signal and depend on the longstanding cAMP synthesis inhibition mediated by $G_{i/o}$ protein coupled to the melatonin receptor. The sensitization of adenylyl cyclase/cAMP signaling system will appear immediately after the ending of the melatonin signal, at the beginning of the day. The distal or lengthy prospective effects depend on the control of the clock genes expression. These genes are the molecular substrate of the circadian rhythmicity in every cell, and their effects are spread throughout the 24-h day mediated by CCGs. [© 2018 Illustration Presentation ENDOCRINE SOCIETY]

An example of these proximal effects is the hypersensitivity or supersensitivity of the intracellular adenylyl cyclase/cAMP/PKA/CREB transduction pathway that is seen after a long-lasting inhibition induced by the interaction of melatonin and its G_i protein–linked receptor, as was demonstrated in several central and peripheral melatonin-responsive systems of mammalian species. Depending on the duration of this interaction, and consequently the duration of a sustained inhibition of the adenylyl cyclase/cAMP/PKA/CREB transduction system, a rebound effect is seen immediately after cessation of the melatonin signal. As a consequence, this proximal effect is maximal during the first hours of the day, when there is a greater effectiveness of the interaction of any agonist of Gsα-linked receptors, increasing the adenylyl cyclase intracellular transduction pathway activation owing to its supersensitization and determining a larger effect than would be observed if melatonin was not present during the preceding night.

This melatonin proximal effect was originally demonstrated in cells of the ovine PT where the effects of different durations of melatonin signal modulated cAMP intracellular signaling sensitization (113, 114). This sensitization effect determined by melatonin, reflecting basal and forskolin-stimulated responses, was demonstrated to gradually intensify depending on the increasing duration of melatonin pre-exposure, being up to 800% higher at the maximal sensitization time as compared with a preparation that was not preincubated with melatonin. The time-dependent sensitization process in this particular system is triggered after 4 hours of melatonin pre-exposure, being maximal after 16 hours; importantly, note that it was not dependent on melatonin concentration or on *de novo* protein synthesis.

In the PT of mice and hamsters, another cellular phenomenon regulated by melatonin proximal effects is the diurnal rhythm of *Per1* transcription and translation (115). This was shown to be a consequence of the nocturnal melatonin $G_{i/o}$-mediated immediate effect that primes sensitization of the adenylyl cyclase/cAMP signaling system that appears when the melatonin signal ends at the beginning of the day. At this precise moment and for a few subsequent hours, pituitary PT cells are sensitized to the induction of *Per1* gene expression by the adenosine A2b receptor–mediated cAMP transduction signal. As a consequence, melatonin, through its proximal effects, is fine-tuning the proper expression of the clock genes transcription/translation inhibitory loop that is critical for the circadian function of this system. Importantly, note that the same kind of proximal effect controlling clock genes expression during the day following a long-lasting melatonin signal is also seen in pancreatic β cells *Rev-erb* (reverse erythroblastosis virus)α and *Bmal1* (brain and muscle ARNT-like 1) gene expression (116).

The same kind of melatonin proximal effect resulting in sensitization of the adenylyl cyclase/cAMP

signal was determined in several other central and peripheral systems. In rat Leydig cells, LH-induced testosterone production was 30% to 40% higher after 16 hours of preincubation with melatonin compared with non–pre-exposed Leydig cells (117, 118). In Chinese hamster ovary cells expressing human MT_1 receptor, the pre-exposure to a physiological concentration of melatonin for a length of time that mimics the period of darkness induced supersensitization of the cAMP-dependent signaling cascade during the withdrawal period, as determined by a potentiation of forskolin-mediated stimulation of cAMP formation, activation of PKA, and CREB phosphorylation (119). This sensitization effect was time-dependent, being maximal at 4 hours after preincubation with melatonin for some parameters (*e.g.*, cAMP) and lasting for at least 16 hours after melatonin withdrawal for other parameters (*e.g.*, phosphorylated CREB).

Among the peripheral systems where the melatonin proximal effect of cAMP signaling sensitization is best studied are the pancreatic β cells and pancreatic isolated islets, with evidence also found in humans. In this system the melatonin proximal effect is shown to be involved in β cell survival, function, and circadian rhythmicity (116, 120–122).

Melatonin may also play a direct facilitatory role of the β cell function by sensitizing the cells to respond to glucagon-like peptide 1 (GLP-1) that results in increased insulin secretion, as shown in the INS-1 rat insulinoma cell line and isolated pancreatic islets (120). Moreover, there is some evidence that a long-duration overnight exposure to melatonin might prevent cAMP-dependent GLP-1 receptor rapid homologous desensitization (123), allowing the receptors to be fully available (if not upregulated) for mobilization the next morning. More recently, it was shown that melatonin preincubation of rat insulinoma cell line INS 832/13 (in a time frame that mimics the typical night exposure), in addition to significantly enhancing the activation of the cAMP-dependent signal transduction pathway as previously demonstrated, attenuated proteotoxicity-induced β cell apoptosis, decreased activation of stress-activated protein kinase/c-Jun N-terminal kinase, and diminished the oxidative stress response (121). Moreover, activation of this kind of melatonin proximal effects (12 to 14 hours of preincubation) in hyperglycemia chronically exposed isolated human islets was shown to significantly reduce the oxidative stress as well as to partially restore the glucose-stimulated and incretin-stimulated insulin response. Similar proximal effects of melatonin were demonstrated in postmortem islets obtained from patients with type 2 diabetes mellitus, except for the incretin-mediated insulin release (121).

Distal or lengthy effects. Distal or lengthy effects are primed during the night and expressed at any time or during the following day, in the absence of circulating melatonin. These effects will be reset by the new episode of melatonin secretion during the following night and are, in general, mediated by the regulation of gene expression and protein translation and degradation, mainly involving clock genes. The clock genes products regulate expression of the tissue-specific output of clock-controlled genes (CCGs) to control the cellular/tissue/organ circadian function. Such rhythmic CCG expression is directly responsible for the daily oscillation of cell metabolism and organ/system function. Therefore, ultimately, melatonin is able to regulate several functions expressed for 24 hours of the day by regulating the dynamic of the clock genes–CCGs interaction (Fig. 2). In other words, through these distal lengthy effects melatonin modulates the period, amplitude, and/or phase of daily expression of these rhythmic genes, participating as an active determinant of the daily functions of central and peripheral systems. In some of them, the absence or amplitude reduction of melatonin (*e.g.*, pinealectomy, light at night, aging) or the absence of receptor-mediated melatonin function (as in single or double MT_1/MT_2 knockout mice or, eventually, single nucleotide polymorphisms) might result in clock genes arrhythmicity. In several cases, adequate melatonin replacement therapy reverts the observed effects on clock and CCGs expression. In addition to these *in vivo* observations, *in vitro* melatonin incubation was shown to be able to trigger, block, and/or modulate clock genes transcription, depending on the cell type and experimental design considered.

A detailed description of these melatonin distal effects is given in the next section.

Chronobiotic effects

A chronobiotic agent is defined as an agent that is able to synchronize and reset biological oscillations (124). The first convincing evidence that melatonin was able to synchronize the behavioral activity/rest circadian rhythm (see Box 1) in mammals came from studies in free-running nocturnal rats that were entrained by 1 mg/kg melatonin when it was injected at or immediately before the beginning of the active circadian phase (125). In addition to showing the chronobiotic property of melatonin, exogenous melatonin entraining action was shown to be critically dependent on the phase of the circadian cycle. A complementary study (126) demonstrated that the entrainment of free-running rats under constant light was achieved at even lower doses of melatonin (~5 μg/kg), pointing to a putative participation of the daily physiological pineal melatonin production in activity/rest circadian rhythm synchronization and stability. It was even demonstrated that melatonin is able to entrain the restored circadian activity rhythms of hamsters bearing fetal SCN grafts (127). In humans, the chronobiotic way of action of melatonin is classically seen in the entrainment of the non–24-hour sleep–wake rhythm disorder either in blind or sighted patients, as discussed below (128, 129).

Owing to these functional characteristics, melatonin is properly defined as a chronobiotic or internal zeitgeber (124, 130–132). As would be expected from a zeitgeber (133), melatonin must act on oscillators according to a well-defined phase-response curve (PRC) (134). A PRC shows the magnitude of response, in terms of phase advance or phase delay, derived from the action of the zeitgeber on the oscillator, being directly dependent on the moment (defined as phase) of its incidence along the intrinsic period of oscillation of the internal clock. Figure 3 shows a classic PRC of circadian activity/rest cycle responses to light pulses. Melatonin PRCs were demonstrated for lizards (135), birds, and mammals (125), besides being clearly and completely demonstrated for humans (110), as shown in Fig. 4. In this case, a melatonin PRC is characterized, as expected, by two opposing regions (phase-advance and phase-delay zones) and a nonresponsive dead zone. The dead zone occurs during the night when endogenous levels of melatonin are usually high. The phase-advance zone is usually located early in the evening, 2 to 7 hours (maximum effect at 3 hours) before the beginning of the nocturnal episode of melatonin production that is characterized by the dim-light melatonin onset (DLMO) or, as in the blind patient, the melatonin onset (110, 136). The phase-delay zone is located in the late night/early morning hours, around (±1 hour) the usual endogenous melatonin offset. This melatonin PRC guides the clinical administration of melatonin as a chronobiotic agent or in replacement therapy. Depending on the desired effect (phase advance, phase delay, or no phase shift), such should be the moment of melatonin administration to the patients.

The chronobiotic effect of melatonin (see Box 1) depends on its putative action in several levels of the circadian timing system. The circadian timing system is composed of a number of structures of the CNS, mainly the hypothalamic SCNs, defined as the central oscillator, that times the peripheral oscillators through neural and/or humoral/hormonal mediators, determining their phase, amplitude, and period. At the cellular level, one basic mechanism of the circadian timing process, present in almost every cell, either central or peripheral, is the cellular molecular oscillatory system represented by the so-called clock genes.

The clock genes oscillatory system is characterized by interconnected negative, positive, and regulatory feedback loops (137). The core clock genes are part of the primary positive/negative transcription/translation molecular loop. In the positive feedback limb, the protein products of the genes *Clock* (circadian locomotor output cycles kaput) and *Bmal1* heterodimerize, and the dimer acts as a transcription factor that positively regulates the transcription of two other groups of core clock genes, *Per* (from Period; *Per1*, *Per2*, and *Per3*) and *Cry* (from *Cryptochrome*; *Cry1* and *Cry2*). In the negative feedback limb of the primary molecular loop, the protein products of these genes, PERs and CRYs, heterodimerize and translocate into the nucleus to inhibit the positive transcriptional activities of CLOCK/BMAL1, resulting in inhibition of their own transcription, causing the cycle to rerun from a low level of transcriptional activity (138). It should be taken into account that the inactivation or degradation of the negative limb proteins PER and CRY is required to terminate the repression phase and restart a new cycle of transcription, setting the period of the clock (139).

The second molecular loop, which is regulatory and stabilizes the circadian period of the first loop (140), is composed of two families of nuclear receptors, REV-ERB α or β and ROR α, β, or γ. The CLOCK/BMAL1 dimer also initiates the transcription of both *Rev-erbα* or β and *Ror* α or β. The REV-ERB and ROR proteins then compete for ROR response element binding sites within the promoter of *Bmal1* where ROR proteins initiate *Bmal1* transcription and REV-ERB proteins inhibit it. One should consider at least a third, more complex transcriptional loop involving several regulatory elements such as DBP (D-box binding protein), PARzip protein (proline

BOX 1. Circadian Rhythms

Circadian rhythms are endogenously generated ~24-hour biological rhythms. The circadian timing system is organized in several levels, from the molecular one, represented by the clock genes, to the systemic regulatory timing neuroendocrine system. This circadian oscillation is directly synchronized by external environmental cycles, mainly the typical light/dark cycle of the geophysical day and night. These external rhythmic events that are able to synchronize biological rhythms are called *zeitgebers* or synchronizers. Synchronization between oscillators is obtained when the phase relationship between them is kept constant. "Entrainment" is defined as the particular case of synchronization between oscillators (with different but similar periods) that occurs when one of the oscillators imposes its period on the other. That is the case of the circadian rhythms. The endogenous period (shown as free-running rhythms in constant conditions) is always slightly different from 24 hours. The day/night light/dark cycle presents a 24-hour period. During the synchronization, the endogenous master oscillator assumes the 24-hour period of the external *zeitgeber*, thus being entrained by it. This entrainment process depends on phase advances and phase delays of the endogenous oscillator that are strictly dependent on the moment of incidence of the external stimulus, defining what is called the phase-response curve (see Figs. 3 and 4).

Excellent books about circadian rhythmicity are the *Handbook of Behavioral Neurobiology*, Vol. 4, *Biological Rhythms*, edited by Jurgen Aschoff (New York, NY: Plenum Press, 1981), in particular chapters 5 through 7, and the classic *The Clocks That Time Us*, by Martin C. Moore-Ede, Frank M. Sulzman, and Charles Fuller (Cambridge, MA: Harvard University Press, 1982).

and acidic amino acid–rich basic leucine zipper), and NAD^+-dependent SIRT1 that add further regulation to the main two clock genes transcription/translation loops (141).

As can be deduced from the above, virtually every stage of the aforementioned gene expression should be considered nodal points for the circadian control of clock genes and CCGs expression. In this context, melatonin, owing to its functional pleiotropic ways of action that regulate basic cellular metabolism, chromatin integrity and gene transcription and translation, nuclear export, microRNA regulation, and mRNA and protein degradation, might modulate almost every stage in clock genes and CCGs expression and function (61, 142–145).

In the SCN, the central circadian pacemaker, *in vivo* experiments and quantitative *in situ* hybridization analysis showed that melatonin systemic injection in rats immediately prior to the light–dark transition is able to modify the mRNA expression of *Rorβ* (preventing the otherwise decreasing expression) and *Rev-erbα* (provoking its phase advance) and does not modify *Rorα* expression (146). Note that although the above-mentioned effects were noticed in the night immediately after melatonin injection (melatonin immediate effects), phase shifting of *Bmal1* mRNA expression is only observed in the following night (melatonin distal and lengthy effect).

