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Melatonin receptors: latest insights from mouse models

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Abstract

Summary—Melatonin, the neuro-hormone synthesized during the night, has recently seen an unexpected extension of its functional implications towards type 2 diabetes development, visual functions, sleep disturbances and depression. Transgenic mouse models were instrumental for the establishment of the link between melatonin and these major human diseases. Most of the actions of melatonin are mediated by two types of G protein-coupled receptors, named MT_1 and MT_2, which are expressed in many different organs and tissues. Understanding the pharmacology and function of mouse MT_1 and MT_2 receptors, including MT_1/MT_2 heteromers, will be of crucial importance to evaluate the relevance of these mouse models for future therapeutic developments. This review will critically discuss these aspects, and give some perspectives including the generation of new mouse models.

Keywords

melatonin; melatonin receptors; sleep; circadian rhythm; sleep; diabetes; retina; photoperiodism

Introduction

Melatonin is a neuro-hormone primarily synthesized by the pineal gland, but other cell types [e.g., retinal photoreceptors] are also capable of synthesizing it [1]. In the vast majority of organisms so far studied, melatonin synthesis occurs during the night, and duration of the synthesis is related to the length of the dark period [2].

In mammals, pineal melatonin synthesis is under the control of the master circadian pacemaker, which is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN controls the timing of pineal melatonin synthesis via a sympathetic pathway that involves the activation of Arylalkylamine N-acetyltransferase (AANAT, the key regulatory enzyme in melatonin synthesis] via the cAMP-CREB pathway [1], Figure 1).

Two types of G protein-coupled receptors (GPCRs), named MT_1 and MT_2 [3-4], mediate the action of melatonin. These receptors are expressed in many different organs and tissues, and therefore melatonin modulates multiple aspects of human physiology. Consequently, dysfunction of the melatonergic system is often associated with sleep and circadian

dysfunction [5], diabetes [6-8], retinal diseases [9-10], depression [11-12], autism spectrum disorders [13-14], and many neurodegenerative diseases, such as Alzheimer and Parkinson diseases [15-16].

In addition to the action mediated by MT_1 and MT_2 receptors, melatonin also can act as a free-radical scavenger, and thus as an antioxidant [8]. The action of melatonin as an antioxidant is believed to play an important role in protecting cells from aging and some neurodegenerative diseases [8].

Melatonin is currently used by millions of people around the world as a natural supplement for circadian and sleep disturbance. However, the mechanisms responsible for the beneficial effect of melatonin are still not fully understood. A significant advance in understanding the role of melatonin in the modulation of different physiological functions has been obtained by the development of transgenic mice lacking melatonin receptors. This review will focus on two aspects of melatonin receptors biology: 1) recent advances in MT_1 and MT_2 receptors pharmacology, including MT_1/MT_2 heteromers, and 2) the role that melatonin-receptor knockout (KO) mice has played over the last decade in improving our understanding of the numerous effects of melatonin on circadian rhythm regulation, sleep, vision, glucose homeostasis, and reproduction.

Melatonin receptor pharmacology is becoming more diverse

The availability of transgenic mouse models for melatonin receptors has concomitantly increased the interest in the properties of melatonin receptors from this species for fully understanding the differences and similarities to their human counterparts. The pharmacological properties of recombinant MT_1 and MT_2 receptors have been determined in several species including humans and mice [4, 17-23]. A recent study revealed strikingly similar overall profiles between recombinant human and mouse melatonin receptors of the same type [19]. This suggests that compounds with high affinity for mouse receptors have a high chance of being readily transposable into humans. However, these similarities might be somehow limited when considering ligands selective for MT_1 or MT_2, as detailed in the next section.

Towards ligands selective for mouse MT_1 and MT_2 melatonin receptors

Identification of selective ligands is an important step toward the development of drugs with improved side-effect profiles. Out of 39 compounds tested on recombinant murine melatonin receptors expressed in Chinese hamster ovary cells, only cis-4-Phenyl-2-propionamidotetralin (4P-PDOT) was selective, showing approximately 100-times higher affinity for MT_2 [19]. Preferential binding of this compound to MT_2 was confirmed in a second study on mouse MT_1 and MT_2 receptors expressed in HEK293 cells, although the affinity for MT_2 was only 22-fold higher than for MT_1 [24]. When compared with the human MT_2 receptor, for which an approximately 300-fold higher affinity was reported [25], the selectivity of 4P-PDOT for mouse MT_2 seems to be lower. More recently, IIK7 (N-Butanoyl 2-[9-methoxy-6H-iso¬indolo[2,1-a]indol-11-yl]¬ethan¬amine) was identified as a highly selective ligand for mouse MT_2 receptors, having more than 1000-fold higher affinity for MT_2 versus MT_1 receptors [24]. For human MT_2 receptors, IIK7 shows only a 90-fold

higher affinity [26], indicating that despite a high overall conservation of pharmacological properties between species, there might exist some important differences between human and mouse receptors for MT_1 and MT_2 selective compounds. While these results on the IIK7 melatonin receptor agonist are encouraging, more systematic studies will be necessary to identify further selective melatonin receptor ligands and, in particular, MT_1-selective agonists and MT_1 and MT_2-selective antagonists.

