MT_1 and MT_2 Melatonin Receptors: A Therapeutic Perspective

Jiabei Liu, Shannon J. Clough, Anthony J. Hutchinson, Ekue B. Adamah-Biassi, Marina Popovska-Gorevski, and Margarita L. Dubocovich

Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, University at Buffalo, State University of New York, Buffalo, New York 14214;
email: jl284@buffalo.edu, shannonc@buffalo.edu, ah64@buffalo.edu, eba@buffalo.edu, mgorevsk@buffalo.edu, mdubo@buffalo.edu

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Abstract

Melatonin, or 5-methoxy-*N*-acetyltryptamine, is synthesized and released by the pineal gland and locally in the retina following a circadian rhythm, with low levels during the day and elevated levels at night. Melatonin activates two high-affinity G protein–coupled receptors, termed MT_1 and MT_2, to exert beneficial actions in sleep and circadian abnormality, mood disorders, learning and memory, neuroprotection, drug abuse, and cancer. Progress in understanding the role of melatonin receptors in the modulation of sleep and circadian rhythms has led to the discovery of a novel class of melatonin agonists for treating insomnia, circadian rhythms, mood disorders, and cancer. This review describes the pharmacological properties of a slow-release melatonin preparation (i.e., Circadin®) and synthetic ligands (i.e., agomelatine, ramelteon, tasimelteon), with emphasis on identifying specific therapeutic effects mediated through MT_1 and MT_2 receptor activation. Discovery of selective ligands targeting the MT_1 or the MT_2 melatonin receptors may promote the development of novel and more efficacious therapeutic agents.

INTRODUCTION

Melatonin: a molecule produced in the pineal gland following a circadian rhythm with high levels at night

Circadian rhythms: the endogenous rhythms generated in constant conditions (i.e., in the absence of external cues) with a defined amplitude and period

Melatonin receptors: G protein–coupled melatonin receptors, termed MT_1 and MT_2

The rhythmic release of melatonin (5-methoxy-*N*-acetyltryptamine) from the pineal gland (for a review, see Reference 1) and retina (2) helps coordinate circadian rhythms and neuroendocrine processes via activation of two G protein–coupled receptors, termed MT_1 and MT_2 (3) (**Figure 1**). The circadian production of pineal melatonin is controlled by endogenous oscillators within the suprachiasmatic nucleus (SCN) and entrained by daily and seasonal changes in the environmental light-dark cycle (4). Endogenous melatonin released from the pineal gland at night may feedback onto the SCN and activate MT_1 and MT_2 receptors to phase shift local and overt circadian rhythms (**Figure 1**). This review thus focuses on the actions of exogenous melatonin and melatonin drugs on functional melatonin receptors associated with therapeutic effects, specifically those affecting the central nervous system and cancer (**Figure 1**). Emphasis is placed on drugs currently on the market targeting MT_1 and MT_2 melatonin receptors for the treatment of insomnia, circadian sleep disorders, major depression, and cancer (**Table 1**). Furthermore, we discuss MT_1 and/or MT_2 receptor–mediated responses to be considered as potential targets for the treatment of learning and memory deficits, neurodegeneration, and drug addiction.

Melatonin acts on the MT_1 (formerly called Mel1a or ML_{1A}) and MT_2 (formerly called Mel1b or ML_{1B}) receptors (3) (**Figure 2**). The human MT_1 and MT_2 (hMT_1 and hMT_2) melatonin receptors show distinct molecular structures (5), pharmacological characteristics (3, 6, 7), and chromosomal sites (4q35.1 for MT_1, 11q21–q22 for MT_2) (8). Human MT_1 and MT_2 receptors are 350 and 362 amino acids long, respectively, with molecular weights of 39–40 kDa and 55% amino acid homology overall (70% within the transmembrane domains) (5). The MT_1 melatonin receptor couples to pertussis toxin–sensitive G_i and –insensitive $G_{q/11}$ G proteins and inhibits

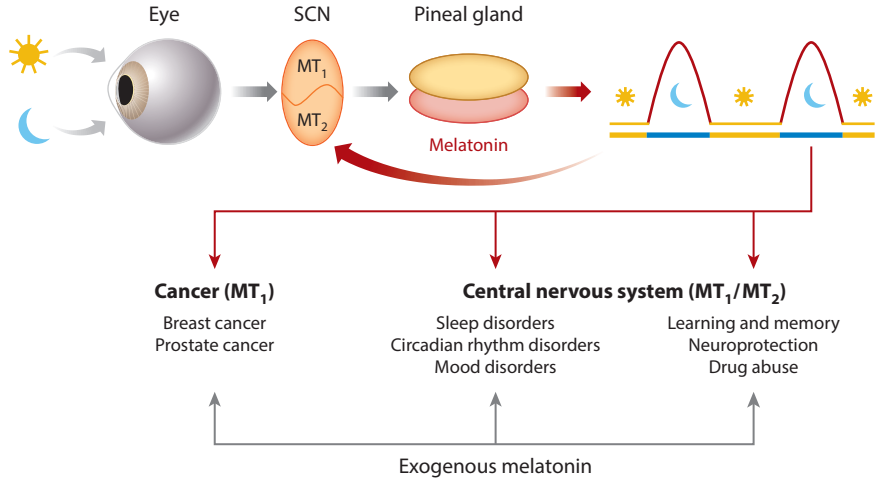
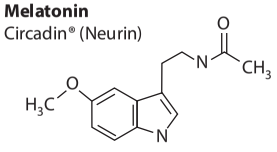
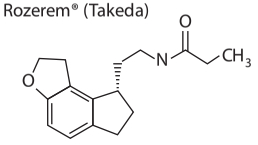


Figure 1

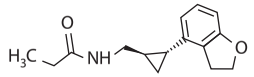
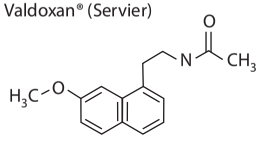
Therapeutic implications of exogenous melatonin. Melatonin production in the pineal gland and locally in the retina follows a circadian rhythm, with the highest levels produced during the dark phase. The rhythmic production of melatonin is controlled by endogenous circadian oscillations and entrained by the light-dark cycle with a 24-h period. Pineal melatonin activates MT_1 and MT_2 melatonin receptors in the SCN, discrete brain areas, and peripheral tissues to signal photoperiodic information and regulate physiological functions. Exogenous melatonin modulates processes and responses in the central nervous system via activation of the MT_1 and/or MT_2 melatonin receptors. Further melatonin activation of MT_1 receptors decreases breast and prostate cancer cell growth. Abbreviation: SCN, suprachiasmatic nucleus.