In addition to that, melatonin administration to mice at the light–dark transition, in a short-photoperiod regimen, is able to modulate the amplitude (and not the phase) of *Per1*, *Per2*, *Bmal1*, and *Clock* expression in the SCN (147). The classic *in vitro* rat's brain slice preparation containing SCNs was used to study the effect of melatonin on neuronal electrical activity and clock genes expression, and the results demonstrated that melatonin being present at the subjective day-to-night transition alters the SCN clock phasing via the regulation of *Per1* and *Per2* clock genes in an immediate effect mediated by PKC (148).

Additionally, considered that melatonin is able to control the circadian timing of the SCN through prospective proximal effects. The circadian rhythm of SCN neuronal excitability presents two temporal domains that are characterized by distinct sensitivity to specific signal transduction pathways [reviewed in (149)]. The nighttime domain is characterized by the predominance of elevated cGMP level and subsequent activation of protein kinase G, the cGMP-dependent protein kinase. Alternatively, the daytime domain can be characterized by its sensitivity to direct activation of the pituitary adenylate cyclase-activating polypeptide/cAMP/PKA pathway. The presence of MT_1 and/or MT_2 melatonin receptors in the SCN is undisputed (109, 150–152), and considering the ability of both receptors to inhibit adenylyl cyclase, it is conceivable that the long-lasting nocturnal melatonin signal may be considered one important factor determining, through its proximal effects, the daytime domain predominance of supersensitivity to the adenylyl cyclase/cAMP/PKA pathway in the SCN (79). In this context, the role played by the regulator of G protein–signaling protein 16 (RGS16), that functions as a GTPase-accelerating protein for G_i (153), should be additionally considered, as it promotes GTP hydrolysis and arrests GTP-bound G_i signaling. It is shown that melatonin might upregulate the RGS16 gene expression (154) and that RGS16 protein accumulation peaks during daytime (155), acting to overcome the melatonin G_i-mediated effect and contributing to the putative daytime domain of cAMP signaling sensitization.

In addition to the control of clock genes transcription and translation in the central circadian pacemaker, it is noteworthy that melatonin is able to modulate clock genes expression in several other CNS structures as other hypothalamic nuclei, hippocampus, and striatum (156–158).

As far as peripheral oscillator clock genes expression and circadian function control are concerned, pancreatic β cells and islets and the metabolically most relevant tissues (liver, muscle, and adipose tissue) are all targets for melatonin. Mouse pancreatic islets present self-sustained clock gene and protein oscillations that are directly involved with growth, survival, glucose metabolism, and insulin synthesis (159). Ramelteon®, a melatonin receptor synthetic agonist that mimics the melatonin receptor–mediated cAMP signaling sensitization proximal effect, controls *Rev-erbα* and *Bmal1* expressions in rat pancreatic INS-1 β cells (116), illustrating the role played by melatonin in the daily regulation of glucose-stimulated insulin release (160). In rodents, visceral white adipose tissue core clock gene expression (*Clock*, *Bmal1*, *Per1*, *Per2*, *Cry1*, and *Rev-erbα*)

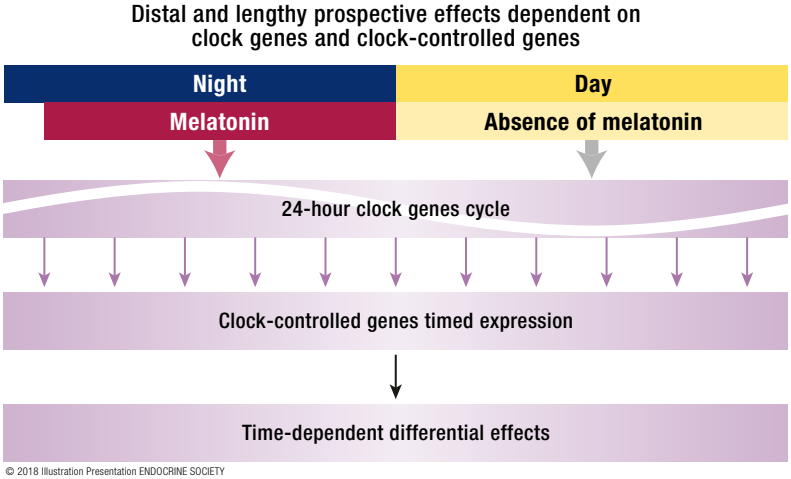
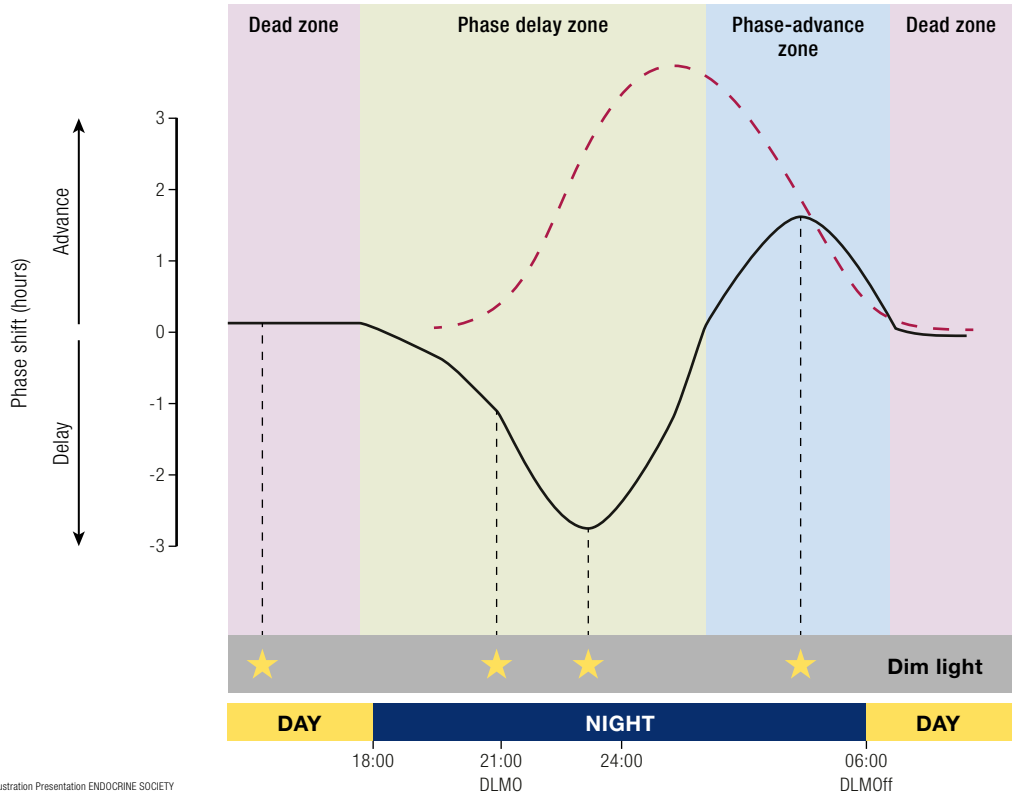


Figure 2. Distal and lengthy prospective effects are dependent on clock genes and CCGs. Melatonin during the night, by immediate effects, might stimulate and/or inhibit the expression of the clock genes. During the day, in its absence, the inverse occurs. Either way, the clock machinery will cycle in accordance to melatonin regulation of the expression of genes either from the positive or the negative loop. Once the 24-hour cycle of the clock genes is defined, the clock genes will, by themselves, control the expression of the CCGs at different phases of the daily cycle. These genes are the effector genes of the cell and will trigger different functions according to the time of the 24-hour cycle. [© 2018 Illustration Presentation ENDOCRINE SOCIETY]

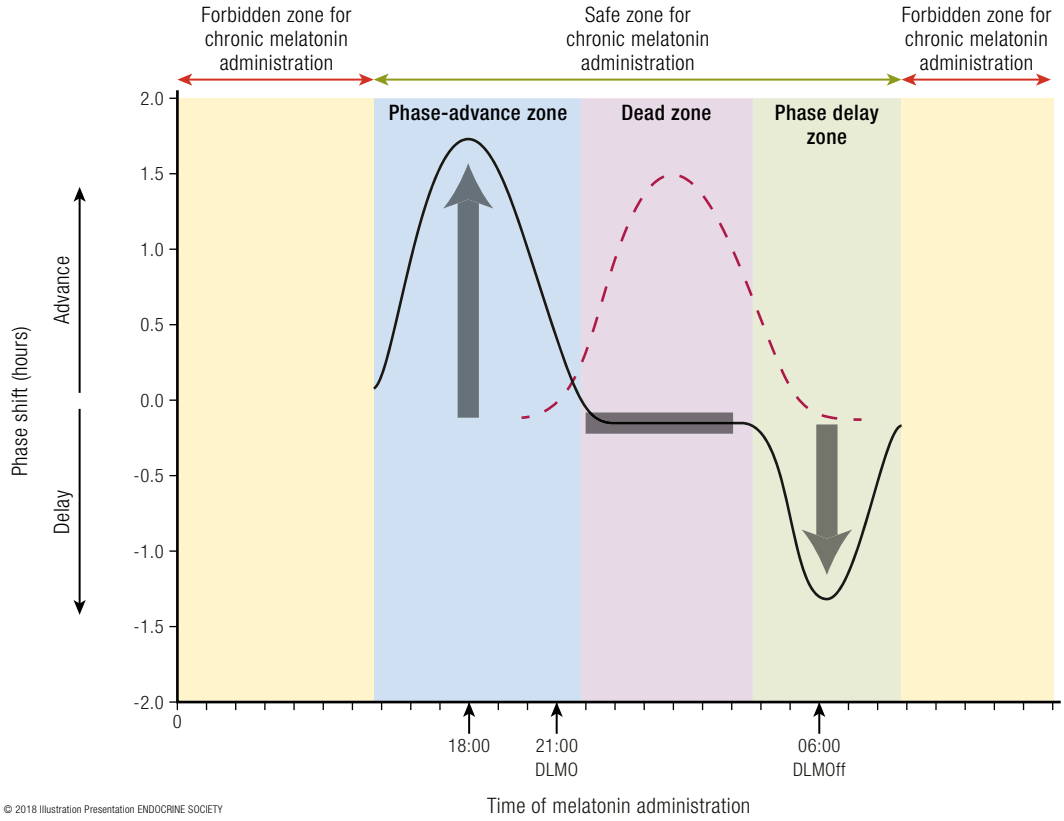
Figure 3. Light PRC. A PRC shows the magnitude of response, in terms of phase advance or phase delay, derived from the action of the *zeitgeber* on the oscillator, being directly dependent on the moment (defined as phase) of its incidence along the intrinsic period of oscillation of the internal clock. The figure shows the PRC (black curve) derived from light stimulation (importantly, note that the PRC depends on the wavelength, intensity, and duration of the light pulse) of young adults under dim light (with gray bar and yellow stars representing light pulses). In this free-running condition, the DLMO or the moment of occurrence of minimal core body temperature is taken as the internal circadian reference time to plan the moment of incidence of the light pulses (measured in units of circadian time—that is, the free-running period divided by 24). DLMO is usually attributed to circadian time 14. The difference between the instant of occurrence of the internal marker (beginning of melatonin secretion or moment of minimal temperature) on the control day and on the day or days after the light pulse defines the phase shift induced by light exposure at that particular circadian time. Despite a light PRC being derived in an experimental free-running condition, the same effects of light on phase-shifting the internal circadian clock can be seen in a day-by-day entrained situation represented by the yellow/dark blue bar. Also shown is the light PRC referenced to the endogenous melatonin rhythm (red dashed curve). As shown, the beginning of the night until about 1 to 2 hours after the DLMO (stated at 2100 h) defines the time zone (green) when the light pulses provoke the maximum phase delays of the circadian clock. Alternatively, light pulses given at the end of the night, near the DLMOff (stated at 0600 h), evokes the maximum phase advance responses (blue). Notably, there is a time zone, usually corresponding to the middle of the day, when light pulses do not phase-shift the circadian clock, which is defined as the dead zone (purple). [© 2018 Illustration Presentation ENDOCRINE SOCIETY]



is under the control of melatonin as well as the daily distribution of its functions such as lipolysis, lipogenesis, leptin production, and adipocyte proliferation (161–164). The same daily organization of functions and clock genes expression is seen in humans (165–168) and seems to be, as well, under melatonin regulation (169). In skeletal muscle, melatonin acting through MT_1 seems to control the amplitude of the clock genes and CCGs *Reverbα* and *Dbp* and to control the phase of *Bmal1* expression (163). In sheep, liver clock genes expression is under photoperiodic control. Under short nights, *Per2* expression peaks at the end of the night, whereas under long nights there is a phase advance of *Per2* and the peak occurs at the beginning of the night (170). In mice liver, a significant reduction of *Per2* daily amplitude in MT_2 knockout animals was demonstrated (163). Moreover, melatonin importantly participates in the daily distribution of liver gluconeogenesis (112, 171).

