Revealing Melatonin's Mysteries: Receptors, Signaling Pathways, and Therapeutics Applications

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ABSTRACT

Melatonin (5-methoxy-acetyl tryptamine) is a sleep-inducing hormone, and the pineal gland produces it in response to the circadian clock of darkness. In the body, MT1 and MT2 receptors are mostly found, having an orthosteric pocket and ligand binding determinants. Melatonin acts by binding on melatonin receptors, intracellular proteins, and orphan nuclear receptors. It inhibits adenyl cyclase and activates phospholipase C, resulting in gene expression and an intracellular alteration environment. Melatonin signaling pathways are also associated with other intracellular signaling pathways, i. e., cAMP/PKA and MAPK/ERK pathways. Relative expression of different proteins depends on the coupling profile of G protein, accounting pharmacology of the melatonin receptor bias system, and mediates action in a Gi-dependent manner. It shows antioxidant, antitumor, antiproliferative, and neuroprotective activity. Different types of melatonin agonists have been synthesized for the treatment of sleeping disorders. Researchers have developed therapeutics that target melatonin signaling, which could benefit a wide range of medical conditions. This review focuses on melatonin receptors, pharmacology, and signaling cascades; it aims to provide basic mechanical aspects of the receptor's pharmacology, melatonin's functions in cancer and neurodegenerative diseases, and any treatments and drugs designed for these diseases. This will allow a basic comparison between the receptors in question, highlighting any parallels and differences that may exist and providing fundamental knowledge about these receptors to future researchers.

Introduction

Melatonin, chemically 5-methoxyacetyl tryptamine, is a sleep-inducing hormone, and in 1958, it was extracted from a pineal gland [1]. Its concentration is high at night time in all species. Animals' endogenous circadian clock, which secretes the hormone melatonin at night, is synchronized by light and dark cycles in the suprachiasmatic nucleus of the hypothalamus. In a 24-hour cycle, the pineal gland releases the hormone melatonin. When the retina detects stimuli of darkness, it is perceived by the suprachiasmatic nucleus, which generates a signal in the form of a nerve impulse and sends it to the upper thoracic cord of the intermediolateral column, then perceived by the superior cervical ganglion. After adrenergic stimuli, pinocytes of the pineal gland synthesize the melatonin hormone intracellular. Neurological and physiological processes are regulated by melatonin. Photoperiodic species show seasonal changes regulated by the melatonin hormone in the hypothalamus, the pituitary pars tuberalis. It exerts direct action on the suprachiasmatic nucleus and entrains the circadian clock.

Circadian rhythm disorders, that is, jet lag, shift work, blindness, and delayed or advanced sleep phase syndromes, are treated by the response exerted by the melatonin hormone [2]. Dopamine synthesis is repressed from the retinal amacrine cells by melatonin [3] and can promote vasoconstriction in the rat tail artery [4]. Melatonin has also played a well-established hypnotic action. It triggers the opening of the sleep gate, which is circadian-dependent and initiates sleeping [5]. Melatonin meditates action as an antioxidant and influences immune function. Sleep and wake rhythm are induced by melatonin [6]. The physiological processes, such as regulating the cardiovascular system, are controlled by melatonin [7] and buffering of the immune system and neurodegenerative disorders [8]. In drug designing, the therapeutic agents are designed that target melatonin targets. Melatonin plays a role in cancer protection, confirmed by research, also bone formation and glucose maintenance [9].

Synthesis of melatonin hormone

The synthesis of melatonin involves a two-step process from the pineal gland

- Arylalkylamine is used for serotonin acetylation by using acetyltransferase enzyme to produce acetylserotonin,
- Melatonin is synthesized by the enzyme hydroxy indole-*O*-methyltransferase by methylation of the 5-hydroxy group (▶ **Table 1**,▶ **Fig. 1**).

Excretion of melatonin

Mainly in the liver, melatonin is metabolized most efficiently but to some extent in the kidney. Melatonin is oxidized into 6-hydroxymelatonin in the liver by the cytochrome P 450 enzyme, and 6-hydroxymelatonin is then conjugated with sulfuric acid to 6-sulfatoxymelatonin [10]. In the form of 6-sulfatoxymelatonin, it is eliminated from the body through urination (▶ **Table 2**), which is a major melatonin metabolite used to access melatonin concentration in the plasma [11].

Mechanism of melatonin effects

In mammals, the effects of melatonin are revealed by four different mechanisms:

1. Melatonin acts as an antioxidant
2. Melatonin binds to plasma membrane receptors
3. Interaction of melatonin to intracellular proteins, that is, calmodulin
4. Orphan nuclear receptors are targeting [12]

Intracellular proteins, that is, calmodulin, calreticulin, and tubulin, interact with melatonin. The binding of calcium to calmodulin, an intracellular second messenger, is antagonized by melatonin [13]. Melatonin shows regulation of antiproliferative effects in cancer. Melatonin shows immunomodulatory effects, which is mediated by retinoid-related orphan nuclear receptor (▶ **Fig. 2**). Mononuclear cells secrete interleukins IL-2 and IL-6 owing to this modulation [14].

Melatonin receptors

Melatonin receptors are present on the plasma membrane of different cells, that is, cells of the immune system, cells of the coronary artery, cells of the cardiac ventricular wall, cells of the cardiovascular system, appendix vermiform, hepatocytes, gallbladder, duodenal enterocytes, aorta cells of the large intestinal cecum, colon, skin cells, fetal kidney, kidney, platelets, brain, retina, parotid gland, cells of cerebral arteries, exocrine pancreas, breast and prostate epithelial cells, placenta, epithelial cells of breast, cells of the ovary, myometrium, and brown adipocytes. The morphology of white adipocytes is different from each other [15]. Jejunal and colonic mucosal cells possess melatonin receptors. Melatonin receptors are of four different types in living organisms in different cells as shown in ▶ **Table 3**. Three receptors are on the plasma membrane, while one is the nuclear receptor.

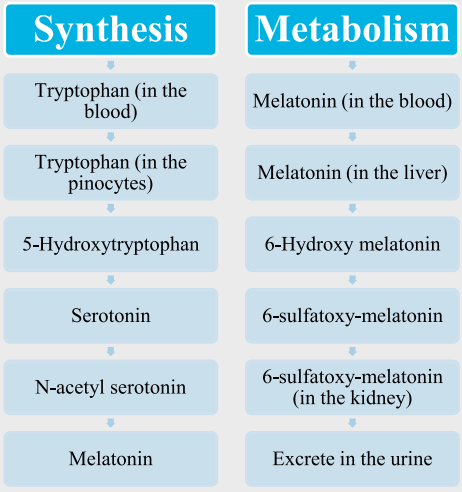
Type 1a receptor

These are primarily present in human skin cells, consisting of 351 amino acids and encoded by a gene on chromosome #4. It has five different receptor subtypes: MT1, MTNR1A, Mel1a, ML1a, and ML1 [16]. The binding of type 1 melatonin receptors to different types of GPCRs inactivates adenyl cyclase [17]. These receptors' expression is decreased in the cortex and suprachiasmatic nucleus during Alzheimer's and aging [18], suppressing protein secretion and neuronal discharge in the suprachiasmatic nucleus [19].