Table 1 Clinical and preclinical effects of currently marketed drugs targeting MT_1 and MT_2 melatonin receptors

Marketed melatonin receptor agonists	Approved use	Affinity and functional responses			Clinical studies	Preclinical studies
		MT_1 (K_i)[a]	MT_2 (K_i)[a]	5-HT_{2C} (K_i)[b]		
Melatonin Circadin® (Neurin) 	Insomnia in the elderly	0.08 nM	0.38 nM	NE	Insomnia in the elderly (65) Sleep disorders in children with neurodevelopment abnormalities (67) Circadian rhythms entrainment in blind subjects with non-24-h sleep-wake disorders (50, 52, 68) Seasonal affective disorder (53) Breast and prostate cancer (135, 137, 150)	Inhibition of SCN neuronal firing (MT_1) (9, 14) Phase shift of circadian rhythms in primates (49) and rodents (MT_1) (61–64) Modulation of REM (MT_1) and NREM (MT_2) sleep (30, 76, 77) Antidepressant-like effects in rodent model of depression (81–85) Inhibition of LTP (primarily MT_2) (110, 111) Neuroprotection in Huntington's disease (MT_1) (118) and ischemic strokes (MT_2) (120, 121) in rodent models Oncostatic properties in cell lines and rodent models of breast and prostate cancer (MT_1) (140, 142, 145, 150)
Ramelteon Rozerem® (Takeda) 	Primary chronic insomnia	0.014 nM	0.11 nM	NE	Primary chronic insomnia (71) Phase advance when given before bedtime (72)	Accelerates re-entrainment of circadian rhythms after an abrupt phase advance of dark onset in rats (73) Phase advances neuronal firing rhythms in mouse SCN slices (MT_1 and MT_2) (62, 63) Phase advances onset of circadian activity rhythms in C3H/HeN mice (MT_1) (62, 63)

(*Continued*)

Table 1 (*Continued*)

Marketed melatonin receptor agonists	Approved use	Affinity and functional responses			Clinical studies	Preclinical studies
		MT_1 (K_i)[a]	MT_2 (K_i)[a]	5-HT_{2C} (K_i)[b]		
Tasimelteon Hetlioz® (Vanda) 	Non-24-h sleep-wake disorder in totally blind individuals	0.30 nM	0.07 nM	NE	Sleep-wake disorder in totally blind individuals (74) Transient insomnia after phase shift (40)	Phase shifts circadian activity rhythms (75)
Agomelatine Valdoxan® (Servier) 	Major depressive disorder	0.01 nM	0.12 nM	708 nM	Major depressive disorder (37) Improves disturbed sleep patterns and circadian/seasonal rhythm entrainment (99–101)	Increases adult hippocampal neurogenesis (91, 92, 103)

Abbreviations: 5-HT_{2C}, serotonin; LTP, long-term potentiation; NE, no effect; NREM, non-REM; REM, rapid eye movement; SCN, suprachiasmatic nucleus.

[a]K_i values for competition of melatonin, ramelteon, tasimelteon, and agomelatine for 2-[^{125}I]-iodomelatonin for the human MT_1 and MT_2 melatonin receptors stably expressed in Chinese hamster ovary cell lines were obtained from References 42, 42, 41, and 7, respectively.

[b]K_i values for competition of melatonin, ramelteon, tasimelteon, and agomelatine for radioligand binding to 5-HT_{2C} receptors were obtained from References 43, 42, 41, and 43, respectively.