Reproductive organ circadian rhythms and physiological functions are also under the control of melatonin. In rat Leydig cells (172) the absence of pineal melatonin abolishes *Per2* daily rhythm and increases the daily amplitude of *Per1* gene expression. In this case, melatonin replacement was able to revert the deleterious effects. It was also demonstrated (173) that pinealectomy altered the daily mRNA expression profile of several clock genes in the rat cumulus–oocyte complex. The absence of melatonin abolished the daily rhythm of *Clock*, *Per2*, *Cry2*, and *Rorα* in cumulus cells, altered the amplitude of *Clock*, *Bmal1*, and *Cry2* in oocytes, and phase shifted *Per1* and *Cry1*

Figure 4. Melatonin PRC. The melatonin PRC is derived from the phase shifts obtained by the difference between the DLMO moment of occurrence in a control situation and after oral melatonin exposure, usually taken by individuals in the day-by-day entrained condition. Melatonin PRC (black curve) is characterized by two opposing regions (phase-advance and phase-delay zones) and a nonresponsive dead zone. Melatonin pulses in the dead zone (purple area) occur during the night when endogenous levels of melatonin are usually high (red dashed curve). The phase-advance zone (blue area) is usually located late in the afternoon and early in the evening, 2 to 7 hours (best seen around 3 to 4 hours) before the beginning of the nocturnal episode of melatonin production. The phase-delay zone (green) is located in the late night/early morning hours, ±1 hour around the usual endogenous melatonin offset. The figure also shows the best (safe zone) and the worst (forbidden zone) times to use melatonin in chronic conditions, depending on the desired effects (phase advance, phase delays, or no phase shift at all). It is interesting to compare the melatonin PRC to the light PRC in Fig. 3. At the beginning of the night, light pulses evoke the biggest phase delays; alternatively, melatonin administration would cause the biggest phase advances. The opposite is seen at the end of the night, when light evokes phase advances and melatonin evokes phase delays. [© 2018 Illustration Presentation ENDOCRINE SOCIETY]



in oocytes and *Bmal1* in cumulus cells. Melatonin replacement therapy was able to counteract several of the above-mentioned effects.

The adrenal gland seems to be another peripheral target for the chronobiotic role of melatonin, as it was shown to synchronize and trigger circadian clock genes oscillation. In the capuchin monkey, for example, it regulates the daily adrenal function and *Bmal1* and *Per2* circadian expression peak (174).

Also, note that the literature demonstrated that melatonin controls peripheral clock genes and CCGs oscillation in several other systems such as retina (175, 176), skin fibroblasts (177), and the CVS (178), particularly in cardiomyocytes (179), as well as PT (115, 180) and human myometrial smooth muscle cells (181). As clock genes are well demonstrated in several human cells (166, 167, 182–186), it is conceivable that melatonin might act as one of their synchronizers (181, 187).

Even the mammalian fetus is entrainable by the maternal melatonin signal. It was shown (188) that maternal melatonin regulates SCN *Bmal1* and *Per2* clock genes expression in the primate capuchin monkey fetus. Corroborating the importance of maternal melatonin circadian rhythm as a key signal for the generation and/or synchronization of the circadian rhythms in the mammalian fetus, the absence of maternal melatonin from day 10 to day 18 of gestation in rats markedly affected the mRNA expression level of clock genes and CCGs in the fetus adrenal gland so that *Bmal1* and *Per2* circadian oscillations were abolished and, additionally, the fetal adrenal circadian rhythm of corticosterone synthesis was also abolished (189, 190). All of these effects were overcome in the adrenal glands from fetuses whose mothers received melatonin replacement therapy.

Finally, even the organisms that cohabitate the human body may be synchronized by the host's melatonin profile, as was shown for the malarial

Plasmodium parasite (191) and for a commensal bacterium from the human gastrointestinal system, the noncyanobacterial prokaryote *Enterobacter aerogenes*, of which the circadian clock and daily activity pattern are synchronized by the host's pineal and gastrointestinal melatonin profile (192, 193).

In addition to controlling clock genes/CCGs expression, there are at least two other ways that melatonin can regulate/induce circadian rhythmicity. The first one is regulating the cellular redox state either in central or peripheral oscillators because it is well known that that there is an important functional interplay between the cellular molecular circadian clock machinery and the cellular redox state (194, 195). The other way is through the previously defined supersensitization of adenylyl cyclase and cAMP signaling that appears when melatonin levels decline at dawn. As demonstrated in the PT (115), this prospective effect of melatonin might amplify clock gene expression rhythms, providing an additional mechanism for reinforcing rhythmicity in central and peripheral tissues.

The appropriate interpretation of the chronobiotic effect of melatonin should additionally consider that the effectiveness of a chronobiotic agent or zeitgeber (and melatonin is not an exception) is directly dependent on two other factors: the regularity of the daily repetition of its signal (110, 147, 196, 197) and its strength (198), which is represented here by the contrast between nocturnal and diurnal melatonin concentration values.

In summary, given its periodic circadian release driven by the SCN, the great contrast between night and day circulating concentrations, in addition to the pleiotropic mechanisms of action controlling central and peripheral oscillators, melatonin acts as a powerful chronobiotic hormone and ultimately participates as one of the most important unifying agents that is responsible for the synchrony between the multitude of circadian rhythms at several levels (cell, tissue, organ, and system). Therefore, pineal melatonin hormone is an important player in the determination and stabilization of the internal circadian temporal order, being crucial to the physiological and therapeutic prevention and treatment of chronodisruption (132, 199–201). Moreover, as stated previously, pineal melatonin, due to its SCN-controlled regularly timed synthesis, is mainly linked to the external light/dark cycle rather than to the activity/rest cycle (as are cortisol or corticosterone) of the mammalian organism. Therefore, acting as a photo-neuroendocrine mediator of the light/dark cycle and as an internal zeitgeber, its chronobiotic effect guarantees the adequate daily rhythmic physiological and behavioral fluctuations that are fundamental to the proper circadian temporal relationship between the organism and the environmental day/night cyclic changes.

Seasonal effects

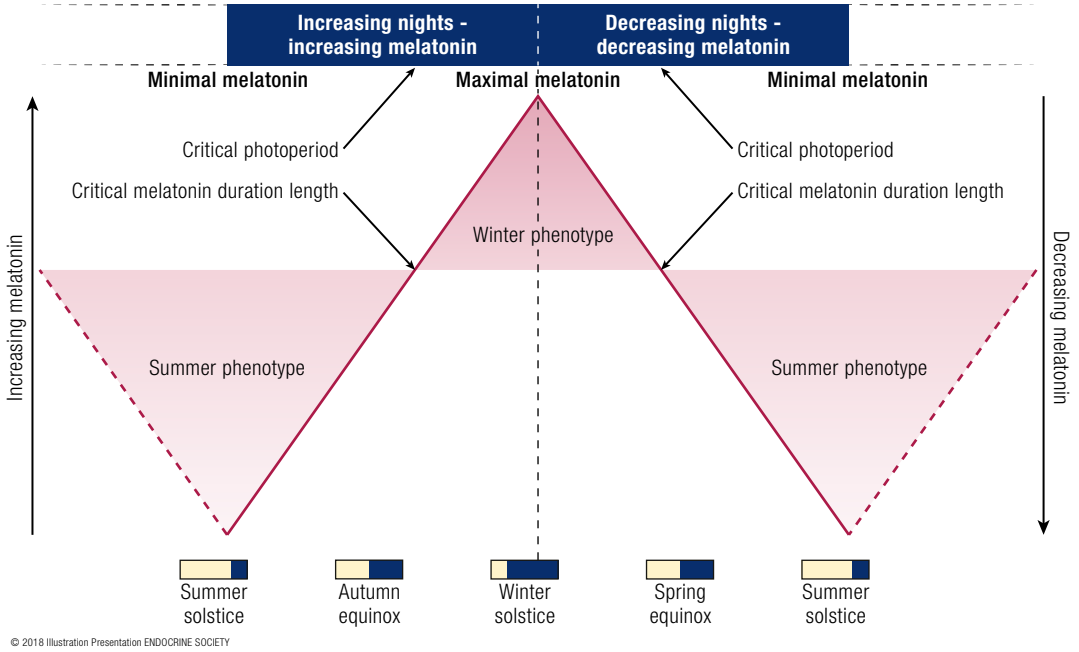
As the chronobiotic circadian effect of melatonin is important for the adequate daily relationship of the organisms and their ecological niche, the seasonal effect mediated by melatonin is fundamentally important to synchronize physiological and behavioral seasonal adaptations to the expected changes in external environmental conditions that are typical of the seasons of the year. These adaptations include annual cycles of reproduction and metabolism, as well as, for example, the consequent growth and body weight control, thermogenesis and brown adipose tissue function, hibernation, migration, and immune responses.

The cyclic photoperiodic annual changes in the duration of day and night are the most important environmental factor for synchronization of circannual rhythms. As the nocturnal melatonin profile varies according to the duration of the night, it internally encodes the photoperiodic annual change in the day and night length. In this manner, melatonin is the internal hormonal mediator that was "sculpted" by the environmental photoperiodic changes so that any seasonal physiological and behavioral adaptations are dependent on this predictable annual regular variation on the daily duration of melatonin signal (202–205), which is also present in humans (see below).

As is well known, there is a critical day/night length ratio (critical photoperiod) that most members of a certain animal population detect as a signal of changing season and switch from one physiological state to another, which will be fundamental for future adaptation to the upcoming season (206, 207). As the duration of the melatonin nocturnal profile encodes this environmental photoperiod, it is well known that there is a critical duration of the nocturnal episode of melatonin secretion capable of triggering the seasonal physiological changes (*e.g.*, brown adipose tissue activation and recruitment, increasing food intake and body weight, modification of the reproductive axis according to the species reproductive profile) that are necessary for the proper adaptation to the seasonal fluctuations of the external environment (*e.g.*, temperature changes, food availability, day and night duration) (208).

Moreover, another fundamental element in signaling seasonal changes is the direction of the daily change in photoperiod (increasing or decreasing day length) and, consequently, the direction of the daily change in the duration of the nocturnal episode of melatonin production. Therefore, the history of the photoperiodic changes and the consequent melatonin nocturnal secretory profile are the major determinants of the seasonal physiological changes that are fundamental for the adaptation of the organism to the expected future seasonal environment. The importance of the history of the annual changes to melatonin secretory nocturnal profile is shown in Fig. 5. As depicted, any given duration of the nocturnal melatonin signal occurs at two different phases of the annual cycle, one in the direction of the winter solstice (increasing night length/increasing duration of

Figure 5. Melatonin seasonal profile. This figure shows the annual seasons and the correspondent photoperiodic change in the duration of the day and the night. At the same time, it is possible to see the annual historic evolution of the duration of the daily episode of melatonin production. The first observation is that the classic calendar of four seasons is reduced to the biological point of view of two seasons, one determined by increasing duration of the melatonin nocturnal episode and the other defined by the increasing reduction of the nocturnal episode. The second point to be observed is the importance of the historical perception of the annual evolution of the environmental photoperiod. Any given duration of the nocturnal melatonin signal occurs at two different phases of the annual cycle, one in the direction of the winter solstice and another in the direction of the summer solstice. However, from the biological point of view, the first one triggers physiological and behavioral preparation for the following winter and the second one for the following summer, depending on how the signal is read and translated by the PT/third-ventricle tanycytes. Finally, note that there is a critical day/night length relationship—the critical photoperiod that is detected as the signal of changing season and switching from one physiological state to another—that is internally represented by the critical duration of the melatonin nocturnal profile. [© 2018 Illustration Presentation ENDOCRINE SOCIETY]



nocturnal melatonin signal) and another in the direction of the summer solstice (decreasing night length/decreasing duration of nocturnal melatonin signal). However, from the biological point of view, the first one triggers physiological and behavioral preparation for the following winter and the second one for the following summer, depending on how the signal is read and translated by the PT, transmitted to the pituitary pars distalis, and, via the third-ventricle tanycytes, transmitted to the hypothalamus (see below) (209–211). Accordingly, the different adaptive effects of melatonin depend on the different phases of the year and are ultimately determined by the hypothalamic detection of the history of the melatonin secretory episode duration, day after day, during the annual photoperiodic cycle. This was exemplified by analysis of two groups of Suffolk ewes that were previously adapted to 16 or 10 hours of light per day (212). These animals are reproductively active during the winter (long night length). The 16-hour light/8-hour dark cycle–adapted group presented reproductive system inhibition whereas the 10-hour light/14-hour dark cycle–adapted group presented reproductive system stimulation. Both groups were transferred to an intermediate 13-hour light/11-hour dark cycle. The first one (previously adapted to 16 hours of light and 8 hours of dark per day) became reproductively active and the other one (previously adapted to 10 hours of light and 14 hours of dark per day) became reproductively inhibited. In other words, in the first group the CNS read the 11-hour melatonin signal as "preparation for the winter" (moving from 8 hours of dark to 11 hours of dark; longer night length), whereas in the second group, the same 11-hour melatonin signal was read in opposite direction and was translated as "preparation for the summer" (moving from 14 hours of dark to 11 hours of dark; shorter night length).

According to the previous discussion, in mammals, the annual sequential variation of melatonin profile duration is the internal representative of the environmental photoperiod (day/night length ratio) that is the main synchronizer of the circannual rhythmicity but not the only one (213). Similar to the circadian chronobiotic effect, the seasonal effect of melatonin depends on its putative action as an "internal circannual zeitgeber or synchronizer" acting on several levels of the circannual timing system that is composed of a number

of structures, mainly the PT, third ventricle tanycytes, and several hypothalamic nuclei (180, 211, 214–216).

The mechanism involved with the synchronizing effect of melatonin that will trigger the physiological adaptation according to the respective seasonal environment depends on melatonin interaction with MT_1 receptors (through immediate and prospective effects as inhibition of cAMP synthesis and its subsequent supersensitization and clock genes daily transcription/translation cycle) at the PT- specific thyrotroph cells (115, 216–218). These specific PT cells express both the α and β subunits of TSH, but they are not under the control of the hypothalamic TSH-releasing hormone, as are pars distalis thyrotroph cells classically. Instead, PT TSH synthesis and release do not respond to TSH-releasing hormone (nor to thyroid hormones) and is under strict control of melatonin, mainly mediated by its MT_1 membrane receptor and clock genes expression regulation (180, 219–222). At night onset, melatonin, through immediate effects, induces *Cry1* gene and CRY1 protein expression (210, 223–226). Alternatively, PER1 protein is induced immediately after melatonin signal offset depending on its prospective effects (primed during the previous night) mediated by cAMP supersensitization (115, 227–229). Therefore, the PER1–CRY1 phase relationship critically determines the amount of PER–CRY dimer—an essential element of the inhibitory limb of the clock genes circadian cycle—being a direct reflex of the seasonal changes in the duration of the nocturnal melatonin signal (210, 230). In this way, the clock genes machinery seems to be the link between melatonin and TSH expression by PT-specific thyrotrophs (231, 232).