Type 1b receptor

The gene for this receptor is present on chromosome #11, encoding a polypeptide of 363 amino acids. It has three different receptor subtypes: MTNR1B, ML1b, and MT2 [6]. The binding of this receptor to different GPCRs inactivates adenyl cyclase and guanylyl cyclase [17]. cAMP synthesis is decreased by inactivating adenyl cyclase [20]. These receptors are located in sweat glands and malign melanocytes [21]. These receptors inhibit gamma amino butyric acid A receptors in rat hippocampus [22]. Showing antidepressant properties reveals that their expression decreased in Alzheimer's disease [15], depression and sleep diseases are associated with abnormal melatonin receptors, and pharmacology and pathophys-

▶ **Table 1** Synthetic and Metabolic Pathway of Melatonin.



iology of Alzheimer's and anxiety diseases are associated with abnormality in these receptors. These receptors are the new target for hypotonic agents. Anxiety and sleep cycles are regulated by these receptors. MT1/MT2 does not possess hypotonic effects as compared to these receptors [17].

MTNR1C and Mel1c

These receptors are present in fish, birds, and amphibians but not in humans. The chicken MT1 and MT2 receptors are antagonistic to this receptor's circadian rhythm. In the daytime, it is present in high and low concentrations at night [6].

MT3

This receptor shows antioxidant properties due to the quinone reductase-2 enzyme and inhibits the electrons transfer reaction of quinone. Melatonin type 3 receptors and detoxification quinone reductase 2-enzyme are present on the plasma membrane of muscle, brown fat tissue, liver, kidney, heart, lung, and intestinal cells. Intraocular pressure is regulated by it [18].

RZR/RORα

These nuclear receptors help bind melatonin to transcription factors in the nucleus and belong to the retinoic acid receptor super T family [23]. This receptor consists of 618 amino acids encoded by chromosome #28. This receptor does not bind to melatonin and is present in all mammals; it helps bind melatonin to MT [6].

A Melatonin as receptor

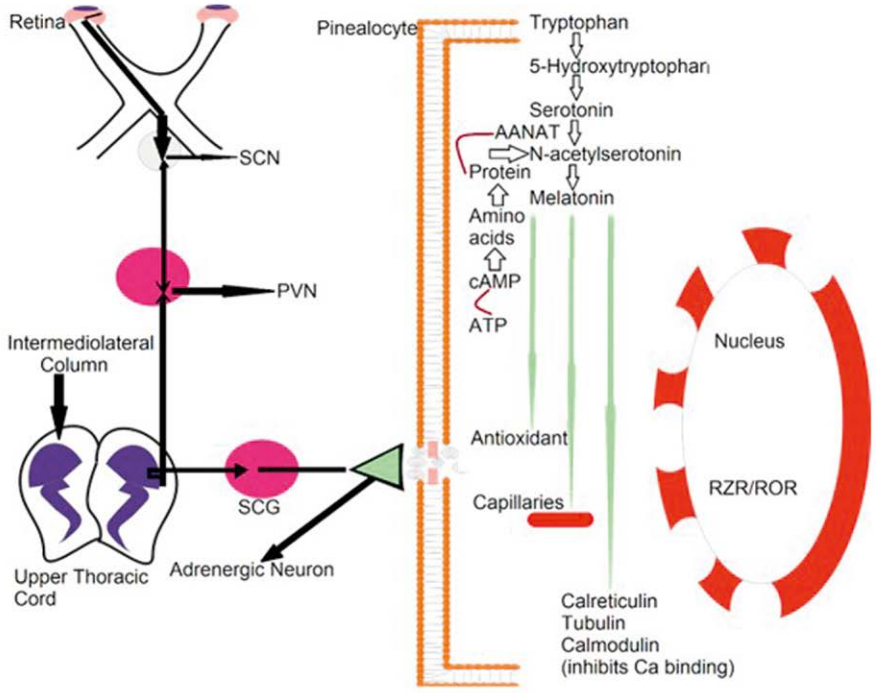
Ligand selectivity determinants and orthosteric

The orthosteric pocket created by TM3, TM5, TM6, TM7, and ECL2 in the structures of both MT1 and MT2 binds ramelteon and 2-iodomelatonin (▶ **Fig. 3, 4a,c**). It is possible to superimpose the ramelteon's binding pose with that of inactive structures in active

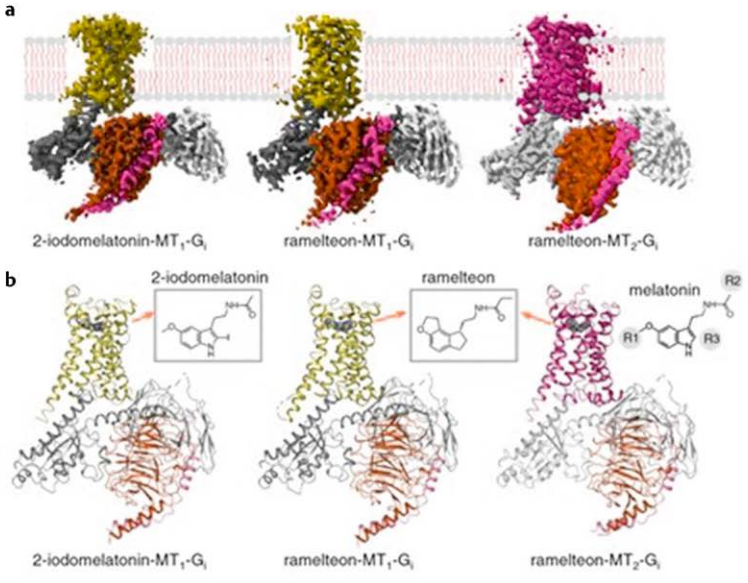
▶ **Table 2** MT1 and MT2 Receptor's distribution in Human.

Receptor type		Tissues	Reference
hMT1	Brain	Cerebellum	[23]
		Occipital cortex	[24]
		Parietal cortex	[23]
		Temporal cortex	[23]
		Thalamus	[23]
		Frontal cortex	[23]
		Hippocampus	[23]
	Peripheral tissues	SCN	[25]
		Retina	[27]
		Brown and white adipose tissue	[28]
		Fetal kidney	[29]
		Coronary artery	[30]
		Granuloma cells	[31]
		Myometrium	[32]
		Pancreatic alpha and beta cells	[33]
		Testis	[34]
hMT2	Brain	Cerebellum	[24]
		Hippocampus	[25]
		SCN	[26]
	Peripheral tissues	Retina	[27]
		Brown and white adipose tissue	[28]
		Fetal kidney	[29]
		Granulosa cells	[30]
		Placental tissues	[31]
		Myometrium	[32]
		Pancreatic alpha and beta cells	[33]
		Testis	[34]

MT1 or MT2. But the active form of 2-iodomelatonin changes slightly from the inactive form, especially where the alkyl amide tail is concerned, where it approaches the W6.48 residue in functioning MT1, which acts as a "toggle switch." ECL2 consistently occupies the pocket's top position in both conformations, blocking ligand accessibility through the extracellular side (▶ **Fig. 4b, d**). The only access point to the orthosteric-binding site in the active conformation has been discovered to be the lateral channel between TM4 and TM5. It was discovered that the only access point to the orthosteric-binding site in the active conformation is the lateral channel between TM4 and TM5 (▶ **Fig. 4b, d**). The orthosteric pocket is more constrained in the center of active structures, but TM3, TM4, and TM5 in dormant structures make a large "longitudinal channel" that this fiber bundle grows to join. While the residues around the iodine group and alkyl amide tail (referred to as the R3



▶ **Fig. 1** Synthesis of melatonin through the neurologic pathway from the pineal gland and its effects.