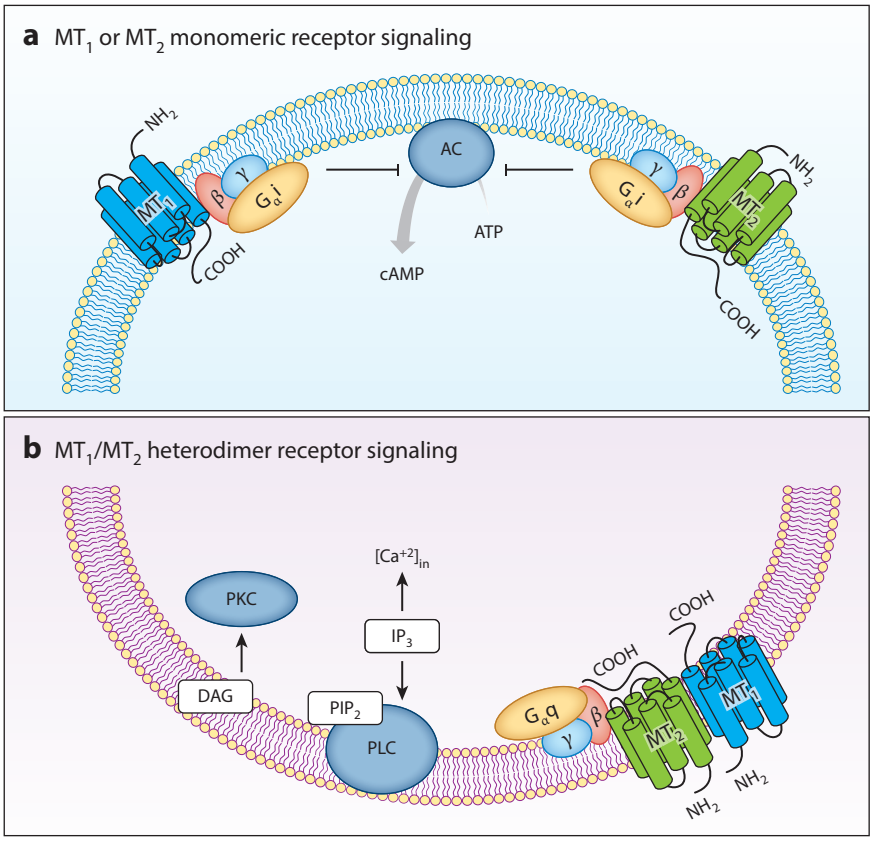


Figure 2

Melatonin receptor signaling. (*a*) MT_1 or MT_2 monomeric receptor signaling. Both MT_1 and MT_2 melatonin receptors couple to pertussis toxin–sensitive $G_{\alpha i}$, β, γ and –insensitive G_q, β, and γ proteins, inhibiting forskolin-stimulated cAMP, protein kinase A signaling, and CREB phosphorylation (see Reference 3). (*b*) MT_1/MT_2 heterodimer receptor signaling. Human MT_1 and MT_2 receptors form homo- and hetero-oligomers between themselves, altering the pharmacological properties of the individual receptors. Formation densities of the MT_1/MT_2 heterodimer and the MT_2/MT_2 homodimer are similar and 3- to 4-fold lower than the MT_1/MT_1 homodimer. Native functional MT_1/MT_2 heterodimers have been characterized in mouse rod photoreceptors, where they mediate the enhancement of scotopic light sensitivity by melatonin through a heterodimer-specific PLC and PKC pathway (21). Abbreviations: AC, adenylyl cyclase; DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C. Figure modified with permission from Reference 151.

forskolin-stimulated cAMP, protein kinase A signaling, and CREB phosphorylation. The MT_1 receptor also increases phosphorylation of mitogen-activated protein kinase 1/2 and extracellular signal–regulated kinase 1/2, as well as increasing potassium conductance through K_{ir} inwardly rectifying channels. MT_2 melatonin receptor activation inhibits both forskolin-stimulated cAMP production and cGMP formation, activates protein kinase C (PKC) in the SCN, and decreases calcium-dependent dopamine release in the retina (3).

Assessing the pharmacology and function of melatonin receptors is challenging, as their native binding site densities in animal tissues are low or undetectable, particularly for the MT_2 receptor (9). The MT_1 and MT_2 receptor sites have been localized to discrete areas of the rodent and human

nervous system—including the SCN, cerebellum, thalamus, hippocampus, as well as peripheral tissues—using receptor autoradiography with 2-[^{125}I]-iodomelatonin, in situ mRNA hybridization techniques, and immunohistochemistry (10–13). MT_1 and MT_2 receptor function has been established using mice with genetic deletion of either the MT_1 or MT_2 melatonin receptor (9, 14) or pharmacological approaches through the use of prototype competitive melatonin receptor antagonists such as luzindole and 4-phenyl-2-propionamidotetralin (4P-PDOT) (15, 16).

Receptor antagonist: a ligand that produces no response and blocks the response of an agonist

Inverse agonist: a ligand that binds to a receptor and induces a response opposite that of the agonist in the absence of activating ligands

Receptor agonist: a chemical that binds and activates a receptor to produce a maximal biological response

Partial agonist: a ligand that activates a given receptor, reaching partial efficacy relative to full agonists even at maximal receptor occupancy

A ligand is considered selective on a specific receptor when its affinity, potency, or both are at least 100-fold higher with respect to the other receptor type under consideration (3). Luzindole, a nonselective ligand with 15- to 25-fold higher affinity for the MT_2 melatonin receptor (6), and 4P-PDOT, a selective MT_2 ligand with 300- to 1,500-fold higher affinity for this receptor (6), are considered the gold standards for pharmacological characterization of melatonin receptors. Luzindole and 4P-PDOT competitively block MT_1 melatonin receptors at concentrations of 300 nM or more, and both act as inverse agonists in systems endowed with constitutively active MT_1 receptors (17–19). *N*-butanoyl-2-(2-methoxy-6H-isoindolo[2,1-a]indol-11-yl)-ethanamine (IIK7) is a selective MT_2 melatonin receptor agonist at recombinant hMT_2 receptors with an affinity ratio of approximately 90 when compared with hMT_1 (20). However, its affinity for the mouse MT_2 receptor is 1,000-fold higher than for the MT_1 receptor (21), suggesting considerable species differences in melatonin receptor pharmacology.

Researchers have been successful at discovering high-affinity and selective ligands for the MT_2 receptor (3); however, there is a lack of selective MT_1 receptor ligands with high efficacy (22). The MT_1 selective ligands available are either antagonists or partial agonists with high selectivity for human recombinant MT_1 receptors in competition for 2-[^{125}I]-iodomelatonin binding; this selectivity is significantly reduced in functional studies (23). S26131, a dimer formed by linking two molecules of agomelatine, shows over 200-fold higher affinity for MT_1 and blocks melatonin-mediated stimulation of ^{35}S-GTPγS binding to receptors expressed in heterologous mammalian cells with less than 30-fold selectivity (23). None of these selective MT_1 antagonists have been tested in vivo.

The efficacy of ligands targeting G protein–coupled receptors in target tissues is dependent on the cellular milieu, G protein type and/or scaffolding molecules, spare receptors, and homodimers and/or heterodimer formation, as well as receptor structure among various species (24, 25). These changes in efficacy lead to species-dependent fluctuations in in vivo ligand selectivity. For example, the affinity of our prototype competitive melatonin receptor antagonist luzindole (MT_1/MT_2-nonselective) and 4P-PDOT (MT_2-selective) varies considerably between human, ovine, rat, and mouse receptors (26–28). 4P-PDOT is considered a selective ligand on hMT_2 receptors when compared with hMT_1; however, the aforementioned properties make selective occupancy of MT_2 receptors in vivo difficult, yet authors often conclude that in vivo functional responses are mediated through MT_2 receptors using 4P-PDOT doses that will raise drug blood concentrations to readily occupy the MT_1 receptors as well.

When establishing ligand efficacy and selectivity, investigators must also consider the observation that simultaneous activation of both MT_1 and MT_2 melatonin receptors expressed in the same or different cells could lead to additive, synergistic, or opposing responses that amplify or diminish each other (29–31). Although the minimal functional unit seems to be monomeric, we know from bioluminescence resonance energy transfer experiments that hMT_1 and hMT_2 receptors form homo- and hetero-oligomers between themselves and other G protein–coupled receptors. Formation of MT_1/MT_2 heterodimers is 3- to 4-fold lower than the formation of MT_1/MT_1 homodimers and is similar to the formation of MT_2/MT_2 homodimers (32) (**Figure 2**). These dimers are functionally relevant and distinct from receptor monomers, as demonstrated by Baba and colleagues (21). These authors reported MT_1/MT_2 heterodimers in mouse rod photoreceptors modulating

light sensitivity via a phospholipase C (PLC)/PKC pathway not normally triggered by activation of monomeric units (21) (**Figure 2**). Monomeric forms of the MT_1 or MT_2 receptors, however, are differentially desensitized by exposure to melatonin depending on concentration (physiological versus supraphysiological), time of exposure (e.g., short versus long) (33–35), cellular background (7), and receptor state (quiescent versus constitutive) (33–35). Thus, ligands with higher affinity or selectivity for the MT_1 or the MT_2 receptor could conceivably tip the MT_1/MT_2 heterodimeric receptor selectivity balance, providing a therapeutic advantage over melatonin and current nonselective synthetic ligands by potentiating or facilitating responses mediated by the target receptor.