In addition to TSH, melatonin regulates neuromedin U (NMU) expression and release in PT-specific thyrotrophs (233). NMU seems to be an important mediator of the seasonal effects of melatonin on energy metabolism as it is detailed below and elsewhere (234). It is interesting to observe that obesity in humans is related to genetic variants of the NMU encoding gene but not of the NMU2 receptor encoding gene (235, 236).

Physiological and behavioral adaptations to the seasonal changing environment are ultimately determined by the hypothalamus. The retrograde functional connection between PT ("season sensor or decoder") and the hypothalamus includes another fundamental element, the third-ventricle tanycytes. Tanycytes are radial glial cells whose cell somas are interposed between the vascular bed of the median eminence and the third-ventricle ependymal cells, whose cellular processes project to the PT and to several hypothalamic nuclei (paraventricular, dorsomedial, ventromedial, arcuate) (237, 238). Tanycytes seem to be key elements, as they are settled at the interface between blood, CSF, and brain tissue. In fact, tanycytes express cell membrane receptors for TSH, NMU, GPR50, FGFR1, IL-6, and LPS, and they are able to transport peptides and hormones by transcytosis, playing the role of sensors of nutrients, hormones, and immune and inflammatory mediators (239–245). Therefore, these cells seem to be the functional link between PT (and, for extension, melatonin-mediated environmental photoperiodic changes), CSF, blood, and hypothalamus, being an important player in the seasonal regulation of reproduction, energy metabolism (ultimately, body weight), and immune function (234, 238, 246, 247).

PT-specific thyrotroph TSH released in the extracellular space acts as a paracrine factor on tanycytes TSH receptors, inducing an increase in the expression of type 2 iodothyronine deiodinase (DIO2) and reducing the expression of type 3 iodothyronine deiodinase (DIO3). DIO2 converts T_4 in T_3 and DIO3 converts T_3 in reverse T_3, degrading T_3 to diiodothyronine. The coordination of both enzyme activities will regulate the availability of the active thyroid hormone in the hypothalamus, modulating several of its nuclei and neuroendocrine systems functions and, consequently, the interplay between the physiological and behavioral functions that are necessary for the organism to cope with the environmental changes (215, 223, 248). Long days (short duration of daily nocturnal melatonin episode) enhance TSH production in PT-specific thyrotrophs, which increases DIO2 expression and suppresses DIO3 expression, increasing thyroid hormone signaling in the mediobasal hypothalamus. On the contrary, short days (long duration of daily nocturnal melatonin episode) trigger an opposite effect. As far as seasonal reproduction is concerned, this T_3 hypothalamic generating mechanism appears to be the same in both long-day and short-day breeders, and thus it must be complemented by some downstream intrinsic hypothalamic species-specific mechanisms to the proper regulation of the final seasonal breeding status (and, perhaps, all other seasonal adaptations) (249–252).

Accordingly, a restricted seasonal differential hypothalamic gene expression following the above-mentioned hypothalamic thyroid hormone availability was recently shown to be the final step in the propagation of the photoperiodic message from PT to the mediobasal hypothalamus (253).

Note that for certain seasonal physiological adaptations such as reproductive activation and inactivation, in addition to the above-described PT retrograde action in the hypothalamus, there is an anterograde action that regulates prolactin production in the pituitary pars distalis. In this case, the melatonin-driven PT mediators are different from TSH and NMU. There is no consensus about their characterization, but messengers from PT to pars distalis include tachykinins, vascular endothelial growth factor, and endocannabinoids (245). An intrinsic endocannabinoid system was shown to be

important for communication between PT and pars distalis, including in the human brain (254).

In summary, the melatonin seasonal effect is dependent on the regular and predictable change of the duration of its synthesis and blood presence across successive nights and how this message is decoded by PT through retrograde and anterograde paracrine mediators. These mediators convey the photoperiodic information to the mediobasal hypothalamus and pituitary, triggering the adaptive physiological and behavioral responses that anticipate the predictable changes of environmental seasons, guaranteeing health maintenance.

The question of whether the melatonin circannual/seasonal synchronizing effect is important in human physiology and pathophysiology should be considered. The evolutionary history of photoperiodism (255) points to a positive response to this question. Its demonstration, however, is not an easy task. All of the experimental studies described previously depend on an elaborate protocol, using artificial photoperiod condition and its controlled changes or, alternatively, in a more naturalistic study, the observation and measurements of physiological and behavioral parameters for at least 1 full year or more. For obvious reasons this is not directly applicable to humans. Additionally, the human cultural attribute allows the artificial control of the environment conditions by the introduction of nocturnal lighting, temperature control, food availability throughout the year, and other factors. As a consequence, these social conditions are expected to reduce the impact of a seasonal changing natural environment to putative natural human circannual physiological and behavioral responses (256, 257). However, using adequately designed epidemiological studies, an adequate choice of communities living in different cultural settings, and proper experimental conditions, it is possible to show that several parameters of human physiology (*e.g.*, birth, puberty, metabolism, body weight, eating behavior, glucose homeostasis, hormonal production, thermoregulation, immune responses, sleep duration) show circannual rhythms so that humans might be characterized as seasonal as any other photoperiodic mammal (246, 258–273). Moreover, humans show seasonal changes in melatonin production (272, 274–276), and melatonin seems to act as a circannual synchronizer in humans, despite no direct demonstration (277–279).

Transgenerational and programming effects

The daily maternal plasma melatonin rhythm typically shows an increase in amplitude from the first to the second and to the last third of pregnancy, reaching a maximum at term and returning to basal levels immediately after delivery, including in humans (280–282). This physiological regular increase of melatonin concentration during pregnancy seems to be dependent, in rats, on the number of fetuses and is under control of some placental factors such as the vasoactive intestinal polypeptide, progesterone, estradiol, and others (281, 283).

Alternatively, it is well known that maternal melatonin is freely transferred to the fetus via the placenta (284–286), and this maternal–fetal transfer of melatonin is the only fetal source of this hormone. Moreover, melatonin concentration in fetal umbilical circulation reflects the day–night difference and nocturnal duration as seen in the maternal circulation (281, 286–290).

This transplacental melatonin has several effects on fetus physiology, including the coordination of peripheral organs and tissues development, neural development and neural plasticity, and metabolic, cardiovascular, and immunological programming, an anticipatory biological response that prepares the offspring to cope with forthcoming environmental demands. Among these programming effects of maternal melatonin on the developing fetus, one remarkable effect is the timing of the fetus future physiology and behavior, another time domain action of hormonal melatonin. Maternal melatonin sends a temporal circadian (time of the day) and seasonal (photoperiod and its history) signal to the fetus so that its CNS is able to properly deal with the environmental day/night fluctuation after birth. It was particularly shown for the onset of puberty that takes the photoperiodic history during gestation time into account to be adequately triggered (211, 289, 291–293).

This phenomenon might be called maternal circadian predictive adaptive programming or maternal photoperiodic predictive adaptive programming. This transgenerational timing programming effect of melatonin is an example of the so-called predictive adaptive responses (294–297). The only difference to the general concept of predictive adaptive responses is that, thanks to the earth rhythmic geophysical temporal organization, the photoperiod and day/night cycle of the future environment is almost confidently predictable and, with a great degree of certainty, there will be no chance of mismatch between the predicted environment and the real one. Therefore, the developmental plasticity determined by maternal melatonin is able to generate an almost perfect temporal adaptation of the offspring to the future day/night cycle and the evolving seasons of the year.

In 1988, an elegant paper showed for the first time the effect of maternal melatonin in timing the circadian behavior of hamster pups (293). They used SCN-lesioned arrhythmic dams (absence of circadian pineal melatonin rhythm) and injected them with melatonin in antiphase (2300 hours and 1100 hours, different groups), using different concentrations, for 4 to 7 days during gestation. The pups wheel-running activity period and acrophase were measured at weaning, and the average acrophases differed between groups by ~10 hours and were correlated to the time of prenatal

"In general, the immediate and prospective effects of melatonin determine the same events both in nocturnal and in diurnal animals, but in opposite phases of the daily cycle."

melatonin injection to the pregnant dams. Moreover, the different peaks of activity of the offspring were exclusively determined by the time of melatonin injection and were not dependent on the dose (10 to 100 μg) or the number of repeated days of injection (4 or 7). A similar set of experiments was done in rats to study the effects of maternal pinealectomy or superior cervical ganglionectomy, with or without melatonin replacement for 5 days in late gestation, on the offspring circadian drinking behavior evaluated for 3 weeks immediately after weaning and during free-running condition (constant darkness) (298). The results show that the maternal melatonin circadian rhythm is also the determinant of the drinking behavior circadian rhythm of the offspring, even though it was evaluated nearly 1 month after weaning. It is noteworthy that scattered drinking behavior acrophases are present both in pups born to pinealectomized dams and in pups born to ganglionectomized ones. It is well known that pinealectomized mammals present no circulating melatonin and that ganglionectomized ones present a residual circulating melatonin throughout the 24-hour cycle of ~12% of the amount of intact animals (299). This might indicate that the critical factor determining the transplacental circadian timing of melatonin is the maternal pineal melatonin circadian fluctuation. Therefore, as observed for the chronobiotic effect of melatonin, the transgenerational timing effect depends on the daily repetition and on the contrast between day and night concentrations of maternal melatonin. This transgenerational timing effect of melatonin was explored at molecular levels (188), in different organs and physiological systems and in both altricial and precocious species, and it is addressed in published reviews (291, 300).

The first convincing demonstrations of *in utero* transplacental transfer of daylength information came from research articles that studied the growth and reproductive development in voles (*Microtus montanus*) (301, 302). Studies of gonadal development in Djungarian hamsters and the effects of prepubertal photoperiodic responses showed for the first time that maternal transfer of photoperiodic information influences prepubertal responses to postnatal different daylengths (303). Additionally, this maternal–fetal transfer of photoperiodic information was so skillful that vole offspring (*Microtus pennsylvanicus*) expressed different postnatal responses depending on the night duration experienced by their mothers: pups born in the autumn (long nights of shorter duration) have much thicker coats than do those born in late winter (long nights of longer duration) (304).

The study of maternal–fetal transfer of photoperiodic information in Djungarian hamsters showed for the first time that the maternal pineal melatonin daily profile was fundamental for the proper maternal–fetal intrauterine transference of photoperiodic information by using pinealectomy, programmed nocturnal infusion of melatonin, and exposure to different environmental postnatal photoperiods (305). In a complementary study, using the same experimental model and animal, the melatonin signal was efficient in transferring photoperiodic information dependent on the daily repetition of the maternal melatonin signal (at least 2 consecutive days) and it also showed an intrauterine-sensitive window to the melatonin timing effect between 3 and 6 days before birth (289).

The mechanism of this prenatal melatonin-induced photoperiodic programming was recently elucidated (211) as it was shown that in Siberian hamsters, the maternal organism primes the fetal PT/hypothalamic tanycytes system so that TSH gene expression in the neonatal PT-specific thyrotroph regulates tanycytes deiodinase gene expression in accordance with the photoperiodic history experienced during pregnancy. As shown, this intrauterine melatonin programming effect uses the same mechanism that is used to regulate the seasonal effect in adult animals.

Melatonin and the Conundrum of Diurnal vs Nocturnal Species: Similarities and Differences

The preceding discussion of the ways of action and effects of melatonin should be taken into account to understand the similarities and differences in the physiological effects and regulation of melatonin on diurnal or nocturnal species. When considering this, it is reasonable to recognize that some of the immediate and prospective effects of melatonin might be different according to the daily distribution of the activity/rest cycle of the studied species. In fact, in nocturnal species such as rats, the immediate effects of melatonin, deduced from melatonin receptor knockdown, pinealectomy, and/or melatonin replacement experiments, result in increased insulin sensitivity, higher glucose tolerance, and increased activity, temperature, and energy expenditure, in addition to resetting the baroreflex so that the rising of blood pressure is limited. Alternatively, the prospective effects of melatonin in nocturnal species include, for example, daytime hepatic insulin resistance and gluconeogenesis.

Conversely, melatonin immediate effects in diurnal species involve, for example, the induction of sleep, blood pressure and temperature dipping, cortisol secretion blockade, and induction of insulin resistance and glucose intolerance. The prospective effects are the induction of daytime insulin sensitivity, pancreatic higher sensitivity to glucose and incretins-induced insulin secretion, regulation of daytime blood pressure, and energy balance.

As can be seen, despite several immediate effects being different and opposite in direction, the biological

role played by melatonin is to trigger at the nocturnal phase, by its immediate effects, the proper adaptive mechanisms to the considered species for that phase of the day and, additionally, to prepare, by its prospective effects, the physiology and behavior to be adaptive when the complementary daytime phase arises.

In other words, in general, the immediate and prospective effects of melatonin determine the same events both in nocturnal and in diurnal mammals, but in opposite phases of the daily cycle.

Unfortunately, the immediate effects of melatonin are the only ones usually considered and discussed in the literature, as is the case with the discussion about the role played by melatonin in the regulation of energy metabolism, particularly in insulin production, secretion, and action. It is well demonstrated in nocturnal mammals (*e.g.*, rats, mice, bats) that the immediate effects, caused by the actual presence of melatonin, are the sensitization of the organism to the action of insulin, either by potentiating the insulin receptor transduction pathways or its peripheral action, mainly increasing GLUT4-dependent glucose uptake in muscle and adipose tissues. It is also shown that the absence or reduced production of melatonin during the night induces insulin resistance and glucose intolerance. The same effects were demonstrated in MT_1 and/or MT_2 knockout mice.