▶ **Fig. 2** MT1-Gi and MT2-Gi complex structures: The complexes are shown by Cryo-EM density maps. Panel **a**: Blue color represents melatonin type 1 receptors; green color represents melatonin type 2, green; purple represent Gαi in MT1; yellow represent Gαi in melatonin type 2; scFV16, violet is color code. Gβ, teal; Gγ, light green. Panel **b** shows the cryo-EM structure of MT1-Gi and MT2-Gi. The left side shows 2-iodomelatonin-bound MT1-Gi; the middle side shows ramelteon-bound MT1-Gi; the right side shows ramelteon-bound MT2-Gi. The top right side shows the structure of ligands and melatonin molecules.

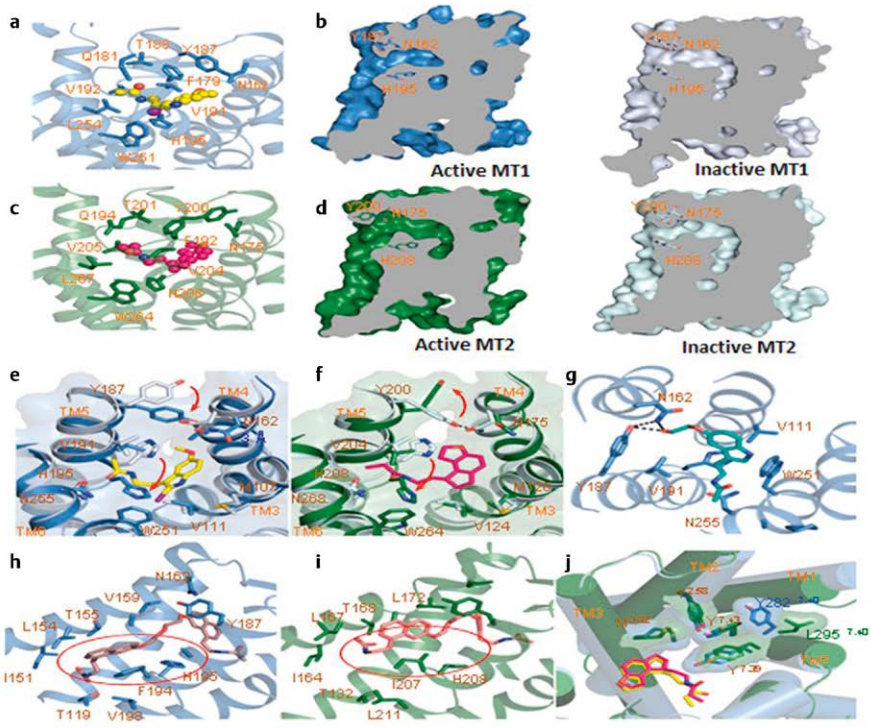
position in melatonin, ▶ **Fig. 5**) match up well, the active pocket's structure might vary depending on the conformations of the residues flanking the solvent channel (▶ **Fig. 4e, f**). A hydrogen bond is formed between the aromatic residue Y1875.38 in MT1 and N1624.60 in the active structure by rotating from the inactive structure's solvent-facing conformation toward TM4.

▶ **Table 3** Mysteries of Melatonin hormone reported in various human systems and processes.

Mysteries of Melatonin	Reference
Circadian Rhythms and Melatonin	[8]
▪ Regulate sleep and wake cycle ▪ Influence on circadian rhythms	
Melatonin Receptors Functions	
▪ **Type 1a and 1b receptors** expression is decreased in the cortex and suprachiasmatic nucleus during Alzheimer's and aging, suppressing protein secretion and neuronal discharge in the suprachiasmatic nucleus and inhibiting GABA showing antidepressant.	[15, 16, 18–19]
▪ **MT3 receptor** shows antioxidant properties due to the quinone reductase-2 enzyme and inhibits the electrons transfer reaction of quinone.	[22]
▪ **RZR/RORα nuclear receptors** help bind melatonin to transcription factors in the nucleus and belong to the retinoic acid receptor super T family.	[15]
Melatonin Receptors as a Drug Target	
▪ **Type 1a and 1b receptors** are the new target for hypotonic agents. Anxiety and sleep cycles are regulated by these receptors. ▪ **Ligand Selectivity Determinants and Orthosteric of MT2** has N4.60-Y5.38-H5.46 motif, the longitudinal channel, and the larger subpocket could all be used as targets for the designing of melatonin subtype-selective drugs. ▪ **Therapeutic agents** are designed that target melatonin targets	[11, 18]
Therapeutic Applications	
Melatonin and Immunomodulation	[14]
▪ Secrete interleukins IL-2 and IL-6	
Melatonin and Cardiovascular System	
▪ Reduces high serum total cholesterol and triglyceride levels in the blood ▪ Improve lipid metabolism by decreasing low-density lipoprotein ▪ Decrease systolic and diastolic blood pressure at night ▪ Decrease nocturnal systolic blood pressure ▪ Reduce the severity of cardiac marker injury and myocardial infarction size ▪ Lower heart rate by increasing ejection fraction ▪ Improves blood pressure, glycemic index, and lipid in patients suffering from chronic heart diseases ▪ Reduce cardiac fibrosis in nonischemic heart failure. Its intake prevents myocardial infarction	[67–69]
Melatonin and Nervous System	
▪ Decrease infarct volume and brain edema and improving neurologic score ▪ Improve sleep quality and cognitive function in the brain ▪ Treats sleep disorders associated with amnesia, sundowning, and Alzheimer's diseases ▪ Delay the degeneration of dopaminergic neurons in the substantia nigra in the treatment of Parkinson, Cognitive dysfunction, anxiety, depression ▪ Improve sleep behavior in epilepsy patients ▪ Treat migraine and prophylaxis in both adults and children ▪ Improve sleep quality and prevents headache and tension ▪ Treat sleep disturbances in patients with traumatic brain injury	[71–77]

▶ **Table 3** continued.