MT_1 and MT_2 receptors also form heterodimers with the serotonin ($5\text{-}HT_{2C}$) receptor (36). This is of particular interest in understanding the antidepressant mechanism of agomelatine as both a melatonin receptor agonist and a $5\text{-}HT_{2C}$ receptor antagonist (36, 37). The formation of $MT_2/5\text{-}HT_{2C}$ heterodimers is more efficient than the formation of $MT_1/5\text{-}HT_{2C}$ heterodimers and $5\text{-}HT_{2C}$ homodimers. The $MT_2/5\text{-}HT_{2C}$ receptor heterodimer exhibits a G_i signaling response to melatonin and agomelatine, i.e., inhibition of forskolin-stimulated cAMP production (36). However, melatonin transactivates the G_q pathway through $MT_2/5\text{-}HT_{2C}$ heterodimers increasing inositol phosphate production. Interestingly, melatonin receptor antagonists, luzindole, and 4P-PDOT also transactivate the $5\text{-}HT_{2C}$ receptor–mediated G_q pathway. Moreover, MT_1 and $5\text{-}HT_{2C}$ heterodimeric receptor formation increases $5\text{-}HT_{2C}$ receptor trafficking to the cell surface and potentiates β-arrestin recruitment induced by melatonin and 5-HT (36). Thus, melatonin receptor heterodimerization (**Figure 2**) with $5\text{-}HT_{2C}$ receptor and possibly other receptor types generates additional signaling responses with therapeutic potential that should be explored as new melatonin-like drugs are developed.

Melatonin [Circadin® (38)] and several melatonin analogues are currently used clinically {i.e., ramelteon/Rozerem® [insomnia (39)], agomelatine/Valdoxan® [depression (37)], and tasimelteon/Hetlioz® [non-24-h sleep-wake disorder (40, 41)]} (**Table 1**). These compounds modulate circadian rhythms and neuroendocrine function in rodents and humans. However, all are nonselective agonists and compete for hMT_1 and hMT_2 receptors with almost equal affinities (6, 42, 43). Ramelteon shows a 10-fold higher affinity for the hMT_1 than the hMT_2 melatonin receptor and a 17-fold higher affinity than melatonin (42). Agomelatine and tasimelteon have a slightly higher affinity for the hMT_2 receptor even though both are nonselective agonists (7, 41). Ramelteon (42), agomelatine (44), and tasimelteon (41) have remarkable selectivity for melatonin receptors, as they show no affinity for many other G protein–coupled receptors, enzymes, and neurotransmitter channels (42, 45, 46). The only exception is agomelatine, which shows high affinity for MT_1 and MT_2 melatonin receptors but is also an antagonist at the $5\text{-}HT_{2C}$ serotonin receptor, a property believed to contribute to its antidepressant effects (for a review, see Reference 37). The following sections describe the therapeutic potential of melatonin, agomelatine, ramelteon, and tasimelteon in the treatment of insomnia and circadian sleep disorders, depression, and cancer, and they discuss potential new melatonin receptor targets to treat conditions affecting the central nervous system.

MELATONIN RECEPTORS AS THERAPEUTIC TARGETS IN THE CENTRAL NERVOUS SYSTEM

Sleep Disorders

In humans and nonhuman primates, acute melatonin treatment promotes sleep onset, maintenance, or both and induces sleep-like brain waves independent of time of day (47–49). Melatonin and related analogues phase shift circadian rhythms when given at clock-sensitive times following

Circadian time (CT): standard time based on the free-running period; activity onset defined as CT0 or CT12 for diurnal or nocturnal species, respectively

phase response curves (PRCs) conserved in mammals (50, 51). Melatonin can be used effectively to entrain free-running circadian rhythms in totally blind individuals not perceiving light (52) and to phase advance delayed rhythms in individuals suffering from seasonal affective disorders, with concurrent improvement of depression scores (53). These two distinct effects of melatonin (i.e., sleep promotion and phase shift of circadian rhythms) resulting from actions at melatonin receptors in the central nervous system are reviewed below in relation to the therapeutic targets of marketed melatonin drugs (**Table 1**).

Insomnia, or sleep-wake disorder, is defined as difficulty initiating and/or maintaining sleep or as nonrestorative sleep and is generally associated with daytime impairment or distress. Insomnia is a highly prevalent disorder affecting about 10% of the world's population (54, 55). Current treatments for insomnia include benzodiazepines and related nonbenzodiazepine drugs, with considerable side effects resulting in impaired cognitive and psychomotor skills, increased risk of falls, rebound, and dependence or abuse potential (56). The search for molecules with improved safety profiles led to the development of a slow-release melatonin preparation (i.e., Circadin) (38) and synthetic melatonin analogues (i.e., ramelteon, tasimelteon, agomelatine) (37, 39–41) (**Table 1**). Insomnia is related to other comorbid disorders, particularly mood and circadian rhythm disorders (56). Melatonin and the synthetic melatonin agonists are generally devoid of the common side effects frequently observed with sleep medication (i.e., impairment of learning, memory, or motor function).

About 80 million Americans suffer from some form of circadian rhythm disorder resulting in depressive mood or sleep alterations, as reported by the US National Institutes of Health. Melatonin and melatonin receptor agonists find therapeutic applications in various circadian disorders including jet lag, shift work, delayed-sleep and advance-sleep phase syndrome, seasonal affective and non-24-h sleep-wake disorder, and major depression (37, 53, 57, 58). The mammalian SCN expresses MT_1 and MT_2 receptors as demonstrated using 2-[^{125}I]-iodomelatonin and in situ hybridization with mRNA probes (3, 59). Melatonin receptor activation decreases neuronal firing through activation of the MT_1 receptor in the SCN and areas of the limbic system, which may mediate the sleep-promoting properties of melatonin (9, 14).