Alternatively, in humans, the immediate effect of melatonin is the opposite: insulin resistance and glucose intolerance. Moreover, nocturnal reduction of melatonin production or reduction of its effects due to putative single nucleotide polymorphisms of its receptors induces daytime defective insulin release in response to an overload of glucose, owing to insulin resistance and glucose intolerance.

In fact, the nocturnal production of melatonin is one of the major determinants of the physiological insulin sensitivity during the day in humans, and during the night in nocturnal rodents. The overall effect of melatonin is the same in both species. If only the immediate effects of melatonin were taken into consideration, melatonin and insulin would be classified as having opposite effects, which is not the case as far as regulation of carbohydrate metabolism is concerned.

The adequate therapeutic use of melatonin is highly dependent on the proper understanding of its immediate and prospective effects. For rodents, nocturnal administration of melatonin increases nocturnal insulin sensitivity and daytime hepatic insulin resistance. In humans, nocturnal administration of melatonin induces nocturnal insulin resistance and diurnal insulin sensitivity. So, in both species the accurate replacement of melatonin during the night induces insulin sensitivity in the respective activity/feeding circadian phase. Therefore, the adequate nocturnal presence of melatonin is essential for the determination of the daily cycle of insulin action.

Additionally, in both species, melatonin acts as a powerful chronobiotic. It means that the consecutive and repetitive daily nocturnal presence of melatonin and the contrast between high nocturnal concentration and diurnal absence or very low concentration help to properly set the circadian clock so that the typical circadian physiology and behavior of the considered species is synchronized to the environmental light/dark cycle of the day and night. So, in addition to considering the immediate and prospective effects of melatonin, its chronobiotic effects should be accounted for to better understand its physiological action and resulting effects to establish a proper putative therapeutic application.

Melatonin, Physiology, Pathophysiology, and Clinical Application

The accurate understanding of melatonin physiological and clinical effects is challenging, as several aspects should be taken into consideration and properly perceived so that its functional characteristics will be adequately interpreted in any considered system or function. The present literature often brings limited highlights of few functional aspects of melatonin, for example, mainly emphasizing the immediate effects, or the supersentization effect, or only the chronobiotic or seasonal effects. However, one should keep in mind that melatonin physiology is integrative *per se* and is dependent on the ontogenetic, daily, and seasonal history of its secretion profile, and on its vastness of actions and resulting effects.

"Experimental studies show that melatonin might affect the sleep mechanism itself, in addition to circadian control."

Owing to its special characteristics, pineal melatonin is a privileged molecule acting through several mechanisms and in almost all levels of the physiology of the organism. As previously stated, it acts by regulating basic cell biology phenomena (59, 61, 62, 145, 306–308) in almost every cell type. As far as its functional integrative action is concerned, melatonin acts both centrally and peripherally in almost every level of several physiological systems: the CNS, every component of the CVS, the energy metabolism system, the reproductive system, the immune system, the hydroeletrolytic regulation system, the respiratory system, the endocrine system, bone, and others. The ways of action and integrative role of melatonin allow the amplification and diversification of its functional action, mainly in the time domain [time domain molecule, "time messenger" (309)] so that it enables the organism's physiology for dealing with the present challenges (immediate effects) and, at the same time, it prepares the organism to deal with future predictive events (prospective effects), in addition to synchronize the organism's physiology and behavior to the daily and annual photoperiodical cycles.

Consequently, it should always be taken into consideration, as far as experimental (even *in vitro* experiments) or clinical studies and treatment are

concerned, that melatonin effects will depend on the time and route of administration, on the concentration and duration of the signal, on the regularity of the daily repetition, and on the traits of the target organ (presence or absence of different melatonin receptors and the associated transduction pathways).

A literature search, early in 2018, showed that there were 3000 to 4000 clinical studies (1000 in the last 5 years) that used melatonin, among which almost 200 were randomized clinical trials. Additionally, from 1996 to July 2017, there were 195 systematic and narrative reviews about the effects of the clinical use of melatonin (96 on melatonin and neurologic and psychiatric diseases, including sleep disorders, and 43 on melatonin and cancer, among others) (310). Additionally, patents and patent applications related to the therapeutic applications of melatonin and its analogs claimed from 2012 to September 2014 predominantly focused on CNS effects (including sleep and circadian disorders, neuroprotection), cancer, and immunological applications (311).

Finally, considering the above-mentioned aspects of experimental and clinical studies and their extent, it follows a selective list of melatonin physiological functions and clinical applications that is surely far from being complete. An embracing approach would require another text exclusively focused on melatonin physiology, pathophysiology, and clinical application. Additionally, to the interested reader, there are several reviews that can be consulted for more embracing aspects of melatonin clinical applications (312–318). The discussion that follows, referenced by melatonin ways of action as described previously, is centered on some specific melatonin functions and dysfunctions, namely, circadian and seasonal rhythmic regulation, sleep–wake cycle, energy metabolism, CVS, and, owing to pineal melatonin privileged access to the CNS, its neural putative action. Melatonin action on these systems show the most consistent clinical effects to date.

Circadian rhythms and sleep are, by far, the most frequently studied areas for melatonin clinical application, which led to almost all of its pharmaceutical-developed analogs. Next in line is ischemia/reperfusion either in the CNS or heart; energy metabolism and diabetes are probably the next important clinical application to be considered; and the CVS pathophysiology, including hypertension, is one of the most promising future areas of melatonin clinical application. It is noteworthy that some areas that show a huge advance in preclinical studies, such as cancer and reproduction, still lack clinical controlled studies to establish, in these cases, a reliable melatonin clinical application.

Melatonin and circadian rhythms

The following discussion is complementary to the above-discussed chronobiotic and seasonal effects of melatonin. Therefore, melatonin acts mainly in humans as a powerful circadian zeitgeber (201, 319).

Based on the melatonin well-defined PRC, its immediate and prospective effects, and its strategically timed repetitive daily prescription, this hormone has successfully been clinically used as a chronobiotic agent. It is used to entrain daily rhythms mainly in clinical syndromes involving circadian rhythm disorders, such as the syndromes involving temporary or permanent circadian misalignment as jet lag, the delayed sleep phase disorder, seasonal affective disorder, and others and in clinical disorders causing circadian free running as it occurs in the non–24-hour sleep–wake rhythm disorder, either in totally blind or sighted patients (128, 129, 319–324).

Importantly, note that melatonin prescription as a chronobiotic agent should rigorously follow the melatonin PRC, taking, in every case, the DLMO as the reference phase to decide the time of melatonin administration in accordance with the desired effect, that is, either phase advance or phase delay (325). Additionally, the duration of the treatment will depend on the case being a transient one (as it is the case for jet lag) or a longer one (as for the treatment of delayed sleep-phase disorder or non–24-hour sleep–wake rhythm disorder).

Melatonin and sleep

The association between melatonin and human sleep started to be studied in the early 1970s (326). In the early 1990s low doses of melatonin, generating near nocturnal physiological plasma levels, were able to reduce sleep-onset latency and oral temperature, triggering the usual polysomnographic patterns of the nocturnal sleep architecture observed in young people (327, 328). By the end of the 1990s it was suggested (329) that the beginning of nocturnal melatonin production is phase locked to the end of the "forbidden zone" and to the opening of the "sleep gate" (330) [zones of increased arousal and induced hypnogogic mechanisms, respectively or, as proposed by Moruzzi (331), the respective appetitive and consummatory sleep behavioral stages], thus triggering the nocturnal circadian episode of sleep. This conception attributes to the nocturnal production of melatonin the property of switching the organism from the biological day (in diurnal species: arousal, energy intake and storage, high cortisol, and active interaction with the external environment) to the biological night (in diurnal species: sleep, low temperature, energy consumption, low cortisol) [as defined in (332)], suggesting that melatonin might promote sleep by regulating the activity–wake/rest–sleep circadian rhythm probably by its actions on melatonin receptors in the SCN (333).

A randomized clinical trial to study disturbed sleep in a population of adults with developmental brain disorders suggested that the efficacy of melatonin treatment (irrespective of the dose) was dependent on

the beforehand amount of endogenously produced melatonin, being that the exogenous melatonin was more effective as the lower was the individual natural nocturnal production (334). Confirming the hypothesis of melatonin regulating sleep by acting on the circadian rhythm, this study showed that melatonin treatment induced an increase in the day-to-night activity ratio (rhythmic amplitude) and a decrease in the fragmentation of the rhythm, increasing its stability, as evaluated by actgraphic records.

The importance of physiological levels of pineal melatonin for human sleep was assessed by studies on pinealectomized patients. Despite some contradictory reports (335, 336), these patients presented a disrupted 24-hour circadian rhythm, a reduction of total sleep time, more nighttime awakenings, poor sleep quality, and, in most cases, all symptoms were reverted by melatonin treatment (337–340).

Experimental studies show that melatonin might affect the sleep mechanism itself, in addition to circadian control. MT_1 and/or MT_2 knockout mice studies showed that the MT_1 melatonin receptor seems to be associated with the incidence of rapid eye movement sleep episodes whereas the MT_2 melatonin receptor was associated with the incidence of nonrapid eye movement episodes (sleep spindles and delta waves) (340, 341). The association of MT_2 to nonrapid eye movement electroencephalographic patterns was demonstrated to be dependent on melatonin action on MT_2 receptors present in reticular thalamic neurons.

Recent reviews and meta-analysis studies of melatonin or its analogs effects on sleep disorders points to its efficacy in reducing sleep latency, increasing total sleep time, and reducing night awakenings, in addition to improving overall sleep quality (323, 324, 342–345). Melatonin, or its analogs, seems to be effective in managing primary insomnia in elderly people, sleep disorders associated with neurologic disorders and neurodegenerative diseases (autism, attention-deficit hyperactivity disorder, Parkinson disease, Huntington disease, Alzheimer's disease), patients with hypertension taking beta-blocker, and rapid eye movement sleep behavior disorder (323, 346–355).

Note that all of the above-mentioned studies are in line with the daily experience in medical practice, where most of the patients report positive results, especially regarding subjective well-being and quality of sleep. Despite that, some guidelines state that the effects of melatonin or its analogs (apart from effects on sleep latency and circadian sleep disorders) are small, of low strength, or insufficient (356–359). These guidelines are mainly based on systematic or narrative reviews that are based on the few randomized placebo-controlled clinical trials. Additionaly, as shown below, the dosage of melatonin should be individually adjusted and would vary among patients. In randomized clinical trials, a fixed and determined dose is usually prescribed to everyone in a relatively large population. The chances of odd results are higher because a greater number of individuals may be inappropriately responsive to the determined dose, differently from what would be expected for a small sample study. This would probably reduce the magnitude of the effect of melatonin in the analyzed outcome. However, one should be cautious, and more adequately planned and controlled studies that take into consideration melatonin ways of action and effects, the melatonin PRC, and, above all, individual differences will surely help to better understand the real therapeutic value of melatonin on human sleep.

Melatonin, energy metabolism, body weight, and diabetes

Melatonin is an important player in the regulation of energy metabolism, including body weight, insulin sensitivity, and glucose tolerance (360).

Melatonin, through its immediate and prospective effects, regulates energy metabolism, acting in every step of the energy balance, including energy intake (eating), energy flow to and from storages, and energy expenditure. Additionally, melatonin, through its chronobiotic and seasonal effects, synchronizes energy metabolism requirements to the daily and annual rhythmic environmental photoperiod.

"The CNS is a privileged target for melatonin physiological action."

Energy metabolism seasonal cycles in mammals are characterized by increased food intake and accumulation of reserves, mainly fat, during spring and summer, followed by reduced appetite and use of stored energy during winter (in addition to, in some cases, a hypometabolic state of torpor or hibernation) (361, 362). These circannual metabolic events are synchronized by the annual change in photoperiod and the consequent nocturnal variation of melatonin profile duration (247).

Alternatively, the circadian distribution of energy metabolism functions allows the synchronization of typical behaviors associated with energy harvesting and eating, which occur during the active/wakefulness phase of the day, with metabolic physiological modifications that assure energy utilization and storage for later use. This daily phase is necessarily associated with high central and peripheral sensitivity to insulin and high glucose tolerance, elevated insulin secretion, high glucose uptake by the insulin-sensitive tissues, glycogen synthesis and glycolysis (hepatic and muscular), blockade of hepatic gluconeogenesis, increased adipose tissue lipogenesis, and adiponectin production. The complementary rest/sleep phase of the day is characterized by fasting associated with the consequent use of stored energy for life maintenance. This phase of the energy metabolism daily cycle exhibits reduced glucose and incretins-induced pancreatic insulin release, insulin resistance, accentuated hepatic gluconeogenesis and glycogenolysis, adipose tissue lipolysis, and leptin secretion. Melatonin is responsible for this daily distribution of energy metabolism

functions (112, 160, 171, 363). Pinealectomized rats show disturbed energy metabolism daily cycles and metabolic disorders resulting in increased body weight associated with increased food intake and reduced energy expenditure (364). Melatonin replacement to pinealectomized rats or supplementation to young, middle-aged, or old rats induces body weight reduction, a decrease in food intake, and an increment in brown adipose tissue energy expenditure (365–367). This energy imbalance toward reducing body weight is likely due to melatonin action on hypothalamic food intake circuits, intensifying the anorexigenic signals and decreasing the orexigenic signals (368, 369).

In postmenopausal overweight women there is a negative correlation between the levels of melatonin urinary metabolite (6-sulfatoxymelatonin) and body mass index (370). Moreover, in a randomized placebo-controlled trial, chronic daily melatonin administration to postmenopausal women induced a reduction in fat mass and increased lean mass (371). In other randomized clinical trials, melatonin treatment was able to counteract the usual metabolic effects of second-generation antipsychotic drugs such as olanzapine and clozapine, including attenuating body weight gain and reducing body fat mass, triglycerides, and total cholesterol levels (372–376). Also note that a reduction in diastolic blood pressure was also demonstrated in some of these studies.