New insights from old and new melatonin receptor tracers

The overwhelming majority of ligand binding data on melatonin receptors have been obtained with the 2-[^{125}I]-MLT radioactive tracer [21]. The tritiated [^{3}H]melatonin tracer has been used less often because of its low specific activity. It is important to note that both radioligands are agonists, and thus are potentially able to sense the low affinity [G protein uncoupled] and high affinity [G protein-coupled] state. These two affinity states have indeed been detected in several studies [27-30]. Depending on the cellular background, the species and receptor type, only 15%–40% of the receptors were in the uncoupled state. These observations are fully compatible with previous observations in native tissues and transfected cells, demonstrating the formation of a stable complex between melatonin receptors and G_i proteins [31-33].

The recent study from Legros *et al.* [30] showed that affinities for ligands were similar for MT_1 and MT_2 receptors in the uncoupled state, the known MT_1 and MT_2 -specific differences [three- to tenfold] were only observed in the G protein-coupled state. This is an interesting point, as it suggests that the differences in binding affinities between receptor types are not due to intrinsic affinity differences between the binding sites of MT_1 and MT_2 receptors, but rather to the formation of different ternary [agonist-receptor-G protein] complexes, which bind melatonin ligands with different affinities. This conclusion is consistent with previous observations showing the formation of differential MT_1 and MT_2 receptor signaling complexes [34-35].

Recently, three further radiolabeled melatonin receptor agonists have been described [27]. The SD6 and S70254 compounds are based on the indole structure of melatonin carrying an iodoacetaminde side chain. The DIV880 compound has a different structure and corresponds to the iodinated analog of a bromo-compound identified in a screening project. Whereas SD6 has equally high affinity for human MT_1 and MT_2, [^{125}I]-S70254 and [^{125}I]-DIV880 bind only to MT_2 receptors with high affinity. Interestingly, different radioligands detected different numbers of binding sites, suggesting that labeling of different receptor subpopulations depending on the radioligand. This supports the notion of the stabilization of ligand-dependent receptor conformations and formation of ligand-dependent signaling complexes.

In conclusion, melatonin receptor tracers with agonistic properties are able to detect different G protein-coupled and -uncoupled receptor complexes. Further studies will be needed to identify the first radiolabeled melatonin receptor antagonist, which will allow the detection of melatonin receptor binding sites independent of receptor activation.

Melatonin receptor oligomers are functionally relevant

Early studies suggested that melatonin receptors have the capacity to form dimers or higher-order oligomers [36-37]. A particularly interesting aspect of these studies was the possibility of MT_1/MT_2 heteromer formation. The potential physiological significance of these in vitro observations is supported by the co-expression of both receptor types in several tissues and the existence of a heteromer-specific pharmacological profile [38]. However, direct proof for formation of MT_1/MT_2 heteromers was lacking until recently. A new study supports the idea that MT_1/MT_2 heteromers do indeed exist in the mouse retina, where they are responsible for the melatonin-dependent increase in light sensitivity at night [24]. Co-expression of MT_1 and MT_2 in photoreceptor cells was shown at the mRNA level and heteromer formation at the protein level by co-immunoprecipitation and proximity ligation assay. By using MT_1 and MT_2 KO mice and transgenic mice overexpressing a dominant negative MT_2 receptor inactive mutant, we could show that activation of both receptor types is mandatory to trigger the effect of melatonin on retinal light sensitivity. This effect was blocked by 4P-PDOT and luzindole, consistent with the idea that these two ligands are antagonist for MT_1/MT_2 heteromers. Injection of a low dose of the MT_2-selective agonist IIK7 activating only the MT_2 receptor protomer was unable to mimic the effect of melatonin. However, a higher dose of IIK7, activating MT_2 and MT_1 protomers, fully recapitulated the effect of melatonin. This study provides the first conclusive evidence for the existence and functional relevance of MT_1/MT_2 heteromers. These results can possibly be extended to humans, for which co-expression of both receptor types has been reported in photoreceptor cells [39-40]. Based on the large panel of tissues co-expressing MT_1 and MT_2 receptors, MT_1/MT_2 heteromers possibly exist in several other tissues and might be linked to other melatonin-related functions that have to be explored in future studies.