Activation of melatonin receptors in the SCN plays a pivotal role in coordinating the phase and amplitude of circadian rhythms throughout the body (60). In humans and mice, melatonin phase advances and delays circadian rhythms, following a PRC with two well-defined periods of sensitivity in late afternoon [advance at circadian time (CT) 8–11] and early morning (delay at CT0–3), respectively (50–52, 61, 62). These periods of sensitivity to melatonin and melatonin agonists (e.g., ramelteon) are conserved in humans (52), nonhuman primates (49), and rodents (61–63). The phase shift of circadian rhythms by melatonin is mediated through activation of the MT_1 melatonin receptor, as demonstrated using mice with genetic deletion of the MT_1 receptor in a model of circadian re-entrainment (64).

Understanding the hypnotic and chronobiotic actions at melatonin receptors in the central nervous system led to the development of novel melatonin formulations and the synthesis of new drugs with melatonin receptor agonist properties for the treatment of primary insomnia and circadian disorders (see **Table 1**). Circadin is a prolonged-release melatonin formulation that mimics the physiological profile of melatonin secretion. It improves sleep quality and latency, as well as daytime function, in elderly insomnia patients (65–67). Circadin also improves sleep quality in children with sleep-wake cycle disorders (67) and in blind adults suffering from non-24-h sleep-wake disorders (68). The clinical use of Circadin is not associated with memory impairment or decreased vigilance and has no significant withdrawal symptoms (56).

Ramelteon, a high-affinity MT_1 and MT_2 melatonin receptor agonist (42), promotes sleep in various mammalian species, including humans, without causing learning, memory, or motor

function impairment or inducing reward (69, 70). In subjects with primary chronic insomnia, ramelteon shows a significant reduction in latency to persistent sleep and an increase in total sleep time, with no apparent next-day residual effects (71). In addition, repeated ramelteon treatment before bedtime phase advances circadian rhythm (72). Ramelteon also entrains circadian rhythms after an abrupt phase advance of dark onset (73), and it phase advances circadian rhythms of neuronal firing in the rat SCN brain slice when applied at CT10 (CT12 is the onset of activity for rodents) (60, 63). Activation of either the MT_1 or MT_2 receptors by ramelteon (10 pM) phase advances the peak of neuronal firing rhythms in C3H/HeN mouse SCN brain slices (63). Further ramelteon phase advances overt circadian activity rhythms in vivo by activating the MT_1 melatonin receptor, as identical shifts are observed in wild-type and MT_2 knockout C3H/HeN mice (63). Together, these findings suggest that ramelteon-mediated phase advances of circadian activity rhythms in vivo are mediated through activation of MT_1 melatonin receptors within the SCN, as previously demonstrated in C57BL/6 mice (64).

Tasimelteon was developed for the treatment of non-24-h sleep-wake disorder, a circadian rhythm disorder affecting totally blind individuals who are unable to entrain their master body clock to the 24-h light-dark cycle (74). Tasimelteon is a high-affinity, non-selective MT_1/MT_2 receptor agonist with no detectable affinity for many G protein–coupled receptors and enzymes (41). This melatonin analogue phase shifts circadian rhythms in the rat with a potency comparable to that of melatonin (75). Phase III clinical trials examined the effects of tasimelteon and a placebo on transient insomnia after a 5-h phase shift of the sleep-wake cycle. Tasimelteon significantly shifted the endogenous melatonin rhythm, reduced sleep latency, and increased sleep efficiency and wake after sleep onset (i.e., sleep maintenance) with no observable side effects (40). In summary, tasimelteon modulates sleep and circadian rhythms, providing a highly effective treatment for a range of symptoms associated with transmeridian air travel, shift work, and other circadian-rhythm sleep disorders, including those observed in blind people.

The melatonin agonists noted above (**Table 1**) are nonselective at the MT_1 and MT_2 melatonin receptor ligands. Recent studies investigated the specific role played by each melatonin receptor type in the modulation of sleep architecture (30, 76). The selective MT_2 partial agonist UCM765 decreased the latency to and increased the amount of non–rapid eye movement sleep (NREM). These effects were blocked by the MT_2 selective receptor antagonist 4P-PDOT (30). Furthermore, the selective MT_2 agonist IIK7 produced similar effects on the latency and amount of NREM (76), suggesting that the MT_2 melatonin receptor mediates some of the effects of melatonin agonists on sleep architecture. Interestingly, sleep recordings in mice with genetic deletion of MT_1, MT_2, or both receptors suggest that each receptor regulates different aspects of the vigilance state, namely NREM for the MT_2 and REM for the MT_1 receptor (77).

Major Depressive Disorder

Major depressive disorder remains the most prevalent mental disorder in the United States and is the leading cause of disability, affecting an estimated 100 million adults worldwide (78). The disorder is characterized by a constellation of symptoms that affect mood, anxiety, neurochemical balance, sleep patterns, circadian and/or seasonal rhythm entrainment, and increased neuronal atrophy. Current treatments primarily include tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs), known for increasing extracellular concentrations of monoamine neurotransmitters. These antidepressants, however, do not treat sleep disturbances or circadian and/or seasonal rhythm dysfunctions associated with depressive disorders. Additionally, long-term use leads to unwanted side effects such as sexual dysfunction, weight gain, and a cluster of cognitive, autonomic, and motor signs that constitute the serotonin syndrome (79, 80).

Thus, researchers urgently need to develop antidepressants with novel mechanisms of action and reduced side effects.

MT_1 and MT_2 melatonin receptors are important targets for the development of novel antidepressants. Early indications of melatonin receptor involvement in depressive-like behaviors were based on antidepressant-like effects of melatonin and its agonists in rodent models of learned helplessness. The melatonin-mediated antidepressant-like effects in the forced swim test were blocked by luzindole, suggesting the potential involvement of MT_1 and MT_2 receptors (81, 82). Chronic melatonin treatment also enhances hippocampal neurogenesis in rodent models, which is considered an important process for antidepressant efficacy (83–85). Mice with deletion of the MT_1 receptor show increased depressive-like behaviors in the forced swim test (86, 87). MT_1 receptor immunoreactivity is upregulated in patients with major depressive disorder, suggesting this receptor type is a potential target for treating some symptoms of depression (88). In summary, melatonin agonists, particularly through activation of the MT_1 receptor, may be effective in attenuating the symptoms, neurochemical changes, or both associated with the clinical manifestations of depressive disorders.