Melatonin regulates the flux of nutrients to and from storages, regulating insulin secretion and insulin action. In nocturnal mammals, such as rats, pinealectomy induces a diabetogenic syndrome that includes glucose intolerance as well as peripheral (hepatic, adipose, and skeletal muscle) and central (hypothalamus) insulin resistance. Melatonin replacement therapy is able to revert all the above-mentioned symptoms [see (360) for a review]. The absence of melatonin results in a reduced total amount of GLUT4 in all of the insulin-sensitive tissues (white and brown adipose tissue, skeletal and cardiac muscle) and impaired central and peripheral insulin signaling (377). Additionally, in rats, nocturnal activation of melatonin immediate effects regulates diurnal insulin sensitivity (112, 171).

In humans (diurnal mammals), acute administration of melatonin induces glucose intolerance both in the morning (decreasing insulin release) and in the evening (decreasing insulin sensitivity) (378). Alternatively, reduction of nocturnal melatonin by photoinhibition induces diurnal whole-body insulin resistance calculated by Matsuda's composite index, and light at night disrupts the expected circadian response to morning GLP-1–induced insulin release (379, 380). These data indicate that even for humans, the nocturnal melatonin profile, despite inducing immediate insulin resistance (what is physiologically expected to occur during the nighttime rest phase of a daytime active species), prepares, through its prospective effects, the daytime insulin sensitivity and β cell sensitization to cAMP agonists insulin-secreting agents. Additionally, nocturnal melatonin secretion is responsible for human β cell survival, contributing to the preservation of β cell mass and function, including in patients with type 2 diabetes (121).

The importance of regular melatonin daily secretion determining daytime high insulin sensitivity is well demonstrated by several clinical and epidemiological studies showing an association between low producers of melatonin and insulin resistance, so that the magnitude of nocturnal melatonin secretion was independently and inversely associated with insulin resistance (381) and lower melatonin secretion was independently associated with a higher risk of developing type 2 diabetes (382). Moreover, there are several research articles showing an association between melatonin receptor dysfunction, resulting from single nucleotide polymorphisms, and type 2 diabetes, gestational diabetes, and insulin resistance associated with polycystic ovary syndrome (383–390).

Additionally, in rats, melatonin participates as an important epigenetic factor regulating fetal and/or neonatal programming of adult energy metabolism (391).

Given the above discussion, several points are stressed as far as melatonin and insulin sensitivity in humans is concerned. First, differently from what might be suggested by the title of some recent papers (392), nocturnal melatonin seems to be an important and necessary determinant of daytime insulin sensitivity provided its signal is restricted to the night and the individual has a normal response to melatonin (*e.g.*, no melatonin receptors genetic variants). Thus, at the very beginning of the day, melatonin production should be interrupted to induce daytime insulin sensitivity and high β cell sensitivity to incretins-induced insulin secretion. When that does not occur for any reason, as when there is a misalignment between the sleep duration and the phase of the waking time and melatonin secretion, it causes an episode of early morning insulin resistance and hyperglycemia, as it is seen in short sleepers, especially in a population showing an *MTNR1B* (MT_2) gene variant (386, 393). Another observation is related to the clinical use of melatonin supplementation. Depending on the melatonin metabolizing characteristics of the individual (see below) and its responsiveness to melatonin, and owing to the nocturnal dose and formulation, the pharmacological-induced melatonin profile (therefore, not amenable to the daytime photoinhibition phenomenon) might extend to the early hours of the morning, resulting in an iatrogenic insulin resistance and hyperglycemia in the morning.

Melatonin and the cardiovascular system

The effects of melatonin on the CVS are well known. Since the 1960s melatonin was shown to be important

in the regulation of the CVS (394, 395) and in particular of blood pressure. Removal of circulating melatonin by pinealectomy, in rats, causes hypertension, and melatonin replacement either prevents or obviates this effect.

In addition to blood pressure, melatonin plays an important role in the regulation of several others parameters of the CVS, including heart rate and vascular resistance (396, 397). Melatonin regulates the CVS using receptor- and non–receptor-mediated effects, immediate, prospective, and chronobiotic ways of action. Among the non–receptor-mediated effects the most important are the antioxidant mechanisms and melatonin action regulating mitochondrial function (398, 399). Melatonin receptors are present throughout the CVS, including the heart (cardiomyocytes, left ventricle, and coronary arteries), blood vessels, and CNS structures involved in CVS regulation (400–402). As far as blood pressure regulation is concerned, in addition to its critical importance in the regulation of blood pressure circadian rhythms (high during the active phase of the day and low during the rest phase of the day) (396, 403, 404), melatonin acts centrally, in the hypothalamic paraventricular nucleus, probably reducing the sympathetic and the hypothalamic–pituitary–adrenal axis outputs (221, 405, 406), in the area postrema (407), regulating the baroreflex set point, reducing the sympathetic tone, and increasing the parasympathetic tone, in the caudal ventrolateral medulla and/or the rostral ventrolateral medulla regulating heart rate (408), and, peripherally, acting in the heart, kidney, and directly in the blood vessels, mediating vasoconstriction and vasodilation (400, 409, 410). Another additional mechanism to be considered is the well-known functional interplay between melatonin and the renin–angiotensin system, particularly involved in the regulation of the blood pressure circadian rhythm (411–413). Moreover, melatonin participates as an important epigenetic factor regulating fetal and/or neonatal programming of adult blood pressure (414, 415).

In humans, in addition to regulating the daily rhythm of blood pressure, melatonin is directly involved, by immediate effects, in the control of the expected blood pressure dipping that occurs during the night (416–418). Despite the need for larger-scale randomized control trials and the existence of some contradictory results, the accumulated experimental and clinical evidence points to the importance of melatonin in human cardiovascular events such as ischemia/reperfusion injury, myocardial chronic intermittent hypoxia injury, pulmonary hypertension, hypertension, valvular heart diseases, and vascular diseases (419–421).

Melatonin and the CNS

The CNS is a privileged target for melatonin physiological action. There is a direct release of melatonin on the CSF due to the particular anatomy of the pineal epithalamic insertion, its vasculature, and the intimate interaction with the third-ventricle pineal recess (422, 423). Additionally, the circulating melatonin might, as well, go to the CSF, increasing the CNS melatonin concentration (424, 425). Melatonin concentration is highest in the third ventricle [where it seems to be directly released by the pineal gland, probably mediated by the tanycytes, as previously suggested (423)], reduces in the lateral ventricle and cisterna magna, and at the lumbar level it is almost equal to the blood, even in humans (426–429). For being present in the CSF, melatonin has a privileged and rapid access to neurons and glia, including the ones located far from the ventricular system and subarachnoid space, mainly by the Virchow–Robin perivascular and extracellular spaces (423, 430). Despite the previously mentioned difference in CSF melatonin concentration, the CSF melatonin profile follows the same circadian pineal production and blood concentration profiles and it is subjected to the same general rule of photoinhibition, free running, and disappearance after peripheral sympathectomy, as is the circadian pineal production (31, 431). Additionally, the CSF melatonin profile/concentration might be correlated with several neurologic disorders such as traumatic brain injury, movement disorders, delirium, and major depressive and bipolar disorders (429, 432–434).

"Melatonin is considered one of the most promising neuroprotective agents to be tested in large clinical trials."

As a complementary factor indicating the importance of melatonin action in the CNS, its receptors are widely distributed in several CNS structures, including neurons and glial cells (435–437).

Considering the privileged access of melatonin directly to the CNS, there is much evidence that it might regulate neural functions in two different ways. In one way, melatonin acts on the general aspects of the neural function, such as neurotransmission and synapse plasticity, neurotrophism, neuroprotection (antioxidant, anti-inflammatory, DNA stability and repair, neurogenesis), and neuroplasticity (neural development, neural stem cell proliferation, neuron maturation, dendritogenesis and axogenesis, and basic mechanisms of long-term potentiation and long-term depression). In the second way, melatonin acts by regulating specific functions, including circadian and seasonal rhythms, reproduction, energy metabolism, sleep, blood pressure, and others.

The following discussion is centered on the general mechanism of melatonin action on neural function regulation.

The role played by melatonin in neurotransmission regulation and synaptic plasticity has been known for a long time (438–443). The cellular basis of this action has been recently revealed (444, 445). Through proteomic and genetic approaches defining protein interactome, in addition to functional studies, the MT_1 melatonin receptor was found at the presynaptic membrane in all studied areas of the CNS

(hypothalamus, striatum, hippocampus, and cerebral cortex), together with the major components of the active zone, as the voltage-gated calcium channel Cav2.2, that, by itself, might connect the melatonin receptor to other synaptic proteins such as SNAP25, Munc-18, and synapsin (444). Moreover, patch-clamp experiments demonstrated that MT_1 receptors interact with voltage-gated Cav2.2 channel and inhibit presynaptic Ca^{2+} entry, providing a molecular and electrophysiological basis for the explanation of a putative general mechanism used by melatonin to regulate neurotransmitter release and, in consequence, to modulate neural function. Moreover, MT_2 receptor interactomes include the presence of several ion transporters and channels, such as the electroneutral potassium chloride cotransporter 1 (SLC12A4), the zinc transporters SLC30A1 and SLC39A6, and the electroneutral Na/HCO_3 cotransporter SLC4A7. In support of this finding, a putative functional association between the MT_2 melatonin receptor and G protein–coupled inwardly rectifying potassium channels was shown to mediate the inhibitory firing rate and the phase-shifting effect of melatonin in the SCN (97, 152). More recently, extending the putative effect of melatonin to synaptic function and plasticity, studies in mice showed that melatonin is directly associated to the hippocampal and striatal daily variation of postsynaptic density of SHANK3 protein (SH3 and multiple ankyrin repeat domains, or proline-rich synapse-associated protein) (445). SHANK3 is a postsynaptic protein associated with dendritic spine growth and maturation, synapse formation and maturation, and, ultimately, brain plasticity (446, 447). Its dysfunction is usually associated with a number of neurologic disorders such as schizophrenia, autism spectrum disorder, and Alzheimer's disease (448).

In addition to this general action on synaptic activity regulation, melatonin regulates neurotrophic factors expression (glial cell line–derived neurotrophic factor, brain-derived neurotrophic factor, conserved dopamine neurotrophic factor, nerve growth factor, persephin, and mesencephalic astrocyte-derived neurotrophic factor) [see (449) for a review] and/or potentiates their neuronal signaling pathway, as for insulin and IGF-1 (377, 450).

Perhaps one of the most important general actions of melatonin in the CNS is neuroprotection. Using several cellular and systemic mechanisms, melatonin protects neuron and glial cells from degenerative actions induced by internal or external insults. Melatonin antioxidant action is an example. Melatonin is highly present in the CNS, as seen before, and due to its special permeability, it might permeate several cellular compartments, in particular the cell membrane and brain mitochondria (451–455) where melatonin is also synthesized (59). The neural tissue of the CNS contains relatively low antioxidant enzyme levels compared with other tissues and contains high concentrations of polyunsaturated fatty acids that importantly contribute to the oxidative processes. Additionally, it is well known that the CNS is a ready target for oxidative stress because the brain represents only 2% of the body weight, receives 15% of the cardiac output, and consumes 20% of the total body oxygen (456), producing more ROS than any other organ and tissue. Therefore, scavenging ROS is a prominent matter because neuronal cells might be injured by oxidative or nitrosative stress, affecting several physiological and behavioral functions and eventually contributing to the origin of neurodegenerative diseases such as Parkinson disease, Alzheimer's disease, and others (457). Melatonin seems to own the most critical importance to accomplish this antioxidant function, most probably contributing to the prevention of neural and consequent systemic disorders (452).

Melatonin is a potent, endogenously produced direct free radical scavenger and broad-spectrum antioxidant. Additionally, mediated by its melatonin receptor–dependent action, melatonin expresses an indirect antioxidant effect upregulating and activating the classical antioxidant enzymes (49, 458). In the antioxidant action of melatonin, it should be considered that one of the main CNS melatonin metabolization process is via N^1-acetyl-N^2-formyl-5-methoxykynuramine and N1-acetyl-5-methoxykynuramine, both potent scavengers of oxygen and nitrogen free radicals (449). This cascade reaction makes melatonin highly effective, even at low concentrations, in protecting the brain from oxidative damage (459).

An important tentative application of the CNS melatonin neuroprotective antioxidant effect is in hypoxic ischemic encephalopathy (HIE), one of the most important reasons for morbidity and mortality of newborns all over the world (460). In HIE, therapeutic hypothermia became a standard treatment of newborns $\geq$36 weeks of gestation, with neonatal encephalopathy related to an intrapartum hypoxic event (461). In a recent prospective trial (462), involving 45 term newborns, 30 with HIE and 15 healthy controls, half of the patients were only treated with hypothermia and the other half were treated with hypothermia and melatonin (10 mg/kg daily for five enteral doses). They found that the melatonin/hypothermia group had better results on the follow-up as far as seizures measured by electroencephalography and white matter abnormalities evaluated by MRI are concerned. This group also improved survival with better neurodevelopmental outcome according to Denver Developmental Screening Test II. In this way, melatonin is considered one of the most promising neuroprotective agents to be tested in large clinical trials (463–468). A more extensive review about melatonin effects on brain ischemia can be read elsewhere (469, 470).

Other general aspects of melatonin actions on the CNS are anti-inflammatory action (471–474), DNA

stabilization and repair (62, 475), neurogenesis, neural development, and neural stem cell protection (476–479), dendritogenesis and axogenesis (480–482), and learning, memory, and long-term potentiation and long-term depression (441, 443, 483–485).

Apart from the regulation of specific functions, melatonin seems to play an essential role in keeping the cellular and systemic integrity of the CNS and preventing neural damage and eventually contributing to prevent the development of neurodegenerative diseases.

Importantly, note that several neurologic disorders (delirium, major depressive and bipolar disorders, autism, attention deficit and hyperactivity disorder, migraines), including the neurodegenerative diseases such as Parkinson disease, Huntington disease, and Alzheimer's disease, present a concomitant and drastic reduction in pineal melatonin production, supporting the routine guidelines to use melatonin as a therapeutic adjuvant in these neurologic disorders (429, 432–434, 486–490).