Signaling pathways: refining the perspective

MT_1 and MT_2 receptors have been shown to activate several signaling pathway [4, 35]. Both receptors are tightly coupled to the Gi/cAMP pathway, which appears to be also the case for MT_1/MT_2 heteromers [24] (Figure 2). Indeed, in vitro studies in transfected HEK293 cells suggest that heteromers inhibit forskolin-promoted cAMP production more potently and with higher efficiency with a more than tenfold difference in EC_{50} for melatonin as compared to homomers. The physiological meaning of this difference remains to be elucidated. Apart from coupling to the G_i/cAMP pathway, the MT_1 receptor has been shown to couple to the G_q/PLC/Ca^{2+} pathway [32]. Studies on mouse MT_1/MT_2 heteromers indicate a significant amplification of the activation of this pathway in cells co-expressing both receptor types with improved amplitude and EC_{50} values as compared to cells expressing MT_1 alone [>tenfold difference], shifting the melatonin dose-response curve more toward physiological melatonin concentrations. MT_2 receptors were completely inactive in this pathway. Thus MT_2 can be considered as a positive allosteric regulator of MT_1 receptors in respect to the activation of the G_q/PLC pathway. Collectively, these data indicate that MT_1/MT_2 heteromers are coupled to G_i-and G_q-dependent signaling pathways, and that heteromers tend to be more potent and efficient in activating these pathways most likely due to positive allosteric interactions occurring at the receptor level [41].

Melatonin has been show to activate other major signaling pathways such as the ERK1/2 and the PI3K/AKT pathways [7, 34, 42-43] (Figure 2). However, a full and detailed picture is still lacking. Information on the respective capacity of melatonin receptor homo- versus heteromers as well as the precise pathway used has still to be clarified. Currently available results suggest cell-type-dependent differences ranging from stimulation and activation of these pathways [44]. Furthermore, inhibition of the guanylyl cylcase pathway, which has been shown to be MT_2-specific [45], also will be interesting to evaluate in the context of MT_1/MT_2 heteromers.

Taken together, research in recent years has revealed that melatonin receptor signaling is much more diverse than initially suspected. Depending on the cellular context, melatonin receptor homo- and heteromeric complexes can be formed. Recruitment of different G proteins and regulatory proteins into these complexes further diversifies receptors' signaling capacity.

Melatonin-proficient versus melatonin-deficient mice

As noted, murine melatonin receptors appear to show a pharmacological profile that is similar to human receptors [19], but a clear understanding of melatonin signaling in mice is still lacking. This lack of data is due to the fact that there is some controversy about the capability of different strains of laboratory mice to produce melatonin. According to many authors, the vast majority of mouse strains are genetically incapable of synthesizing melatonin [46-49], and only CBA and C3H are considered melatonin-proficient strains, whereas most of the other laboratory strains [e.g., C57/BL6, Balb/C, SV129] are considered melatonin-deficient mice. However, it is worth noting that other authors have reported that C57/BL6 and many other strains [OF1 Swiss, BALB/c,] may also produce a small amount of melatonin for a brief period during the night [50-51]. Although the amounts of melatonin produced in these mice is small (10-30 pg/pineal vs. 200-300- pg/pineal of C3H or CBA mice [47, 50-51]), we cannot exclude the possibility that these levels of melatonin are sufficient to activate the MT_1 and MT_2 receptors in these so-called melatonin deficient-mice.

The recent **i**dentification of the *Hiomt* gene in the mouse genome [52] will facilitate the development of a "real" melatonin-deficient mouse [i.e., a mouse without the gene that codify for the enzyme responsible to convert N-acetylserotonin to melatonin]. The development of such a mouse will facilitate first the full understanding of the action of melatonin and then—by back-crossing these mice with melatonin-receptor KO mice—will also allow us to dissect the actions of melatonin as antioxidant from those mediated via the G protein-coupled receptors.

Melatonin receptor knockout mice as a key tool to understand melatonin action

Over the last 20 years, the use of transgenic mice in which specific genes have been ablated has provided important tools for understanding the function(s) of a specific gene. Melatonin-receptor KO mice were first developed by the Reppert laboratory in the late nineties [23,

53], and during the last decade many studies have used these transgenic mouse lines to investigate the effects that melatonin-receptor removal produces on the mouse physiology. These studies have provided clear experimental evidence on the importance of melatonin signaling in the regulation of many biological functions.

Circadian Rhythms

Several studies have shown that melatonin plays an important role in the entrainment of circadian rhythms, and remains the only pharmacological tool used to treat circadian dysfunction in humans [54]. Melatonin receptors are expressed in the SCN [3], MT_1 being the most prevalent receptor. Administration of exogenous melatonin to SCN slices in vitro induces an acute inhibition of the neuron firing rate and phase-shifts the circadian rhythms of neuronal firing [55-57]. However, since most of the MT_1 and MT_2 receptor agonists and antagonists available lack the specificity to fully dissect the action of melatonin [24], this approach has not provided conclusive evidence about the role of the specific role of MT_1 and MT_2 receptors in the modulation of SCN function by melatonin.