The clinical efficacy of melatonin as an antidepressant therapy is limited at best (89). However, interest in targeting melatonin receptors to treat the symptoms of mood disorders was reinvigorated by the discovery of agomelatine, a novel antidepressant that acts through synergistic actions at the melatonin receptors and 5-HT_{2C} receptor (36, 37). In clinical studies, agomelatine is as effective as and better tolerated than SSRIs (i.e., paroxetine, fluoxetine, sertraline) and serotonin/norepinephrine reuptake inhibitors (i.e., Venlafaxine) (90). Agomelatine not only relieves depressed mood, neurochemical imbalance, anxiety, and neuronal atrophy (91–93) but also improves disturbed sleep patterns and circadian/seasonal rhythm entrainment (37, 43). The antidepressant-like effects of agomelatine are not mimicked by individual treatment with melatonin agonists or 5-HT_{2C} antagonists, suggesting the need for simultaneous actions at both receptors for effective antidepressant-like effects (94).

Interestingly, luzindole shows antidepressant-like activity in the forced swimming test through actions at the MT_2 receptor, as mice lacking this receptor do not exhibit this effect (95, 96). Chronic luzindole administration also increases hippocampal neurogenesis in mice (97). The antidepressant-like effects of luzindole, a melatonin receptor antagonist, may appear contradictory to that of MT_1/MT_2 melatonin receptor agonists such as agomelatine. However, MT_2 agonist–mediated desensitization in response to agonist stimulation may lead to a net decrease in its receptor signaling over time, hence mimicking the action of a receptor antagonist (34, 96). In support of this hypothesis, melatonin exerts antidepressant-like effects and increases hippocampal neurogenesis only upon chronic administration (83, 84). We suggest that the rapid MT_2 receptor desensitization kinetics could facilitate melatonin-mediated antidepressant-like effects following treatment with an MT_1/MT_2 receptor agonist (33, 35).

Sleep and circadian disturbances are key symptoms of major depressive disorder, coinciding with onsets of depressive episodes and correlating with depressive severity (98). The ability of agomelatine to normalize disturbances in sleep and circadian rhythms holds a great therapeutic advantage over SSRIs and TCAs, which often exacerbate sleep and circadian disorders. In clinical studies, agomelatine phase advances rhythms in core body temperature when given in the late afternoon (99, 100). Also, agomelatine improves sleep and circadian rest-activity rhythms compared to venlafaxine in patients with depression (101). The chronobiotic properties of agomelatine are likely to be mediated primarily through its agonist action at melatonin receptors in the SCN.

Another important hallmark of depressive disorders is decreased hippocampal neurogenesis resulting from decreased expression of neurotrophic factors (102). Neurogenesis denotes a morphogenetic process that comprises distinct successive steps of progenitor cell proliferation, survival

of immature neurons, and neuronal differentiation (103). Chronic agomelatine administration promotes brain-derived neurotrophic factor expression, leading to a net increase in hippocampal neurogenesis in rodents (92, 104). Melatonin and 5-HT_{2C} antagonists appear to differentially modulate the various stages of adult hippocampal neurogenesis, potentially through heterodimer formation. Chronic administration of 5-HT_{2C} receptor antagonists increases neural progenitor cell proliferation in rodent hippocampus (93), whereas chronic melatonin administration increases neuronal differentiation and immature neuron survival (83, 84, 93).

Learning and Memory

Age-related dementias such as Alzheimer's disease diminish memory and cognition in approximately 5.4 million Americans, indicating a need for new nootropic therapeutics (105). The clinical significance of the melatonin receptors in dementia is strongly supported by postmortem histology studies in hippocampus from Alzheimer's disease patients showing increased MT_1 and decreased MT_2 receptor immunoreactivity (106, 107). Memory formation is driven by long-term potentiation (LTP) in the hippocampus, a process by which associations between neurons are cooperatively and selectively strengthened by increased synaptic activity through *N*-methyl-D-aspartate (NMDA)- and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-type glutamate receptors (108, 109).

Melatonin inhibits LTP in the CA1 dendritic layer of the Schaffer collaterals of mouse hippocampal brain slices (110). This inhibition of LTP was observed in MT_2 knockout but not MT_1 knockout mice, revealing the unique requirement of the MT_2 receptor for this response. Accordingly, Larson and colleagues (111) demonstrated a significantly reduced baseline level of LTP in hippocampus from MT_2 knockout C3H/HeN mice compared to wild-type mice. This link to LTP implies a role for melatonin in memory formation (110). Indeed, melatonin suppresses long-term memory formation in zebrafish, with melatonin receptors being implicated by reversal of the effect via the melatonin receptor antagonists luzindole and K-185 (112). MT_2 knockout mice show learning deficits in the elevated plus maze (111), whereas MT_1/MT_2 double knockout mice show improved spatial and reference learning and memory performance as well as basic memory function, in addition to displaying increased LTP and memory-related neuronal signaling (113). In humans, exogenous melatonin has been linked to decreased activation of the left hippocampus during an autobiographical memory task, and subjects with higher levels of endogenous melatonin demonstrated increased activation of the left parahippocampus (114). Although this study did not address memory performance, it proposes a compelling role for melatonin's contribution to hippocampal activation in humans. Together, these studies suggest an important role for melatonin and its receptors in memory formation, most likely stemming from the underlying process of LTP. Therefore, a melatonin receptor antagonist may lead to improvements in memory-based tasks and may be useful in a prophylactic or therapeutic role in patients showing signs of Alzheimer's disease.

Neuroprotection

Evidence suggests a role for melatonin in protection against neurodegeneration, apoptosis, and ischemia/reperfusion injury (115). The neuroprotective effects of melatonin are often attributed to its free radical–scavenging properties (116); however, recent evidence suggests that activation of the MT_1 and/or MT_2 melatonin receptors may also play a role. In hippocampal slice cultures deprived of oxygen and glucose, melatonin reduces reactive oxygen species to near basal levels, an effect blocked by luzindole (117). This melatonin receptor function may involve the induction of antioxidant genes, such as superoxide dismutase and catalase, through receptor-mediated

transcriptional signaling. Thus, melatonin receptors may be viable targets for novel agents capable of counteracting the oxidative stress components of neuroinflammatory processes.