Description and Characterization of Clinical Syndromes Involving Melatonin Dysfunction

Unhealthy low or high hormonal synthesis and actions are classically described in human pathophysiology. Considering melatonin as a hormone, the present section aims to define the syndromes characterized by hypohormonal or hyperhormonal production and by impaired receptor signaling.

However, as the time domain is one major determinant of melatonin action (duration, daily repetition, immediate and prospective effects, chronobiotic effects, seasonal effects), there are some putative melatonin-related syndromes that are characterized not by hyperproduction or hypoproduction but by the temporal displacement or extension of melatonin's daily profile. Adequate melatonin measurement is a crucial point, and several observations should be made relative to this point.

First, it is possible to measure blood levels of melatonin, saliva melatonin, and 6-sulfatoxymelatonin in the urine (Fig. 6). Differently from any other hormone measurement, where the biological samples are usually collected during the morning hours, either for blood or saliva melatonin, samples should be collected during the evening and the night, at very low level of illumination, preferably avoiding the blue spectrum of light. Ideally, the patient will be in a dark room or under red light of very low intensity (<50 lux) and have the sample collected every 30 minutes or hour during the evening for DLMO determination (110), one of the best indexes of the internal circadian timing, or, even during the entire evening/night for other purposes. Also note that blood collection should not affect the patients' sleep, as it may lead to flawed internal circadian phase determination. That is why saliva is usually collected at the beginning of the evening and should not be collected during the night. Alternatively, urinary 6-sulfatoxymelatonin excretion is directly proportional to the total amount of melatonin produced in a given night, provided that the patient rests under a low level of environmental illumination. Urine collection from dusk to the first urine excretion in the following morning (*e.g.*, the patient should discard the urine excretion at 1800 hours or 1900 hours and start collecting all of the produced urine until 0600 hours or 0700 hours the next morning) is an excellent index of the total nocturnal production of melatonin. The amount of melatonin produced during the night is directly proportional to the 6-sulfatoxymelatonin excreted load that is calculated as concentration times the total nocturnal urine volume. The ratio between 6-sulfatoxymelatonin and urinary creatinine is usually calculated for the normalization by the patient's renal function.

The values of nocturnal melatonin or 6-sulfatoxymelatonin vary individually and according to sex and age. In any case, despite the great variability of the values, comparison with a control population is feasible, provided it was sampled in accordance with the desired clinical design, always paired by sex and age.

Hypomelatoninemia

Hypomelatoninemia is defined by decreased melatonin nocturnal peak value or total production when compared with what is expected for the age- and sex-paired population. Putatively, several symptoms may derive from this syndrome that will vary according to the basal underlying pathology: circadian and sleep disorders (insomnia, chronic daytime fatigue or somnolence, delayed sleep onset, non–24-hour sleep–wake syndrome); hypertension; insulin resistance and glucose intolerance; dyslipidemia; obesity; metabolic syndrome; higher risk of type 2 diabetes; higher risk of cancer, mainly breast and prostate cancer; low-quality aging process, such as frailty syndrome, and others. However, differently from other classical hormonal hypofunction syndromes, hypomelatoninemia is usually not found in isolation, as it can be seen below, being either a participant of a complex genetic disease syndrome or associated with several other diseases, the aging process or environmental disruptors. As a result, the full picture of the putative hypomelatoninemia syndrome is rarely seen. More frequently, fragments of it such as sleep disturbances and circadian rhythms disorders are the more prominent clinical hallmarks, and those should not be seen as modest abnormalities but rather as very serious ones with systemic repercussions that interfere with every other aspect of human physiology and behavior, jeopardizing health, quality of life, and even longevity.

Sleep deprivation is a well-known cause of metabolic disorders such as obesity, insulin resistance, diabetes, and metabolic syndrome in both children and adults (491–493). A subtle daily decrease of 30 minutes in the night sleep episode, for example, may not be perceived by the patient or by the physician, but it increases insulin resistance and body weight in early diagnosed patients with type 2 diabetes, worsening the metabolic picture (494).

Sleep disturbances derived from melatonin reduction are clearly seen in pinealectomized patients (pineal surgical removal usually as a consequence of pineal tumors or cysts) (see "Melatonin and sleep"). However, in the case of these patients who present a clinical situation where the unique hypomelatoninemia syndrome is represented, there are no complete, prospective, and embracing studies so far, and the only available ones are restricted to certain aspects of the expected syndrome such as sleep, circadian rhythms, and eventually some hormonal secretion such as GH, cortisol, prolactin, and ACTH disturbances (338, 495–497).

Alternatively, in aging, some of the typical clinical aspects of the expected hypomelatoninemia syndrome are seen, such as sleep and circadian disorders, insulin resistance/diabetes, hypertension, obesity, immunodeficiency, and a higher incidence of tumors. Some of these signals and symptoms could be obviated by melatonin replacement therapy, probably indicating the pathophysiological role played by the natural reduction in melatonin production in aging.

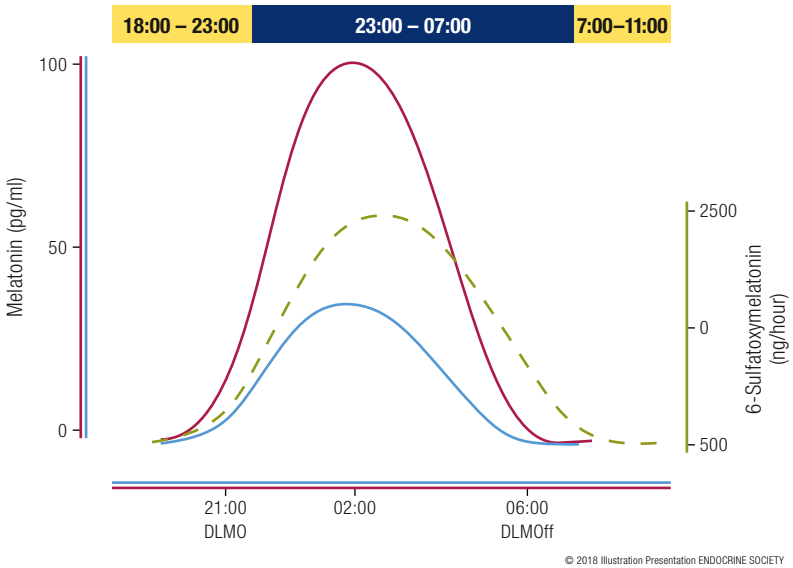


Figure 6. Daily curves of blood and saliva melatonin and urinary 6-sulfatoxymelatonin. Any of these parameters can be measured to show the daily profile of melatonin production. This figure shows the curves deduced from values typical for young adults. The DLMO is best obtained by plasma melatonin (red curve and red axis) analysis, followed by saliva (blue curve and blue axis) assay. 6-Sulfatoxymelatonin (green dashed curve and green axis) is frequently assayed for urine sampling, being the least invasive procedure for sample collection. Blood and saliva samples show melatonin concentrations of the precise time of sampling, whereas urine measurements show the accumulated amount of 6-sulfatoxymelatonin during an interval of time, being usually expressed as the excreted amount of 6-sulfatoxymelatonin per time unit, as shown. Additionally, it is possible to estimate the entire nocturnal melatonin production by measuring the concentration of 6-sulfatoxymelatonin in the evening/night accumulated urine collected in the early morning. It is advisable to refer the 6-sulfatoxymelatonin excretion rate to the renal function evaluated by the concomitant assay of urinary creatinine. [© 2018 Illustration Presentation ENDOCRINE SOCIETY]

Primary hypomelatoninemia

Primary hypomelatoninemia is dependent on factors that directly affect the pineal or its innervation, embryonic formation, or pineal melatonin synthesis as a result of a genetic or innate disease. It might be dependent on pineal agenesis or hypoplasia, sympathetic pineal innervation agenesis, and biochemical defects in pineal melatonin synthesis, as is the case in gene polymorphisms linked to the enzymes involved in the melatonin synthesis pathway (tryptophan hydroxylase, AANAT, or acetylserotonin *O*-methyltransferase) (498–511). The absence of circulating melatonin that follows surgical pinealectomy should be considered primary hypomelatoninemia as well (512).

Secondary hypomelatoninemia

Secondary hypomelatoninemia develops as a consequence of a primary event, such as another disease, or as a consequence of environmental factors, including medications (iatrogenic). Examples of diseases and situations causing secondary hypomelatoninemia are: spinal cord cervical transection, resulting in tetraplegia; cervicothoracic sympathectomy, aging, neurodegenerative diseases (Parkinson disease, Huntington disease, Alzheimer's disease, depression), genetic diseases not directly linked to the origin of the pineal gland and its innervation (*e.g.*, sepiapterin reductase deficiency leading to reduced serotonin synthesis and drastic melatonin synthesis reduction without daily rhythm; fatal familial insomnia and Morvan syndrome); hyperglycemia associated with diabetes; obesity; exposure to light at night; use of drugs that reduce melatonin production (*e.g.*, beta-blockers, calcium channel blockers, inhibitors of angiotensin synthesis and action) and shiftwork (46, 411, 429, 432, 433, 486–490, 513–524).

Hypermelatoninemia

Medical syndromes associated with hyperproduction of melatonin are rare, and there are five clinical situations described so far: spontaneous hypothermia hyperhidrosis, hypogonadotrophic hypogonadism, anorexia nervosa, polycystic ovarian syndrome, and Rabson-Mendenhall syndrome (which is a rare genetic disorder that shows pineal hyperplasia associated with a high level of plasma melatonin and urinary 6-sulfatoxymelatonin) (525–531). Finally, the iatrogenic hypermelatoninemia is characterized by high nocturnal values usually associated with extended duration resulting in high morning levels of circulating melatonin, which are determined by

inadequate control of prescribed melatonin. The reported symptoms in hypermelatoninemia are diurnal sleepiness, sleep episodes, low body temperature, dizziness, and hypotonia (532). In the spontaneous hypothermia hyperhidrosis syndrome, associated with high levels of circulating melatonin also during the day (>1000 pg/mL), it is described by an altered level of consciousness, even complete loss, and syncopal attacks with sweating and hypothermia (body temperature 33 to 34°C). These symptoms associated with a high level of melatonin ameliorate with phototherapy and the patients are successfully treated with beta-blockers (530).

Circadian displacement

In this putative syndrome, the magnitude of the daily melatonin peak is usually not altered but the blood melatonin nocturnal curve is displaced in time. It might be completely displaced to daytime as in the Smith-Magenis syndrome, delayed as in attention-deficit/hyperactivity disorder, or it is extended to the morning, surpassing the nocturnal sleep episode. It also may not be synchronized to the light/dark cycle at all, and it would rather be free running, as observed in some circadian rhythms disorders (*e.g.*, totally blind people). Depending on the case and the phase-shifting desired effect, patients should be adequately treated with melatonin given at the right phase and dosage as discussed elsewhere (325). The symptoms associated with a circadian melatonin displacement are a result of the consequent chronodisruption and include sleep and awake states assigned to unusual times, daytime somnolence or even sleep episodes, low diurnal performance, nocturnal insomnia, chronologically misplaced eating behavior, insulin resistance or sensitivity not temporally related to the sleep–wake cycle, and others (386, 393, 533).

Inappropriate melatonin receptor–mediated response

In the case of inappropriate melatonin receptor–mediated response, pineal melatonin production is adequate, temporally restricted to the night, and controlled by the circadian clock and the annual photoperiod. However, mainly due to genetic variations of melatonin receptors (either MT_1 or MT_2), usually single nucleotide polymorphisms, the central and peripheral responses of the target organs to melatonin are impaired. The symptoms will be defined based on the affected tissues and can virtually include any of the above-mentioned ones.

The first point to be emphasized is that melatonin receptor polymorphisms do not necessarily generate nonresponsive receptors. There is evidence in the literature pointing to the fact that single-nucleotide polymorphism–bearing receptors may inappropriately respond to the melatonin signal, either as a hyposensitive system or a hypersensitive system. This inadequate receptor response may render the physiological system nonresponsive to the regulatory role exerted by melatonin and, in consequence, it will express a nonadaptive response to the daily and annual rhythmic behavioral and physiological demands. The immediate consequence is chronodisruption, with the symptoms described previously. The proposed pathologies associated with melatonin receptor variants are type 2 diabetes, gestational diabetes, sleep and circadian disorders, Graves disease, impaired metabolic response to a hypocaloric diet, metastasis, polycystic ovarian syndrome, and others (383–387, 390).

Therapeutic Use of Melatonin, Its Pharmacokinetics and Toxicology

This section addresses general criteria to be considered when medical doctors intend to prescribe melatonin to their patients. It is more a series of questions to be considered rather than the determination of specific dose values, formulation, and time of administration [see (315–317) for a thoughtful review]. Moreover, in spite of the several available melatonin receptors agonists [*e.g.*, Ramelteon® (Rozerem™, Takeda Pharmaceuticals America, Inc., Deerfield, IL), Agomelatine® (Valdoxan™, Servier Laboratories, Paris, France), Tasimelteon® (Hetlioz™, Vanda Pharmaceuticals Inc., Washington DC)], the present discussion is restricted to melatonin, the natural biological product [note that Circadin® (Neurim Pharmaceuticals Inc., Tel-Aviv, Israel) is pure melatonin in a slow-release formulation].

The time domain is a critical factor to be considered in chronic melatonin treatments, as this molecule has a unique characteristic of being a hormone that regulates the timing of the organism physiology and behavior.