The use of the melatonin receptors KOs has provided a clearer picture on the mechanisms by which melatonin can influence the circadian mammalian system. In the initial study using MT_1 KO ($MT_1^{-/-}$), it was reported that the inhibitory effect of melatonin on SCN neuronal activity was no longer present, whereas the phase-shift response to melatonin appeared to be normal [23]. In addition, the phase-shifting response to melatonin in $MT_1^{-/-}$ was blocked by pertussis toxin [23]; thus suggesting that MT_2 receptors may be responsible for this phenomenon. Further studies have also shown that melatonin -- via MT_1 receptors -- modulates cAMP responsive element (CREB) phosphorylation in the mouse SCN, since the induction of CREB phosphorylation induced by Pituitary adenylate cyclase-activating polypeptide (PACAP) was inhibited in $MT_1^{-/-}$ mice [58]. This suggests that melatonin interacts with the circadian clock machinery via the cAMP-signaling pathway. However, it is important to note that at high melatonin concentration (100 nM and higher), melatonin can also affect the cAMP signaling pathways in mice lacking MT_1 receptors, and co-application of 4P-PDOT abolished the inhibitory effects of melatonin on CREB phosporylation [58]. This suggests that MT_2 may also be involved in the modulation of the cAMP pathway when melatonin is administered at pharmacological doses.

Indeed, a series of studies have indicated that MT_2 receptors mediate the phase-shifting effect on melatonin in the SCN [59-60] via the PLC-PKC pathway [61]. To resolve this issue, Jin *et al*., [53] investigated the effect of melatonin administration on the SCN of MT_2 KO mice [$MT_2^{-/-}$]. As expected in these mice, the inhibition of the SCN neural activity by melatonin was not affected, and the reduction in pCREB observed in $MT_1^{-/-}$ at higher concentration is no longer present in $MT_1^{-/-}MT_2^{-/-}$, hence confirming that both receptors are involved in this response.

Finally additional studies have indicated that, although the activation of MT_2 receptors is necessary for the phase-shift of the SCN firing in vitro, activation of MT_1 receptors may be required for the melatonin-meditated phase shift of the circadian rhythms in locomotor activity [62] and the re-entrainment of the circadian rhythm of locomotor activity to six-hour phase advances is significantly slower in mice lacking MT_2 receptors [63].

Sleep

In addition to being a well known player in the regulation of circadian dysfunctions, melatonin is also involved in the regulation of sleep. Although rodents are nocturnal animals (i.e., differently from humans they sleep during the day time when melatonin levels are low) these animal models may still represent an important tool to dissect the mechanisms by which melatonin can modulate sleep. Indeed studies in rats have shown that administration of melatonin can affect several sleep parameters such as rapid eye movement (REM) and non-rapid eye movement (NREM) sleep [64-65], whereas other studies have questioned the effectiveness of melatonin to affect sleep [66-67]. A recent study using a MT_2 receptor agonist (IIK7) has suggested that the action of melatonin on sleep is mediated by MT_2 receptors [68]. However, as noted, most of the melatonin agonists or antagonists available lack the specificity to conclusively demonstrate the type of melatonin receptors involved in the modulation of sleep. Therefore the use of a mouse lacking melatonin receptors is an essential tool for dissecting the contribution of melatonin to the regulation of sleep. Indeed, two recent studies have provided compelling experimental evidence on the involvement of melatonin receptors in the regulation of sleep in mice. In general it can be said that in mice melatonin can promote NREM sleep by acting on MT_2 receptors located in the reticular thalamic nucleus [69]. This idea is supported by experimental data showing that infusion of a MT_2 agonist in this nucleus or systemic administration increased the firing rate of the neurons in this area and such an effect can be blocked by the administration of a MT_2 antagonist [69]. Consistently with these pharmacological data, mice lacking MT_2 receptors showed a decrease in NREM sleep during the light phase [i.e., when mice usually sleep], whereas mice lacking MT_1 increase the amount of NREM sleep during the dark phase. Furthermore, MT_2 KOs increased the time of wakefulness during the light phase, and MT_1 showed a decrease in wakefulness during the dark phase. Finally, in WT and $MT_2^{-/-}$, REM sleep lasted longer in the light phase than in the dark phase, and $MT_1^{-/-}$ spend the same amount of time in REMS during the light or dark phase. Such a result would suggest that MT_1 receptors may be involved in the regulation of the daily rhythm of REM [69]. Finally, an additional study, in which MT_1/MT_2 receptor KO mice were used, demonstrated that these mice showed an increase in wakefulness [probably due to the lack of MT_2 receptors], and a reduction in REM sleep (as a consequence of the MT_1 removal) [70]; this suggests that removal of melatonin receptors affects wakefulness rather than sleep. Thus the use of melatonin-receptor KO mice indicated that melatonin and its associated receptors are involved in the regulation of sleep and wake cycle, and MT_1 and MT_2 receptors differently affect the sleep and wake cycle.

Although these studies have laid the foundation for the dissection of melatonin receptors' contribution to sleep, it must be noted that much work remains to be done. For example, the mice used in these studies were complete KO: therefore the use of inducible conditional melatonin-receptor KO mice in brain areas involved in the regulation of sleep and wakefulness may provide additional clues as to the role of melatonin in the regulation of sleep, and might be helpful in developing new tools to treat sleep disturbances.