Mysteries of Melatonin	Reference
Reproductive System	
▪ Enhance the corpus cavernosum's ability to contract and relax ▪ Improves endothelial density and erectile function ▪ Improves fertilization rate ▪ Increases progesterone production in the corpus luteum	[78–79]
Gastrointestinal System	
▪ Protect against mucosa oxidative damage in different types of gastrointestinal tract ulcers ▪ Reduces relaxation duration, and increases gastrin ▪ Decrease abdominal pain, bloating, and constipation and increases rectal pressure ▪ Lowers liver cholesterol, triglycerides, serum AST and ALT levels in hepatic steatosis patients ▪ Treat Hemorrhagic shock, ischemia-reperfusion injury, liver damage, ionizing radiation, and Schistosoma mansoni infection	[80, 83]
Renal System	
▪ Protect against radiation, folic acid, aminoglycoside, contrast-mediated, and nephrotoxicity induced by these agents ▪ Reduce creatinine and blood urea nitrogen levels ▪ Decreases inflammasome activation	[84–85]
Dermatology	
▪ Protect against UV-light-induced damage by preventing the production of free radicals, erythema caused by natural sunlight, and radioprotective effects ▪ Anti-aging properties, enhances hydration, and lessens the roughness of the skin	[86]
Fibromyalgia	
Improves pain level and fibromyalgia	
Autism Spectrum Disorder	
▪ Lowers mid-sleep awakenings, ▪ Improves sleep quality, and lengthens total sleep time	[88]
Mood Disorder	
▪ Diminishes sleep disorders linked to a depressed mood	[89]
Oncology	
▪ Melatonin adjuvant therapy for ER-positive breast cancer ▪ Provides protection to ovaries and fertility preservation ▪ Lessen radiation-induced lung injury ▪ Modulates the effectiveness of DNA repair in humans as well as the genotoxic activity of irinotecan ▪ Ursolic acid has antiproliferative and pro-apoptotic effects on colon cancer cells	[90–93]
Viral Syndromes	
▪ Reduce the acute lung oxidative injury by respiratory syncytial virus ▪ Preservation of cardiac functions and for repression of virus-induced cardiomyocyte apoptosis ▪ Inhibits apoptosis, regulates the rate of autophagy, and maintains mitochondrial dysfunction ▪ Increases survival rate by reducing virus load in the brain and serum ▪ Treat encephalomyelitis by preventing death and paralysis ▪ Treat viremia and postpones disease ▪ Prolongs survival time by reducing oxidative damage and slowing down the release of cytokines	[94–99]
Circadian Rhythm Disorders	
▪ Treat jet lag, shift work, blindness, and delayed or advanced sleep phase syndromes	[4]

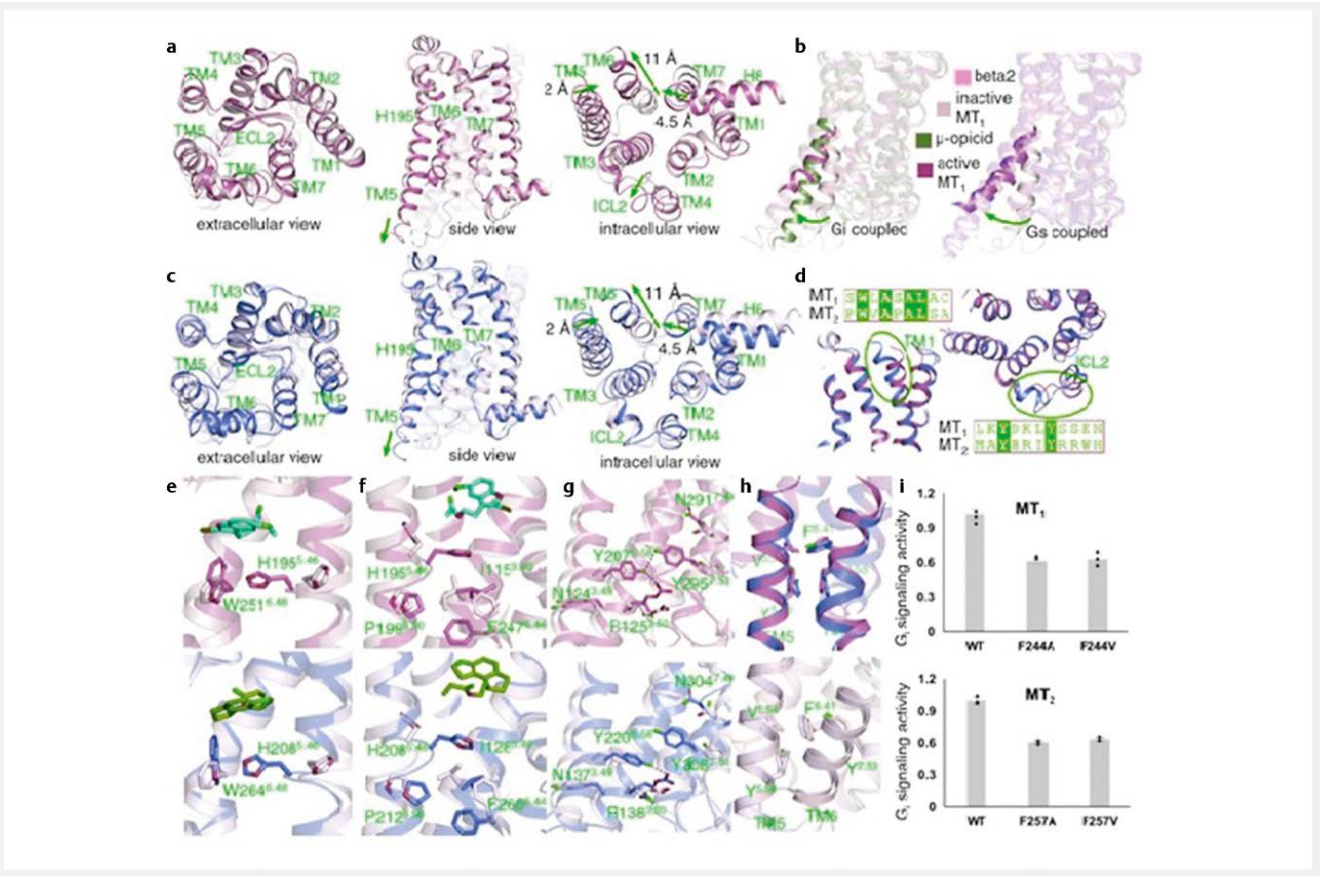


▶ **Fig. 3** Ligand-binding pocket and selectivity of determinants: **a**: MT1 has a ligand-binding pocket (yellow) where 2-iodomelatonin (yellow) is bound (blue). **b**: Active (left) and inactive (right) forms of the ligand access channel MT1 are shown in the slab view (right, light blue, PDB ID: 6ME4). Ramelteon (pink) is attached to the ligand-binding pocket in MT2 (green). Schematic slab views of the active (left) and inactive (right) forms of the MT2 ligand access channel (right, light cyan, PDB ID: 6ME9). Ligand-binding residues of MT1 are shown in blue, while those of inactive ligand-binding pockets are shown in a lighter shade of blue. Red arrows indicate alterations of note. N162 and Y187 form a 3-f orbital distance hydrogen bond. The ligand-binding pockets of active (green) and inactive (light cyan) MT2 are contrasted. Red arrows indicate changes of significance. Active MT1 has g 5-HEAT docked in it (cyan). Key residues that are causing problems in this pocket are shown as sticks. In the open MT1, salmon CTL 01–05-B-A05 has successfully spawned. The red circle denotes the hydrophobic packing of the naphthalene group and F1945.45. MT2 was open when salmon CTL 01–05-B-A05 swam in. The naphthalene group and I2075.45 are packed incompatible, as indicated by the red circle. **j**: Comparison of the sub pockets from MT1 and MT2 that are active and bound to ramelteon (red in MT1, yellow in MT2). Sticks represent important distinct residues.