Melatonin physiological production is precisely timed every day, and the beginning of its synthesis helps to set the circadian time to the central clock, concomitantly with the triggering of the biological night for the CNS and the peripheral targets. The early morning shutting down of its production, timed by the central clock and reinforced by the early morning lights, ceases the biological night and triggers the biological day. The history of the duration of the biological nights throughout the year times the annual calendar of the organism. In addition to this daily and seasonal time domain variation of melatonin signal, every organism has a particular ontogenetic history of the magnitude of the daily peak of melatonin production, as seen previously. Moreover, it should be considered that the melatonin profile is unique for each person (512), showing very large interindividual variation. However, for each individual, "the timing, amplitude, and even the details of the profile are highly reproducible from day to day and week to week rather

like a hormonal fingerprint" (534). As a consequence of this individual variation, the duration of the daily signal and its onset phase (DLMO) vary from person to person, mainly based on the chronotype and sleep duration type (morning or evening types, long or short sleepers). "Early birds" or morning people start the melatonin daily production earlier than the "night owls" or evening people do, and the duration of nocturnal melatonin production in long sleepers is more prolonged than in short sleepers. It should also be considered that a certain melatonin dosage could result in different plasma concentrations in different patients due to interindividual differences in absorption, distribution, metabolism, and elimination of melatonin, which are related to age (from premature babies to elderly people), clinical condition (relatively healthy to critically ill), pathologies, and functional integrity of some physiological systems such as the gastrointestinal tract, liver, and kidney. These substantial differences, if not adequately taken into account, may potentially impact the desired clinical efficacy.

All of the above-mentioned statements point to the fact that a proper chronic melatonin hormonal replacement therapy is only achieved when dosage and formulation are carefully chosen and individually tailored and controlled to accomplish the desired clinical effect.

The first and most important aspect would be to determine the DLMO of each patient and then prescribe melatonin according to this reference time point. As this procedure is not feasible in the everyday clinical practice, another more practical approach is to reference the daily nocturnal time intake of melatonin to the so-called usual time of sleep during the night. The time each person chooses to go to sleep every night is determined, in most cases, by chronobiological type (mainly if allowed by social constraints). As most oral melatonin formulations take ~45 minutes to 1 hour to be bioavailable, it is advisable to prescribe melatonin to be taken about an hour before the reported usual bedtime every day. As melatonin is a powerful timer of the organism physiology, this timed everyday intake should be rigorously maintained. The second and an important aspect to be considered before melatonin is prescribed is that its nocturnal pharmacological profile should be restricted to the natural biological night of each patient. That is to say that the dose and the pharmaceutical formulation (fast, slow, or mixed-release forms) should be wisely considered. If chronic replacement therapy is in question, the preferred formulation should generate an initial blood concentration high enough to set the time of the circadian clock, and the dynamics of the drug release, absorption, and metabolism should also build a nocturnal profile very close to the one that would be physiologically produced by the patient. Moreover, and foremost, the duration of the pharmacological nocturnal profile has to be such that it will end by the usual wake time of the patient. This is a very important point to be observed because the pharmacological melatonin profile is not subjected to the early morning photoinhibition seen for the physiological melatonin production. Daytime somnolence is a good clinical criterion to be considered as an indication of morning extension of the melatonin pharmacological profile. The gold standard to check if that is the case would be to measure melatonin either directly in the plasma or saliva or to measure the urinary metabolite 6-sulfatoxymelatonin early in the morning (we should remember that there is no other plasma hormonal reference to be used as a marker of a higher or lower dosage, as is the case for several of the hormones dependent on the hypothalamus–pituitary axis, as melatonin production is not subjected to the classical feedback phenomenon).

Dosage is another crucial point to be discussed, and there is no general consensus in the literature. Average young people taking ~0.1 to 0.3 mg of melatonin will present plasma concentration in the range of 100 to 200 pg/mL that is considered "physiological," and 1.0 mg would result in plasma concentration of ~500 to 600 pg/mL, far higher than the physiological concentration (535, 536). These values should be taken into consideration when replacement therapy is in question. However, depending on the clinical application (316), one can find a large range of used dosages, as 0.1 mg/d for the central clock synchronization, 0.6 to 5 mg/d for sleep disorders treatment, 300 mg/d for amyotrophic lateral sclerosis treatment (537), or even 2000 mg as a short-period drug administration (538). The same is true for pharmaceutical formulations and routes of administration (oral, nasal spray, skin patch and creams, intravenous and suppositories). However, depending on the primary outcome, the pharmaceutical formulation matters importantly. For example, if the outcome is an acute (for some or a few days) phase displacing, as it is desired for jetlag treatment, a fast-release pulse correctly timed (according to the PRC) is perfectly adequate. However, if the desired effect is a sustained phase displacement as, for example, in non–24-hour sleep disorder or circadian dysfunction in totally blind people, the synchronizing effect requires chronic continuous daily intake of melatonin. In this case, as described previously, the chronobiotic effect requires not only the first nocturnal pulse of melatonin but also an all-night melatonin signal to prime the next day's prospective effects. For this purpose, a slow-release or dual-release formulation is the most appropriate. When the first primary desired outcome is a hormonal replacement therapy, as is the case in aging and several diseases that present melatonin production reduction such as diabetes, autism spectrum disease, neurodegenerative diseases, and others, or pinealectomy, the most appropriate formulation would be, as well, a slow or dual release that would build a pharmacological profile more similar to the natural physiological one.

Another point to be discussed is management of the melatonin daily profile in the displacement situations previously described. The first approach is to use melatonin and/or phototherapy to try to displace the circadian rhythms in the case of circadian rhythms disorders. Classically, the use of melatonin and light should strictly follow the PRCs for each of them (Figs. 3 and 4). As the respective PRCs are in phase opposition (light during the day and melatonin during the night), the approach should be amended accordingly. If the intended goal is to phase advance the rhythms, melatonin should be given at the end of the afternoon or at the beginning of the evening, and light should be applied at the end of the night or at the beginning of the day. Conversely, light in the evening and melatonin at the end of the night should be used if the aim is to phase delay the rhythms. However, in this particular case, it should be carefully considered because, as stated before, the daily extension of the melatonin profile could be a putative chronodisruptive signal. If the intended clinical outcome is to synchronize free-running rhythms, as in totally blind patients, melatonin should be given strictly at the same clock time every day. Finally, for the circadian displacing treatment, as seen in patients with Smith-Magenis syndrome, it is necessary to block daytime melatonin production, which is not subjected to the usual photoinhibition by the melanopsinergic retinal system in these patients (533), using regular β-blocking agents and, additionally, it is necessary to build a nocturnal pharmacological melatonin profile that would replace the absent natural nocturnal one. As a final observation, melatonin ultimate blood concentration and profile depend on the absorption, transportation, and, most importantly, on the hepatic metabolism. In this last case, interaction between melatonin and other drugs should be considered mainly when the drug is metabolized by liver cytochrome P450 enzymes, particularly CYP1A2 (35, 539–542). Depending on drug interactions, the melatonin plasma profile might be abnormally extended, resulting in adverse morning effects.

Exogenous melatonin pharmacokinetics, toxicology, and safety have been studied in a limited manner, but the general conclusion is that melatonin lacks toxic adverse effects, being a safe drug for clinical treatments. Given that the correct timing of administration and the adequate dose and formulation were followed, the most common tested concentrations vary from 0.5 to 5 mg/kg, but a report administered up to 800 mg/kg to rats with no measurable toxic adverse effects (543). The 50% lethal dose for intraperitoneal melatonin injection was determined for rats (1168 mg/kg) and mice (1131 mg/kg), but it was not possible to be determined for melatonin oral administration (tested up to 3200 mg/kg in rats) and for melatonin subcutaneous injection (tested up to 1600 mg/kg in rats and mice) (544). Adverse effects were also not found either in the pups or in the rat dams treated with 200 mg/kg/d (545).

Exogenous melatonin pharmacokinetics and bioavailability are known in experimental animals (546, 547) and in humans (548). Human studies considered different routes of administration and patients of various ages, pointing out that the time to reach maximal plasma concentrations is ~45 minutes for orally administered melatonin, with a generally low bioavailability due to the first-pass metabolism in the liver [reviewed in (549)]. Besides that, age, liver metabolic status, and drug interactions may influence plasma melatonin levels.

Despite the great number of studies about the clinical use of melatonin, few have been designed to be randomized, double-blinded clinical trials that would test for safety and the adverse effects of exogenous melatonin in humans (550). By all means, melatonin administration is shown to be safe, and the adverse described effects are usually either irrelevant or are also described in the placebo groups.

Several studies (551–553) performed in newborns and older children who received different doses (0.5 to 10 mg) of oral or intravenous melatonin for the acute or chronic management of a number of clinical issues (newborn ischemia, respiratory distress, surgery, epilepsy, Smith-Magenis syndrome, autism, attention-deficit/hyperactivity disorder, and other neurologic diseases involving primary or secondary sleep impairment) have proven melatonin safety. It is noteworthy that the pubertal development of children treated with 0.3 to 10 mg/d for an average of 3 years was not different to the observed in nontreated age-matched children (554).

"The general conclusion is that melatonin lacks toxic adverse effects, being a safe drug for clinical treatments."

Melatonin lack of toxicity and clinical safety were also shown in studies (316, 555) involving adults and elder patients who were treated with various doses (0.1 to 300 mg/d) of oral, intravenous, or rectal suppository melatonin for short or long treatment of sleep disorders, jet lag, depressive disorders, attention-deficit/hyperactivity disorder, autism, amyotrophic lateral sclerosis, Huntington disease, diabetes and metabolic syndrome, polycystic ovary syndrome, and frailty, among others.

Finally, two unresolved questions about melatonin clinical therapy regarding its putative seasonal effect in humans and high dosages remain. Given that humans show, despite the social and cultural aspects of environmental modifications, circannual behavioral and physiological rhythms, and that melatonin might play some role in their determination, the question that remains is: Should chronic melatonin treatment, in terms of dosage and formulation, vary according to the annual season?

The second question is related to chronic high-dosage treatment as seen for multiple sclerosis (300 mg every night, at bedtime, for up to 2 years) (537), where the diurnal levels of melatonin were very high, although to a lesser extent than the values observed during the

night. In that study, the authors explicitly stated that the treatment was generally well accepted and "no signs of hangover or increased fatigue during daytime were noted." Is it possible that the organism chronically adapts itself to a new level of melatonin oscillation so that "normal" physiological and chronobiotic responses would be stated? The answers to these two questions will depend on adequately planned clinical and experimental future scientific work.

Concluding Remarks

This review considered melatonin as a pineal hormone. As stated, melatonin is a special hormone that acts in the biological time domain. To deal with these timeline effects, melatonin developed receptor-mediated or nonmediated unique ways of action. The immediate way of action is similar to the classic and well-known hormone–effector interaction. The difference is that, in addition to the immediately measurable effects (*e.g.*, inhibition of cAMP synthesis and its consequences for the considered system), melatonin primes during the night, either by sustained action via $G_{i/o}$ PCR and subsequent supersensitization or by regulating the expression of clock genes and CCGs, effects that will only be seen during the following day, after the melatonin signal ceases. Moreover, owing to its special mechanisms of synthesis and synchronization to the environmental light/dark cycle, melatonin acts as an internal synchronizer of circadian and circannual rhythms, consequently synchronizing the organism physiology and behavior to the environmental day and night and seasons of the year.

Additionally, melatonin is crucial to the transgenerational transplacental circadian and seasonal time transfer. The offspring is prepared in advance to deal with the environmental daily and annual phase after birth, expressing what should be considered a typical time domain predictive adaptive response.

Considering these particular hormonal physiological characteristics of melatonin, it is possible to define some melatonin-related pathological syndromes and discuss general guidelines toward clinical melatonin therapy assessment. In addition to the classical hormonal syndromes related to hypomelatonin and hypermelatonin production, clinical syndromes related to inappropriate melatonin receptor–mediated response and to melatonin characteristic timing effects should also be considered, as they cause the rupture of the internal temporal organization of the organism, resulting in several putative signals and symptoms.

As far as therapy using melatonin is concerned, several cautions should be taken into consideration: restrict the chronic administration to the night; the time of administration should be carefully chosen according to the desired effect; and the dosage and formulation should be individually adapted to build a blood melatonin profile that mimics the physiological one and end by the beginning of the morning.

In addition to that, the present urban society and the industrial production processes as organized should be taken into account, as both depend on the presence of indoor lights during the night and include the profuse use of electronic devices whose screens are rich in blue wavelength light. Light during the night delays the beginning of the secretory episode of melatonin and blunts its peak, causing chronic hypomelatoninemia, sleep deprivation and, eventually, chronodisruption (513).

Finally, after 60 years of melatonin isolation, it is about time to systematize the present knowledge and to introduce a common language in the field. It is also about time for the endocrine and neural sciences to consider the pineal as a gland and melatonin as a hormone, including the addition of these subjects to medical and biological science course programs, and to define clinical syndromes involving alterations in melatonin production, such as described earlier. Concurrently, it is crucial to discuss and establish guidelines for the clinical use of melatonin for treatment purposes. The present review aimed to shed light on some of these topics, stimulating a broader and clearer way of thinking about and perceiving melatonin.

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Abbreviations

AANAT, arylalkylamine *N*-acetyltransferase; *Bmal1*, brain and muscle ARNT-like 1; CCG, clock-controlled gene; *Clock*, circadian locomotor output cycles kaput; CNS, central nervous system; CREB, cAMP response element binding protein; *Cry*, *Cryptochrome*; CSF, cerebrospinal fluid; CVS, cardiovascular system; DIO2, type 2 iodothyronine deiodinase; DIO3, type 3 iodothyronine deiodinase; DLMO, dim-light melatonin onset; GLP-1, glucagon-like peptide 1; HIE, hypoxic ischemic encephalopathy; MT_1, melatonin receptor 1; MT_2, melatonin receptor 2; NMU, neuromedin U; *Per*, Period; PKA, protein kinase A; PKC, protein kinase C; PT, pars tuberalis; PRC, phase-response curve; *Rev-erb*, reverse erythroblastosis virus; RGS16, G protein–signaling protein 16; ROR, retinoic acid–related orphan receptor; ROS, reactive oxygen species; SCN, suprachiasmatic nucleus.