Vision

As we have previously mentioned melatonin is also synthesized in the retina, where it plays an important role in the regulation of retinal physiology by acting on MT_1 and MT_2 receptors that are widely distributed in the retina and in other ocular structures [71-75]. Interestingly, melatonin receptors are abundantly expressed on the photoreceptor cells, thus suggesting that melatonin, in this case, may act as an autocrine signal to regulate its own synthesis.

Removal of these receptors has a profound effect of the retinal physiology, since it appears that melatonin via MT_1 actually controls the daily rhythms of the scotopic and photopic electroretinogram (ERGs) and the scototopic threshold response [73-75]. Furthermore, the circadian regulation of the photopic ERG is also absent in $MT_1^{-/-}$ mice; thus demonstrating that MT_1 receptor signaling is required for the circadian regulation of the photic ERGs [75]. MT_1 receptor removal also affects the viability of the photoreceptors and the retinal ganglion cells during aging [73].

Finally, a recently published study has shown that MT_2 receptors have similar distribution of MT_1 receptors within the retina, but MT_2 mRNA seems to be absent in retinal ganglion cells [24]. Surprisingly, removal of MT_2 receptors phenocopied the effects on the ERGs produced by the removal of the MT_1 receptors [24], thus suggesting that in the mouse retina, and more precisely in the mouse photoreceptors, MT_1 and MT_2 receptors form heterodimers. Indeed, additional studies confirmed the presence of a functional MT_1/MT_2 heterodimer in the mouse photoreceptors [24].

These recent studies on the role played by melatonin and its associated receptors in the modulation of visual function have clearly demonstrated that melatonin is indeed a key player in retinal physiology. Removal of these receptors affects the viability of the photoreceptors and retinal ganglion cells, thus suggesting that melatonin can represent useful tools in the prevention of retinal cell loss that often occurs during the aging process and in some pathological conditions that are associated with aging (i.e., age related macular degeneration and glaucoma).

Diabetes

One of the new and most exciting news of the recent years in the melatonin field has been the discovery that polymorphisms in the genes encoding human melatonin receptors (*MTNR1A and MTNR1B*) may be in involved in the pathogenesis of type-2 diabetes (T2D) [6-8, 76]. These studies have confirmed earlier studies in rats that established a possible link between melatonin and glucose metabolism [77-78, Figure 3]. In more recent years, data supporting a specific role for melatonin in the modulation of insulin secretion has been documented. Indeed, both melatonin receptors are present within pancreatic islets, although there are some discrepancies in their relative distribution. While both receptors were found to be present within β-cells of human and rodent islets [5], a study utilizing islets derived from melatonin-receptor KO mice reported expression of MT_2 solely in β-cells and exclusive expression of MT_1 within pancreatic α-cells [79]. However, regardless of discrepant observations in localization, several studies have arrived at the conclusion that

melatonin receptors exert a predominantly inhibitory effect, at least in rodent β-cells on insulin secretion via receptor-mediated attenuation of adenylate cyclase and guanlylate cyclase [80-83]. In contrast to the insulin secretory response, glucagon is secreted from pancreatic α-cells in response to low blood glucose levels and stimulates hepatic glucose output. In vitro studies utilizing a glucagon producing mouse pancreatic α-cell line, αTC1.9, established that melatonin administration produces a direct stimulatory effect on glucagon secretion via a PLC dependent mechanism [84]. This secretory response was subsequently blocked in the presence of luzindole and 4P-PDOT; thus demonstrating that melatonin receptors within pancreatic islets are coupled to signaling pathways involved in the modulation of both insulin and glucagon secretion.

In addition to studies examining the role of melatonin on the secretion of gluco-regulatory hormones, it has been postulated that signaling through melatonin receptors enhances systemic glucose tolerance via a direct effect on glucose uptake. In support of this notion, in vitro studies have demonstrated that melatonin administration, independent of insulin, is capable of stimulating glucose uptake in both skeletal muscle and adipose tissue [42, 85], and, in mouse muscle cells, the effects of melatonin on glucose uptake were found to be mediated via an IRS-1/PI-3-kinase-dependent pathway [41]. Along similar lines, intra-cerebroventricular infusion of melatonin in rats proved capable of stimulating tyrosine phosphorylation of the insulin receptor, Akt, and IRS-1 [86]. These results support the intriguing possibility of intracellular cross talk between the melatonin and insulin signaling system.

In line with the data obtained in rodents, recent genome-wide association studies also have demonstrated that polymorphisms in both MT_1 and MT_2 are associated with altered glucose metabolism. Variants in MT_2 have been linked to impairments in both insulin secretion and increased fasting glucose levels [6-8], and variants in MT_1 have also been shown to be associated with an increased risk of developing polycystic ovarian syndrome, an endocrine disorder marked by insulin resistance and T2D onset [87]. By re-sequencing the coding region of the *MTNR1B* gene coding for the MT_2 receptor, 40 variants have been identified and functionally characterized. Corresponding mutants with impaired receptor signaling did strongly associate with the T2D risk, indicating that loss of melatonin receptor function is positively associated with disease risk [7].