Furthermore, the hydrogen bond's diameter of the ligand entry is decreased, which may inhibit the unbinding of the bound agonist because the Y1875.38 A mutation caused a high ligand dissociation rate. The functional relevance of this proton pair in MT1 activation was further demonstrated by the fact that the N1624.60 A mutation rendered MT1 inactive. Homologous pair N1754.60 and Y2005.38 also underwent structural changes in MT2 during the transition. This hydrogen bond's absence demonstrates that MT2 does not require an entrance-restricting hydrogen bond similar to the one found in MT1 for activation, which is consistent with the earlier finding that the N1754.60 protein does not need such a hydrogen bond [24, 25]. No functional consequences resulted from a mutation [26]. Because N4.60-Y5.38 can be altered in conformation thanks to a conserved proline (P4.59) located close by in MT1 and MT2, changing P4.59 impairs MT2's ability to bind ligands [7].

Residue H5.46 (H1955.46 in MT1 and H2085.46 in MT2), two helical turns beneath Y5.38, distinguishing the pockets most clearly from the active and dormant structures (▶ **Fig. 4e, f**). The pocket size of the residue H5.46 (H1955.46 in MT1 and H2085.46 in MT2) differs the most between the inactive and active forms (▶ **Fig. 4e, f**). H5.46 avoiding bound ligand forms inactive complexes with TM4 (▶ **Fig. 4e, f**). The toggle switch residue W6.48 (W2516.48 in MT1 and W2646.48 in MT2) and van der Waals contacts with the connected ligand's alkyl amide tail are formed when the ligand moves inside by 2.4 Å and flips its side chain in the active structures (▶ **Fig. 4e, f**). H5.46's new conformation clarifies why the H2085.46 A mutation dampened MT2 activity [27].

Despite the fact that H5.46 experiences similar conformational changes in MT1 and MT2, its functional significance seems to vary between the two receptors, as the H2085.46 (MT2) mutation only slightly decreased MT2 function whereas the H1955.46 A (MT1) mutation drastically impaired MT1 activity. Then, we docked to both receptors using the common ligands CTL 01–05-B-A0527 and 5-hydroxyethoxy-*N*-acetyltryptamine (5-HEAT) [28]. In contrast to melatonin, 5-HEAT and CTL 01–05-B-A05 have substitutions in the R1 position. 5-HEAT was able to keep a position superimposable to that of bound 2-iodomelatonin, thanks to hydrogen bonds to MT1 residues N1624.60 and Y1875.38. (▶ **Fig. 4g**). To conclude that 5-HEAT is an MT1 agonist, we must first determine whether or not its molecular structure is consistent with the expected position of the active pocket necessary to activate MT1.



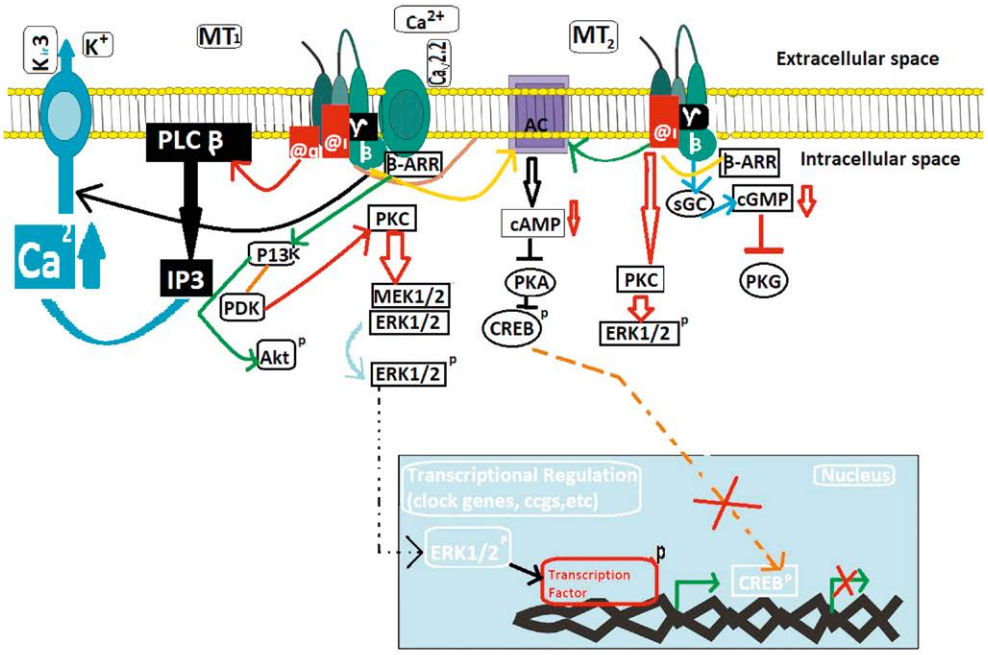
▶ **Fig. 4** Activation of MT1 and MT2 receptors: **a**: The active structure of the MT1 receptor (blue) and an inactive type 1 receptor (light blue) are shown and contrasted. There are three perspectives available. In this image, the TM6 conformation of the Gs-coupled beta2 receptor (right) and the Gi-coupled opioid receptor (left) is compared to that of the MT1 molecule. Structures of active (green) and inactive (light green) MT2 are shown side by side. Three different perspectives are seen here. These images show the differences between MT1's TM1 (left) and MT2's ICL2 (right). **e**–**g**: There are conformational changes in MT1 motifs and other critical residues during receptor activation. Structures of active and inactive melatonin receptors 1 and 2 revealed F6.41 conformations. Gi signaling pathways were visible in I F6.41 from both MT1 and MT2 mutants. The findings are presented as the means standard deviations of three separate experiments in which wild-type receptors were used as a reference.

On the other hand, due to the dissimilar shapes of N1754.60 and Y2005.38, the MT2 antagonist 5-HEAT was not a good fit for docking in the active pocket of MT2. Induced-fit binding is probably used by 5-HEAT. Weak binding of CTL 01–05-B-A05 to MT2 was observed because the side chain of I2075.45 disrupted the stacking contact between these two molecules (▶ **Fig. 4i**). Notably, in light of our findings, further biopic ligand development is required to produce more focused MT1 agonists. In light of our findings, further development of the biopic ligand is required to produce more focused MT1 agonists. A feasible technique for optimizing the fit with the "longitudinal channel" would include specific substituents in the second unit.

The region known as the sub-pocket, which is located around the R3 group of the ligand and was barely distinguishable in the inactive MT1 and MT2 pockets, became more distinct in the active structures. At position 7.40 in MT1, there is a tyrosine (Y2827.40). Lucien (L2957.40) is the equivalent residue in MT2 (▶ **Fig. 5j**). When Y2827.40 is packed against TM1, the two adjacent residues Y2817.39 and Y2857.43 are pushed closer to the pocket's center than the corresponding residues Y2947.39 and Y2987.43 in MT2 (▶ **Fig. 4j**). Since MT2 has a larger sub pocket; as a result, it can accommodate ligands with bulky R3 substituents, which is in line with the chemical architectures of the majority of MT2 selective agonists [27, 29]. MT1 and MT2 receptors are structurally and functionally similar and also have unique features in their ligand binding pockets. The N4.60-Y5.38-H5.46 motif, the longitudinal channel, and the larger sub pocket in melatonin receptor type 2 could all be used as targets for the designing of melatonin subtype-selective drugs.