Diurnal variation: fluctuations in the level of a substance or behavior with a defined peak and trough across a 24-h light-dark cycle

Melatonin inhibits mitochondrial cell death pathways in a mutant striatal cell model of Huntington's disease. Moreover, melatonin inhibits cell death in cell lines as well as primary cerebrocortical and primary striatal neuronal cultures, an effect reversed by luzindole (118). This effect is likely mediated through the MT_1 receptor, as knockdown of this receptor sensitizes cultured neurons to cell death, whereas overexpression is protective (118). In the R6/2 mouse model of Huntington's disease, melatonin slows disease progression and blocks the mitochondrial cell death pathway. Lower levels of the MT_1 but not the MT_2 melatonin receptor are observed in brains of R6/2 mice, a deficit that can be partially reversed by treatment with melatonin (118). These findings imply that development of selective MT_1 receptor agonists might lead to neuroprotective therapeutics capable of treating patients with Huntington's disease.

In contrast, activation of MT_2 receptors has been linked to the protection melatonin affords against neuronal damage that follows ischemic strokes (119). Melatonin treatment in vivo protects against ischemia-related neuronal death in the CA1 region of the hippocampus of the Mongolian gerbil and is associated with increased MT_2 immunoreactivity and protein levels (120). Similarly, melatonin treatment protects against ischemia/reperfusion injury in a mouse model of stroke via receptor-mediated mechanisms blocked by 4P-PDOT or luzindole (121). Furthermore, melatonin promotes neurogenesis and cell proliferation via an MT_2 receptor–dependent mechanism (121). Together, these data suggest a role for the MT_2 receptor in mediating the neuroprotective effects of melatonin following ischemia/reperfusion, as well as an association with a robust increase in neurogenesis.

Drugs of Abuse

Use of illicit drugs is widespread and costly in the United States, with approximately 24.6 million people reporting usage within the past year (122) and a monetary cost in excess of $700 billion per year stemming from increased health-care costs, crime, and loss of productivity (123). However, the development of effective and safe therapies to prevent or counteract the detrimental effects of drug abuse is lacking. Melatonin has been linked to modulation of drug-induced sensitization and reward (124–128). Recent evidence, including data from our laboratory and others, suggests that melatonin receptors modulate responses to drugs of abuse in two well-established models of addictive liability, i.e., locomotor sensitization (124, 125, 127) and conditioned place preference (126, 128).

Locomotor sensitization denotes a progressive increase in ambulatory response following repeated administration of a psychostimulant, which in turn reflects accumulating neuroadaptations in the brain (129). Cocaine induces locomotor sensitization in C3H/HeN mice during the light phase (12-h light-dark cycle), when melatonin levels are low (130), but this effect is absent at night (127) during peak melatonin levels. This diurnal variation is not observed in C57BL/6J mice (127), which are considered melatonin-deficient owing to a truncation of the arylalkylamine *N*-acetyltransferase gene (131). These findings suggest a potential link between nocturnal melatonin and the lack of sensitization during the dark phase.

Researchers have linked melatonin receptors to sensitization induced by the psychostimulant methamphetamine. The development and expression of locomotor sensitization to methamphetamine requires the presence of either MT_1 or MT_2 receptors, as sensitization is significantly reduced only in knockout mice lacking both receptor types (124). In contrast, repeated methamphetamine treatments during the dark phase increase the expression of locomotor sensitization MT_1 knockout mice but not their wild-type and MT_2 knockout counterparts (124). However,

genetic MT_1 receptor deletion completely abolishes the expression of locomotor sensitization in C57BL/6 mice pretreated with a single methamphetamine injection (125). These findings in C3H/HeN and C57BL/6 mice appear to be at odds concerning the functional role of the MT_1 receptor in sensitization. The discrepancy, however, could be attributed to differential levels of endogenous melatonin in the two mouse strains, leading to a difference in the relative level of ligand-dependent versus constitutive MT_1 receptor activity and hence its functional manifestation at the behavioral level. Taken together, data from locomotor sensitization studies imply a unique role for the MT_1 receptor in regulating circadian sensitivity to the psychostimulant properties of methamphetamine and could be targeted to block the onset of addiction-favoring maladaptations in the brain.

Researchers have also linked melatonin to the rewarding properties of drugs of abuse, as evidenced by studies using the conditioned place preference paradigm. C3H/HeN mice develop a place preference for cocaine during the light phase, which is significantly decreased during the dark phase (128). This diurnal variation is abolished by pinealectomy (128). Systemic or intracerebral administration of melatonin to KM mice abrogates the expression of morphine-induced conditioned place preference in a dose-dependent manner, as evidenced by a significant decrease in preference for morphine following treatment with either 25 or 50 mg/kg melatonin (132). These effects are reversed through treatment with luzindole or the selective MT_2 antagonist K-185 (132). Together, these results suggest that melatonin abrogates the rewarding properties of morphine through the MT_2 receptor (132). Results from our laboratory revealed a strong place preference in methamphetamine-treated wild-type C3H/HeN mice during the mid-light phase, which was abrogated during the peak melatonin period of the mid-dark phase (126). Knockout mice lacking either the MT_1 or MT_2 receptor exhibited sharply reduced place preference for methamphetamine at both times of day (126). Thus, melatonin appears to act through its receptors to inhibit methamphetamine-induced reward, and antagonists of the MT_1 and MT_2 receptors may have the capacity to block the induction of the methamphetamine-induced reward sensation.

MELATONIN RECEPTOR–MEDIATED ONCOSTATIC EFFECTS IN BREAST AND PROSTATE CANCER

In humans, the nocturnal serum concentration of endogenous melatonin is inversely associated with the risk of breast, lung, and cervical cancers (133). Melatonin treatment enhances the efficacy of chemotherapy in patients with lung, breast, gastrointestinal tract, head, and neck cancers (134). In addition to its oncostatic effect, melatonin decreases anxiety, depression, and toxicity associated with chemotherapy (134, 135). Breast and prostate cancer account for an estimated 30% of new cancer cases and 26% of cancer-caused death in the United States (136). Melatonin is emerging as a safe and effective treatment for breast and prostate cancer, either alone or in combination with other therapies. Here we summarize the oncostatic properties of melatonin and its receptors in breast and prostate cancers models, with particular focus on the MT_1 receptor.