To date, only a few studies have characterized mechanisms underlying glucose homeostasis in melatonin receptor KO mice [83, 88-89]. The first study examining the effect of melatonin on rhythms in glucose metabolism demonstrated that mice lacking MT_1 exhibited higher mean blood glucose levels than WT, $MT_2^{-/-}$, and $MT_1^{-/-}/MT_2^{-/-}$ mice [87], and a subsequent investigation further established the MT_1 KO mice are glucose intolerant, and insulin resistant, with respect to WT and $MT_2^{-/-}$ animals [89].

Additional support for an inhibitory role of melatonin signaling in the modulation of insulin secretion also can be found in a study in which islets derived from melatonin receptor KO mice were found to have enhanced insulin secretion in the presence of melatonin [1uM] compared to WT islets. However, it is important to note that these results could not be elicited using physiological concentrations of melatonin [10 nM], and in WT islets

attenuation of insulin secretion in the presence of melatonin could not be demonstrated [88]. In contrast to the studies examining insulin secretion, basal glucagon secretion was affected by the presence of melatonin receptors since basal glucagon secretion was significantly reduced in pancreatic islets of $MT_2^{-/-}$ and $MT_1/MT_2^{-/-}$ mice in comparison to WT and $MT_1^{-/-}$ mice [83].

Melatonin, photoperiod, and reproduction

Melatonin is synthesized at night, and the timing of its production correlates with the duration of the period of darkness. Thus melatonin signaling is believed to be the internal signal by which the organisms may perceive the seasonal changes in the photoperiod and thus regulate the reproductive cycles. In some vertebrate species, the length of photoperiod regulates the reproductive season (e.g., hamsters, sheep, and many others), and the *hypophyseal pars tuberalis* (PT) transduces the seasonal changes in the melatonin rhythmic profile into a pattern of prolactin secretion [90]. Although the reproductive system of the mouse is not sensitive to photoperiod, the development of the melatonin-receptor KOs have provided an important tool for dissecting the mechanisms by which melatonin regulates reproduction in photoperiodic species. Recent studies have shown that many of the genes and proteins that are responsible for the generation of the circadian oscillation are not only present in the SCN, but are also expressed in many peripheral tissues [91]. These genes are also found in the PT, and it is believed that melatonin signaling can affect the pattern of expression of these clock genes/proteins. Consistently with this hypothesis, it has been demonstrated that rhythmic expression of the clock gene *Period 1* in the pituitary gland depends on the heterologous sensitization of the adenosine A_{2b} receptors via the activation MT_1signaling during the night [92].

Additional studies have reported that the rhythmic expression of several other clock genes (*Per1, Per 2, Bmal1, and Cry 1*) in the mouse *PT* depend on MT_1 signaling as well [93], and MT_1 and MT_2 receptors are also involved in the control of the activity of lactotroph cells in the *pars dystalis* [94]. Finally, a recent paper has investigated the effect of melatonin, signaling removal on two genes responsible for photoperiod gonadal reproduction (e.g., type 2 and 3 deidodinase). These genes are present in the ependymal cell layer [EC] where they are transcriptionally regulated by the hormone thyrotropin secreted by the PT. In C3H mice with intact melatonin receptors, *Deiodinase 2* (*Dio2*) mRNA levels are low, and no significant changes are observed with a different photoperiod; whereas *Deiodinase 3* [*Dio3*] mRNA levels are significantly increased in a light-dark cycle with a short day. In mice lacking MT_1 and MT_1/MT_2 receptors, photoperiodic changes in *Dio3* mRNA were no longer present, whereas $MT_2^{-/-}$ mice retained the response [95]. Additional experiments using C57/BL indicated that administration of exogenous melatonin down-regulated *Dio2* mRNA and induced *Dio3* mRNA under a long day. These effects were not observed in C57/BL6 mice in which the MT_1 receptors had been genetically removed [95]. Such a result further indicates that MT_1 signaling is crucial for the photoperiodic response of gene expression in the EC and thus for the photoperiodic regulation of gonadal activity.

Conclusions and Outlook

The development of melatonin-receptor KO mice has significantly improved our understanding of melatonin in the regulation of mouse physiology; furthermore, it has helped to elucidate the underlying mechanisms and started to pave the way towards a potential role of melatonin in disease development. Several exciting aspects remain to be elucidated in the future. For example, the mechanism by which this hormone regulates circadian rhythms via MT_1 and MT_2 receptors is still not fully understood, and may differ between different tissues. The same questions remain to be solved for the action of melatonin in the modulation of sleep and glucose homeostasis and reproduction. Furthermore, the full impact of MT_1/MT_2 heteromers remains to be elucidated in the numerous tissues co-expressing both receptors. Finally, emerging experimental evidence indicates that, in human *post-mortem* samples, melatonin-receptor expression seems to be affected by aging [15], depression [11-12], Alzheimer's disease [15], and Parkinson's disease [16]. Therefore, it would be beneficial to explore whether mice lacking melatonin receptors might represent a good model for investigating the pathogenesis of these diseases.