Melatonin type 1 and type 2 structure signaling complex

2-Iodomelatonin, a nonselective agonist [8] and ramelteon [30] are used to obtain stable MT1 Gi-Protein complexes. Both of these compounds show high potency and affinity toward these receptors. Co-expression of G protein and receptor was studied in the insect cells. The resolution of 2-iodomelatonin and ramelteon was determined, showing global resolutions 3.1 and 3.3 Å, respectively. The assembled complex was purified for homogeneity, and cryo-EM studied their complexes for single particles. An atomic model



▶ **Fig. 5** Signaling Pathway of MT2 receptor: Melatonin activates MT1 receptors and activates Gαi decreasing cAMP second messenger and activates PI3K/Akt, PKC, and ERK pathways dependent on Gβγ. Intracellular Ca2⁺concentration is increased. PLC is activated by Gq coupling to melatonin. Potassium and calcium on channels are activated by melatonin and modulate neuronal action mediated and inhibit Ca2⁺entry through Gβγ subunits. MT2 receptors are activated by the ERK signaling pathway and Gαi-dependent cAMP and inhibited cyclic guanosine monophosphate synthesis. Recruitment of α-arrestin is induced by melatonin, down streaming signaling mechanism is still unknown.

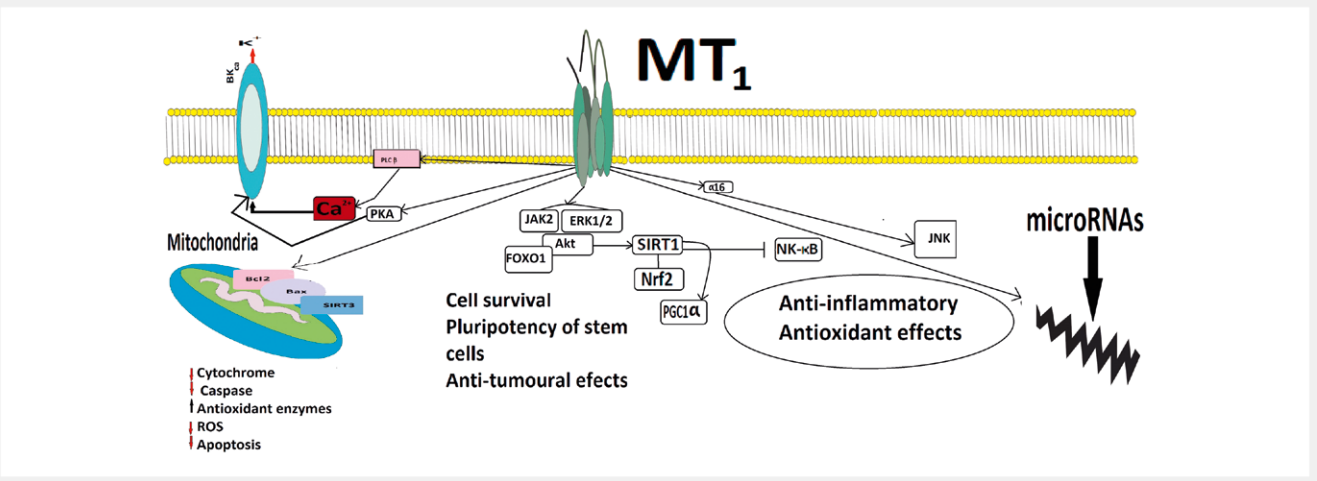
consisting of ligands MT1, Gi, and scFv1627 was built, and relatively high-quality density maps were used. The side chain of melatonin receptor type 1 and G-inhibitory protein was explained in the structure. TM1 and TM7 possess extra density between their N-terminal portions, and as a cholesterol molecule, it was changed. The agonist-bound MT2-Gi complex was studied in the same manner. The reconstituted ramelteon-bound MT2-Gi-scFv16 complex was acquainted by cryo-EM.

For high-resolution maps, the receptor stability was improved. According to previous findings, three thermostable mutations, F1293.41 W, C1403.52 L, and L108ECL1F, were introduced to MT2, which are not contagious to the coupling interface of G-protein and ligand binding pocket. The ligand interaction with receptor and G-proteins coupling interferes minimally with mutations [24]. An EM density map was obtained at 3.5 nominal resolution by using the triple mutant complex of ramelteouun-MT2-Gi-scFv16, enabling to model ramelteon, scFv16, significant portions of the receptor, MT1 and MT2 Gi protein receptors are assembled similarly to Gi protein, GPCRS, and G-protein complexes. The ramelteon and 2-iodomelatonin ligands bound to orthostatic pockets of MT1 and MT2 receptors. MT1-Gi bound to ramelteon bound and 2-iodomelatonin are structurally identical, showing 1 Å root mean square deviation values indicating complexes of Ca atoms and 0.8 Å values indicating the Ca atoms of MT1. Ramelteon-bound MT2-Gi and MT1-Gi are structurally identical showing 1.4 roots mean square deviation of receptors Ca. The regions involved in the engagement of G-protein and the extracellular side are structurally different.

B Melatonin's as signaling pathway

Receptor signaling

The intrinsic melatonin receptor affinity for different types of G proteins is not yet known. The relative expression of different proteins is dependent on the coupling profile of the G protein, accounting pharmacology of the melatonin receptor bias system. MT1 and MT2 receptors inhibit Adenyl cyclase after coupling to G inhibitory proteins. Melatonin receptor type 1 co-immunoprecipitated with Gαi3 and Gαi2 inhibitory proteins, has the least affinity for Gq/11 proteins and does not couple to Gαi1, Gαz, Gαo, Gα12, or Gαs proteins in HEK293 cells. The concentrations of inositol triphosphate, diacylglycerol, Ca2⁺, and cAMP are regulated by melatonin receptors in the cells [31]. Gα16 protein is expressed in hematopoietic cells, which illustrates the bias system [32].Melatonin receptors type 1 and type 2 couple to Gα16 protein through Jun N-terminal kinase. In COS-7 cells, the melatonin signaling pathway is initiated [33]. In tissues and cells, Gq/11 couples to melatonin receptors endogenously in the myometrium, prostate [34], pancreatic cells and epithelial cells [35] and mesenchymal stem cells of humans [36], cells from non-mammalian organisms [37] and cells which express recombinants [38]. Ion channels and multiple pathways are regulated by melatonin. Muscle contraction is modulated by melatonin in arteries [39]. Melatonin controls the myometrium's conductance of K⁺channels that Ca2⁺activates, and the activation of the Gi/cAMP/PKA and Gq/PLC/Ca2⁺signaling pathways modulates the function of these channels. Activation of gene transcription and inhibition of transcriptional factor