In breast cancer, melatonin displays an oncostatic effect both in vivo and in vitro. Coadministration of melatonin and tamoxifen reduces lesion damage size and increases survival rates in breast cancer patients resistant to tamoxifen treatment alone (135, 137). Decreases in endogenous melatonin levels by exposure to light at night (such as what shift workers are exposed to) significantly increases breast cancer risk (138). Decreasing endogenous melatonin levels through exposure to constant light or pinealectomy significantly increases the incidence of mammary tumor formation in rodents. Exogenous melatonin supplementation reverses this process (139). Cancer signaling markers in human breast cancer xenografts implanted in nude rats are significantly increased by perfusion with melatonin-deficient blood obtained by daytime sampling or by exposure to light at

night from healthy premenopausal women (140). In contrast, melatonin-rich blood collected at night inhibits human breast cancer growth, which is blocked by a melatonin receptor antagonist (S20928) (140), suggesting nocturnal endogenous serum melatonin inhibits breast cancer growth by receptor-mediated mechanisms.

Cell culture model systems illustrate the conditions and molecular signaling mechanisms involved in melatonin receptor–mediated modulation of cancerous tumor development and growth. Low nanomolar concentrations of melatonin, which are within the range of the nocturnal physiological serum level, inhibit proliferation of estrogen receptor α–positive MCF-7 human breast cancer cells by 30–50% (141). AMMTC, an MT_1 and MT_2 receptor agonist, inhibits MCF-7 cancer cell proliferation. This antiproliferative effect of melatonin in MCF-7 cells is blocked by CBPT, an MT_1 and MT_2 receptor antagonist (142). Together, these systematic pharmacological studies demonstrate that the antiproliferative effect of melatonin in MCF-7 cells is melatonin receptor–mediated.

Molecular studies demonstrate that mRNA for the MT_1 receptor is expressed in breast cancer cell lines and human breast cancer specimens (142–144). MT_2 melatonin receptor mRNA is either absent or expressed at a low density. The MT_1 receptor mRNA expression level varies among different breast cancer cell lines and primary breast tumors and is modulated by exogenous estrogen and melatonin treatment (143). Interestingly, MT_1 receptor overexpression inhibits MCF-7 cell proliferation and enhances the sensitivity of MCF-7 cells to the antiproliferative effect of melatonin (145, 146). The antiproliferative effect of MT_1 receptor overexpression in MCF-7 cells in the absence of exogenous melatonin suggests the presence of constitutively active MT_1 receptors.

Studies linking melatonin and prostate cancer show a direct antiproliferative effect of melatonin in human prostate cancer cell lines and nude mice (147, 148). Melatonin also slows the early stages of tumor development in castrated prostate cancer patients, as shown by a 23% reduction in the prostate-specific antigen doubling rate (149). Luzindole but not 4P-PDOT blocks the antiproliferative effect of melatonin on hormone-refractory 22Rv1 human prostate cancer cells, suggesting an MT_1 receptor–mediated effect (150).

CONCLUSIONS

This review describes the latest advancements on the role of melatonin receptors as therapeutic targets for the development of drugs aimed at the treatment of sleep and circadian disorders, depression, and cancer. The development of new drugs targeting the MT_1 and MT_2 melatonin receptors is the next challenge for the field. Described below are potential therapeutic targets for the melatonin receptor types.

Sleep and Circadian Rhythm Disorders

Research has firmly established that melatonin and melatonin ligands promote sleep in nocturnal and diurnal species independently of time of day. Activation of melatonin receptors in the SCN phase shift circadian rhythms at specific times of day following a PRC that is conserved among mammals. A slow-release melatonin preparation (i.e., Circadin) and synthetic melatonin ligands (i.e., ramelteon, agomelatine, tasimelteon) are marketed for the treatment of circadian sleep disorders, insomnia, and depression. Discovery of MT_1 or MT_2 melatonin receptor–selective drugs may improve efficacy as compared to nonselective ligands and lead to the discovery of new therapeutic targets.

Depressive Disorders

Agomelatine ameliorates symptoms of major depression by a combined action of MT_1 and/or MT_2 receptor activation and 5-HT_{2C} receptor blockade. Adjuvant melatonin therapy with current antidepressants may thus have additional benefits for depressed patients. Importantly, researchers must remember that these two classes of agents have different processes for modulating hippocampal neurogenesis, sleep or circadian disruption, and synaptic connectivity in the prefrontal cortex. The MT_2 receptor appears to be the most promising new target for the development of new antidepressants, as selective ligands of this receptor modulate antidepressant-like effects.

Learning and Memory

Deletion or blockade of the melatonin receptors results in increased LTP as well as improved performance in learning and memory tasks. Therefore, melatonin receptor antagonists may promote increased learning and memory and may thus be useful in ameliorating the symptoms of dementia, particularly Alzheimer's disease.

Neuroprotection

Melatonin inhibits mitochondrial cell death pathways via the MT_1 receptor, whereas action through the MT_2 receptor protects against ischemia/reperfusion injury. Therefore, melatonin itself and melatonin receptor agonists may be advantageous in protecting against cell death observed in diseases such as Huntington's disease, promoting neurogenesis and protecting against injury following stroke-related ischemia/reperfusion.

Drugs of Abuse

Decreased responses to drugs of abuse are observed during the dark phase, when melatonin is at its peak. Deletion of MT_1 and/or MT_2 melatonin receptors results in abrogation of methamphetamine-induced locomotor sensitization and reward. Thus, melatonin receptor antagonists may provide useful treatments in ameliorating the symptoms of drug abuse.

Cancer

At the cellular level, melatonin and its receptors are poised to influence the pathology of cancer. The MT_1 melatonin receptor mediates the oncostatic effect of melatonin in breast and prostate cancer models. Interestingly, the MT_1 receptor may be constitutively active in breast cancer cells and intrinsically inhibits cancer cell proliferation. As such, selective MT_1 receptor agonists may be effective in the treatment of breast and prostate cancer, alone or as an adjunct to currently available therapeutic approaches.

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