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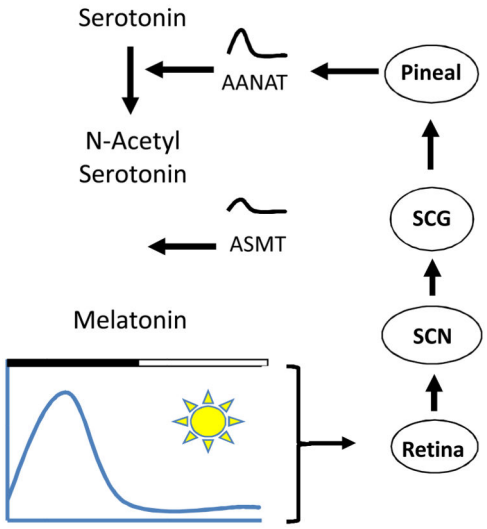
Figure1



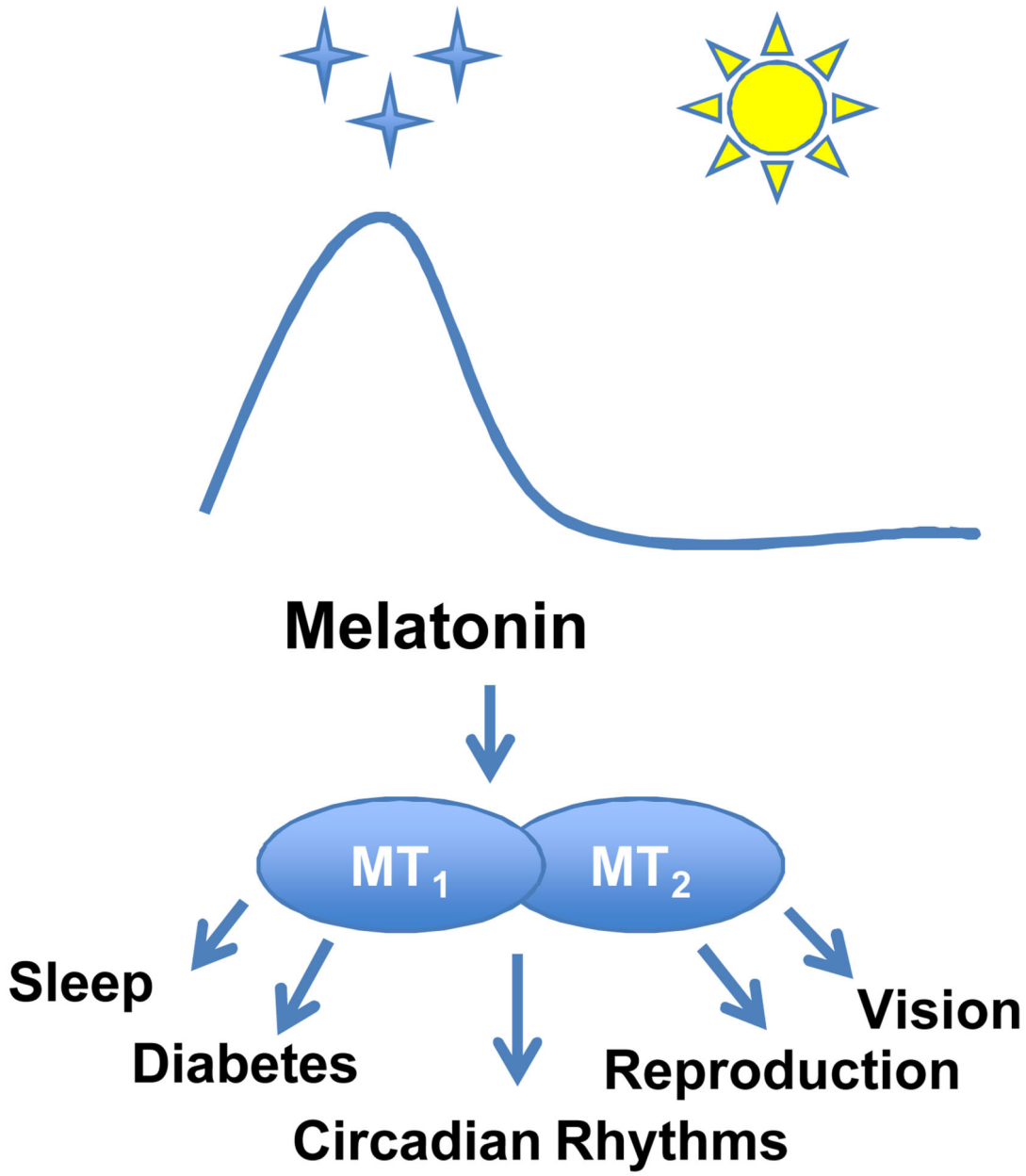


Figure 1.