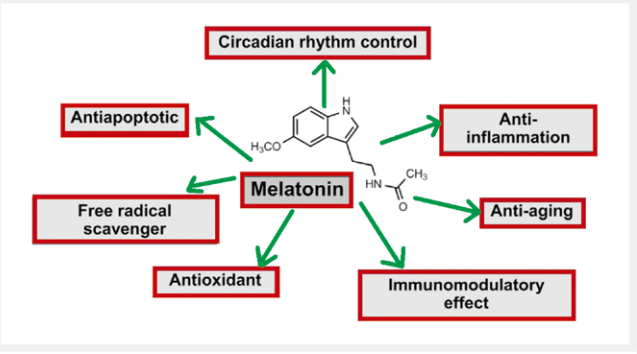


▶ **Fig. 6** Signaling pathways of MT1 and MT2 receptors: Different signaling pathways are activated by melatonin depending on the presence of cell stressors or cell types. MT1 receptors are mainly involved in these signaling pathways, and MT2 receptors also participated in these pathways and were studied in neurodegenerative disorders and under oxidative stress conditions, involving melatonin modulation of mitochondrial signaling mechanisms, that is, translocation of SIRT proteins and Bcl2/Bax is regulated. The Akt/FOXO1, ERK, and JAK2 complexes activated by melatonin induce the survival of cells and regulate stem cell differentiation. These signaling pathways are inhibited by melatonin in cancer cells. Anti-inflammatory and anti-oxidative effects are regulated by the transcription factors, Nrf2, PGC1α, and NF-κB, which depends on the activation of SIRT1. In hematopoietic cells, the JNK pathway is triggered by the coupling of MT1 to G16 protein. Expression of different miRNAs is regulated by melatonin in different types of cells, that is, cancer cells.

cAMP responsive element binding protein takes place through extracellular-signal-regulated kinase pathway at the transcriptional level. Melatonin receptors type 1 and type 2 are different only in the inhibition of cGMP synthesis during signaling. Melatonin receptor type 2 synthesizes cGMP, which is studied in human non-pigmented ciliary epithelial cells [40].

Signaling cascades and effects

Regulation of circadian rhythm has been extensively studied and based on system bias [8]. Melatonin affects the master clock and hypothalamic suprachiasmatic nucleus neurons and mediates in a cAMP-independent manner but a Gi-dependent manner. G protein-coupled receptors are activated, rectifying K-channels, that is, Kir3 in melatonin receptors type 1 [41], and melatonin receptor type-2 mediates action through the PKC signaling pathway [42]. Both receptors modulate neuronal actions through induced cAMP synthesis by pituitary Adenyl cyclase activating peptides (PACAP) in the suprachiasmatic nucleus [43]. Melatonin mediates action in a Gi-dependent manner and modulates gene expression in the striatum (▶ **Fig. 6**) [19]. Melatonin type 1 receptor can affect the rate of activation of cerebellar Purkinje cells by inhibiting P-type $Ca2^+$ channels via Gi/G/PI3K/PKC signaling [44]. Synchronizing effects in the hypophyseal pars tuberalis with SCN are mediated by melatonin. In order to control the production of mPer1, mCry1, clock, and Bmal1 genes, melatonin activates a heterologous repressive mechanism via MT1 and adenosine A2B receptors and sensitizes the cAMP pathway [45]. This signaling cascade is mediated by NPAS4, a transcription factor with a Per-Arnt Sim domain, and G protein regulators [46]. It is not fully understood how melatonin regulates circadian rhythm, but it appears to vary on cell type, including Clock Gene Transcription and Post-Translational Regulation [47]. Melatonin regulates the clock machinery of the retina. The



▶ **Fig. 7** Physiological action of melatonin hormone.

melatonin signaling mechanism in retinal physiology is still unknown [48].

Melatonin receptors of knockout mice showed variations in the expression of genes that control clock rhythm and other genes' expression [49]. Melatonin mediates action dependent on MT1/MT2 heteromers, activating Gq/PLC/$Ca2^+$ pathway and controlling light sensitivity in the retina at night [49]; regulation of photoreceptor is dependent on the Akt/FOXO1 signaling pathway [50]. During pathological and physiological conditions, the viability of neurons is regulated by melatonin. Melatonin shows neuroprotective and antiapoptotic and different signaling pathways. Melatonin helps in cell survival, maturation, and differentiation in the stem cells and is prevented by luz indole, a competitive receptor antagonist [51]. Melatonin stimulates neural development in pluripotent stem cells by activating the PI3K/Akt pathway, while luz indole inhibits this process [52]. Melatonin increases the glucose transporter GLUT1's activity, activating the PI3K/Akt and ERK pathways in ES cells to promote

pluripotency [53, 54]. Luz indole is vulnerable to neurons and is the main cause of neuron cell death in MT1-silenced cells. Melatonin upregulates different antioxidant enzymes, that is, SOD1 and glutathione peroxidase, and plays antiapoptotic and antioxidant roles in ischemia or reperfusion [55]. Neuroprotective effects of melatonin and ago melatonin in cerebral ischemia via upregulation of nuclear factor erythroid related factor 2 and downregulation of reactive oxygen species [56]. Neu-P11 ligand, which acts on both the 5-HT and melatonin receptors, activates multiple pathways critical to neuronal survival. These include the PI3K/Akt, ERK, and JAK2 pathways [57] (▶**Fig. 7**). Mitochondrial function and dynamics are responsible for the antioxidant and antiapoptotic effects of melatonin [15]. It activates caspase-3 and, prevents cytochrome c from being released and regulates Bcl-2 and Back expression [58]. Bax/Bcl-2 translocation is induced by the JAK2/STAT3 pathway in cardiomyocytes [59]. By inducing ERK activation and blocking p38 MAPK in monocytes, an antiapoptotic effect is produced [60]. Sirtuin histone deacetylase (SIRTs) is activated by melatonin through mitochondrial signaling pathways [61], that is, in hepatocytes, AMP-activated protein kinase (AMPK), sirtuin 3 (SIRT3), superoxide dismutase (SOD2), and sirtuin [62]. The transcription factor PGC-1α is controlled by the MT1 receptor in retinal cells [63]. The nuclear factor kappa B (NF-κB) pathway is inhibited by sirtuin 1 (SIRT1), which in turn causes the anti-inflammatory effects of melatonin (▶**Fig. 7**) [64]. Melatonin-induced MT1-dependent regulation of mitochondrial function in mice models is used to treat the neurodegenerative diseases of Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis [6]. Cytochrome c is inhibited by melatonin in brain mitochondria through mitochondrial MT1 receptors [53]. A cell-permeable melatonin receptor agonist was employed to distinguish between the mitochondrial Gi/cAMP cascade generated by MT1 and the rest of the cell [65]. Melatonin's neuroprotective effects are under-studied and is associated with mitochondrial MT1 signaling pathways. In the cancer field, melatonin impacts system bias on melatonin receptor cascades and shows antitumor properties by inducing apoptosis and inhibiting proliferation. MT1 receptors inhibit the phosphorylation of AKT, ERK, and PKC molecules in breast cancer models and show antitumor activity [66]. In these cells, melatonin activates the p53 DNA pathway dependent on the receptor, Akt, p38 MAPK and mTOR pathways are inhibited by melatonin in ovarian cancer.

Therapeutic application

The regulatory effects of melatonin on the sleep-wake cycle and circadian rhythm are crucial for a wide range of melatonin therapeutic applications. Due to its potential therapeutic effects, melatonin hormone is used to treat a variety of disorders, including jet lag, insomnia, circadian rhythm disorders, mood disorders, cancer, cardiovascular diseases, and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, as well as other seasonal affective disorders.

Cardiovascular system

Lipids metabolism

Melatonin reduces high serum total cholesterol and triglyceride levels in the blood. It improves lipid metabolism by decreasing low-density lipoprotein and hence maintains lipid profile [67].

Blood Pressure

Melatonin intake decreases systolic and diastolic blood pressure at night. It also decreases nocturnal systolic blood pressure [68].

Ischemia/reperfusion injury

Consuming melatonin reduces the severity of cardiac marker injury and myocardial infarction size. It lowers heart rate by increasing ejection fraction. Melatonin intake improves blood pressure, glycemic, and lipid in patients suffering from chronic heart diseases [69].

Heart Failure

Owing to antioxidant and antiapoptotic properties, melatonin intake decreases cardiac fibrosis in nonischemic heart failure. Its intake prevents myocardial infarction [70].

Nervous System

Ischemic stroke

Melatonin intake is helpful in decreasing infarct volume and brain edema and improving neurologic score [71].

Alzheimer's disease

Melatonin intake is helpful in improving sleep quality and cognitive function in the brain. Its intake treats sleep disorders associated with amnesia, sundowning, and Alzheimer's diseases [72].

Parkinson's disease

As it delays the degeneration of dopaminergic neurons in the substantia nigra, melatonin intake is beneficial in the treatment of Parkinson's disease. Cognitive dysfunction, anxiety, depression, and sleep quality all improve as a result [73].

Epilepsy

Melatonin improves sleep behavior in epilepsy patients [74].

Migraine

Melatonin is used to treat migraine and prophylaxis in both adults and children [75].

Chronic tension-type headache

Melatonin intake improves sleep quality and prevents headache and tension [76].

Traumatic brain injury and spinal cord injury

Melatonin is used to treat sleep disturbances in patients with traumatic brain injury who report feeling anxious [77].

Reproductive system

Erectile dysfunction

Consuming melatonin enhances the corpus cavernosum's ability to contract and relax. It improves endothelial density and erectile function [78].

Female reproductive system

Melatonin intake improves fertilization rate. It increases progesterone production in the corpus luteum [79].

Gastrointestinal system

Gastroesophageal reflux disease and gastrointestinal ulcer

Melatonin provides protection against mucosa oxidative damage in different types of gastrointestinal tract ulcers, reduces relaxation duration, and increases gastrin [80].

Irritable bowel syndrome

Melatonin decreases abdominal pain, bloating, and constipation and increases rectal pressure [81].

Hepatic steatosis

Melatonin lowers liver cholesterol, triglycerides, serum AST and ALT levels in hepatic steatosis patients [82].

Hepatoprotective effects

Hemorrhagic shock, ischemia-reperfusion injury, liver damage, ionizing radiation, and Schistosoma mansoni infection are all diminished by melatonin [83].

Renal system

Reno protective effects

Melatonin protects against radiation-, folic acid-, aminoglycoside-, contrast-mediated-, and nephrotoxicity-induced by these agents. It reduces creatinine and blood urea nitrogen levels [84].

Sepsis-induced renal injury

Melatonin intake decreases inflammasome activation [85].

Dermatology

Melatonin protects against UV-light-induced damage by preventing the production of free radicals, erythema caused by natural sunlight, and radioprotective effects. It exhibits antiaging properties, enhances hydration, and lessens the roughness of the skin [86].

Fibromyalgia

Melatonin intake improves pain level and fibromyalgia [87].

Autism spectrum disorder

Consuming melatonin lowers mid-sleep awakenings, improves sleep quality, and lengthens total sleep time [88].

Mood disorder

Melatonin diminishes sleep disorders linked to a depressed mood [89].

Oncology

Breast cancer

Due to its antiestrogenic effects, which lessen unwanted side effects, melatonin adjuvant therapy is used for patients who are at risk of developing ER-positive breast cancer [90].

Ovarian cancer

During chemotherapy, melatonin provides protection to ovaries and fertility preservation [91].

Lung cancer

Melatonin lessens harm to the ileum, colon, liver, and lungs. It lessens radiation-induced lung injury and modulates the effectiveness of DNA repair in humans as well as the genotoxic activity of irinotecan [92].

Colorectal cancer

Because urosolic acid has antiproliferative and pro-apoptotic effects on colon cancer cells, melatonin is used to treat this disease [93].

Viral syndromes

Respiratory Syncytial Virus

The acute lung oxidative injury brought on by respiratory syncytial virus is lessened by melatonin [94].

Viral myocarditis

Melatonin is used for the preservation of cardiac functions and for repression of virus-induced cardiomyocyte apoptosis. It inhibits apoptosis, regulates the rate of autophagy, and maintains mitochondrial dysfunction [95].

Venezuelan equine encephalitis

Melatonin intake increases survival rate by reducing virus load in the brain and serum [96].

Encephalomyelitis virus

Melatonin is used to treat encephalomyelitis by preventing death and paralysis [97].

Semliki forest virus

Melatonin lowers viremia and postpones disease and death [98].

COVID-19

Consuming melatonin prolongs survival time by reducing oxidative damage and slowing down the release of cytokines [99].

Future prospects

Future directions in studying the signaling pathways and receptors for the melatonin hormone appear promising. To better comprehend the signaling pathways underlying melatonin action, the structure of the MT1 and MT2 receptors in association with various ligands and signaling molecules must be determined. Recent research has focused on the implicit role of the MT1 and MT2 receptors in a variety of sleep, cancer, and metabolic disorders. Creating new medications and adjusting melatonin's signaling pathways to treat complaints. Just two examples of the signaling pathways that interact with melatonin signaling are the cAMP and mitogen-activated protein kinase (MAPK) pathways. To find new signaling pathways, researchers are examining how the melatonin hormone binds to receptors that are similar to orphan GPCRs. More study is required to determine new therapeutic targets for melatonin and its analogs. More research is required to fully comprehend its mechanisms of action and to maximize its therapeutic application. Downstreaming signaling mechanism is still unknown. All studies about melatonin effects were assessed in in vivo and in vitro testing and preclinical trial but lacked

significant clinical trials. So, there is a huge need to conduct clinical trials to fully understand long-term melatonin's physiological effects. The signaling mechanism of melatonin receptors, their structures and their interaction with the melatonin hormone are still unknown, so there are a lot of unexplored future directions for researchers. Researchers may be able to develop potent new treatments for a variety of diseases and disorders if they learn more about the receptors involved and the signals they send. Melatonin's regulatory effects on several physiological systems make it promising for a variety of therapeutic purposes.

Conclusion

Melatonin, a hormone produced by the pineal gland, controls circadian rhythms, sleep-wake cycles, and other physiological processes. G-protein-coupled receptors (GPCRs), of which two are known as MT1 and MT2, mediate melatonin's effects on the body. Molecular modeling and X-ray crystallography are two methods that have been used to determine the structures of the melatonin type 1 and type 2 receptors. Each of these receptors has seven transmembrane domains that connect the intracellular and extracellular domains together. A conformational change that occurs when melatonin binds to its receptors causes downstream signaling pathways to become active. Recent research continues to focus on the receptors and signaling pathways that melatonin mediates. Due to the multifactorial pathophysiology of melatonin, the majority of the studies were preclinical, in vitro, with small sample sizes, and concentrated on short-term results. Additionally, more research is required because inadequate study methods constrain decision-making regarding this new use for this medication. Before applying this knowledge to clinical practice, more clinical trials with larger sample sizes, precise dosages, and longer durations are required to confirm the long-term side effects of melatonin. More investigations of the receptors and signaling mechanisms that melatonin uses to exert its beneficial therapeutic effects are required.

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Remarks

A previous version of this manuscript has been deposited on a preprint server [100].

Conflict of Interest

The authors declare that they have no conflict of interest.

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