Schematic drawing illustrating the regulation of pineal melatonin synthesis by the light/dark cycles via the SCN. During the daytime, the SCN inhibits melatonin synthesis, whereas at night the SCN sends a signal to the pineal to activate melatonin synthesis by increasing [over 100-fold] the transcription of the *Aanat* and then the activity of AANAT. AANAT converts serotonin to N-acetyl serotonin, and the Acetylserotonin N-Methyltransferase [ASMT] converts N-acetylserotonin to melatonin. ASMT transcription and activity also increases during the night, albeit to a lesser extent than AANAT.

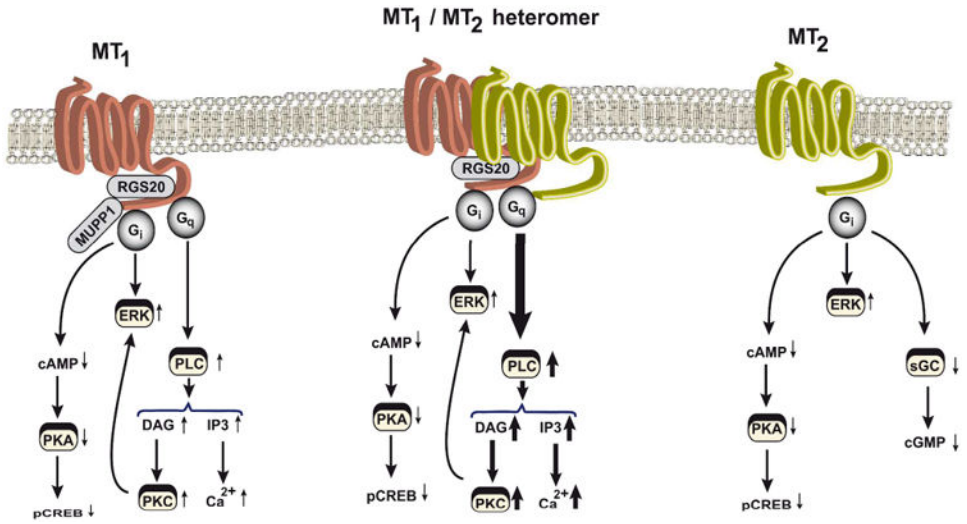


Figure 2.

Principal melatonin-receptor signaling pathways. Depending on the type of melatonin-receptor complexes present in cells (MT_1 homomers, MT_2 homomers, or MT_1/MT_2 heteromers), the depicted signaling pathways are activated upon melatonin stimulation. Thickness of the arrows represents the potency and efficiency of the activation of the pathway. DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; IP3, inositol triphosphate; MUPP1, multi-PDZ domain protein 1; pCREB, phospho-cAMP-response element-binding protein; PLC, phospholipase C; PKA, protein kinase A; PKC, protein kinase C; RGS20, regulator of G protein signaling 20; sGC, soluble guanylyl cyclase.

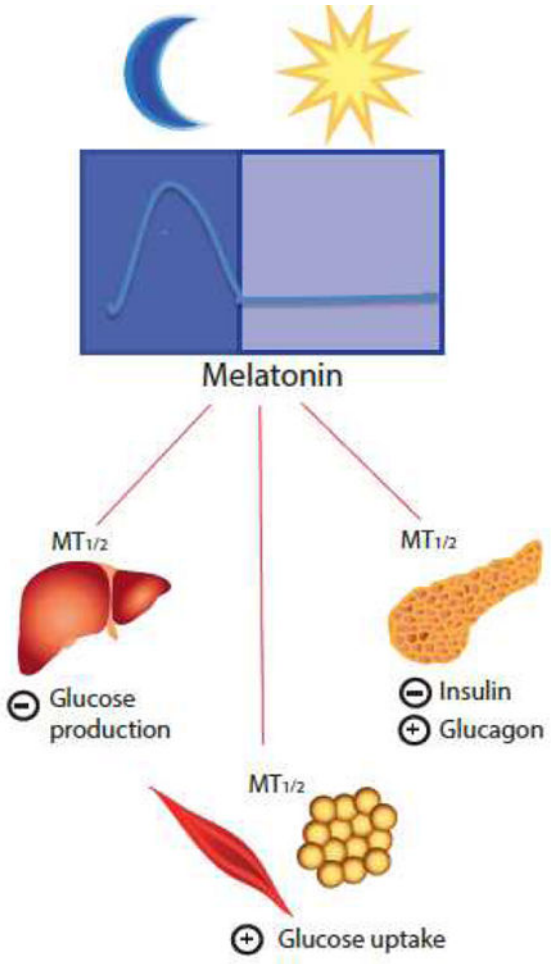


Figure 3.

Melatonin receptors are involved in the regulation of glucose metabolism. Evidence from in vitro and in vivo studies has demonstrated that signaling through melatonin receptors interacts with many facets of glucose metabolism. Within pancreatic islets, melatonin receptors are coupled to signaling pathways, which exert an inhibitory effect on insulin secretion from β-cells and a stimulatory effect on glucagon secretion from α-cells. In peripheral tissues, melatonin receptors appear to positively regulate glucose uptake within skeletal muscle and adipose tissue, and negatively regulate nocturnal glucose production by the liver.